FUNDAMENTALS OF ANATOMY AND PHYSIOLOGY OF FARM ANIMALS

(for Sandwich Agric. Edu. Student)





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First Published: 2015

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ISBN: 978-978-53588-1-0

Printed in Nigeria by:
Tunde-Babs Printers
08166715071, 08056566499

DEDICATION

To Prof A. G. Ologun, D. O. Adejumo and K.G. Aning my dear teachers and the great students of the Faculty of Agriculture, University of Ilorin, Nigeria.

PREFACE

There is no doubt that there are a number of text book available to students on the subject of anatomy and physiology. This book is designed to provide an introduction on animal anatomy and physiology that is suitable for teaching of undergraduate students of agriculture. It is presented in a format and style to make it easier for the readers to understand and to stimulate their interest towards more advanced study of this subject.

However, it is not intended to be a substitute for standard text books in anatomy and physiology of animals. It is meant to serve as an overview of the fields of anatomy and physiology since it contains as much facts as are required for passing the standard undergraduate university examination. The book will serve as a useful tool in anatomy and physiology for students preparing for examination in general agriculture, animal production and endocrinology. The book would have covered a major problem that plagued the students i.e. the acute dearth of readable and student friendly texts. The provision of this hand book is therefore borne out of my desire to fill this vacuum by providing this introductory anatomy and physiology from my lecture notes and other materials in order to touch basic subject areas in physiology.

ACKNOWLEDGMENTS

My first acknowledgment goes to the various authors of textbooks, journals, magazines and other numerous sources that I have made use of at one time or the other to make my lecture notes which culminated into this book. Whether cited or not, I hereby acknowledge every source of knowledge manifestly or remotely reflected here within.

The completion of this book involved the support of many people. I will like to start by acknowledging the support of my numerous students in the Faculty of Agriculture and the Faculty of Education, University of Ilorin.

In the midst of many some stands out clearly for their specific contributions to this book such as Prof. Adeloye A. A. a professor of ruminant nutrition, University of Ilorin; Prof. Osinowo O.A. a professor of animal reproductive physiology, Federal University of Agriculture, Abe-okuta;Prof. (Mrs) Adeyombo F. Bolarinwa a professor of Human Physiology, University of Ibadan and Prof. Adejumo D. O. a professor of animal physiology, University of Ibadan.

I also wish to acknowledge the detailed work of several physiologist and colleagues that edited this book in parts and advised on the order of compilation. I am very grateful to all of you. My special thanks to my dear wife, Mrs C.O. Okukpe for her patience and endurance for the periods spent behind the desk and her useful suggestions.

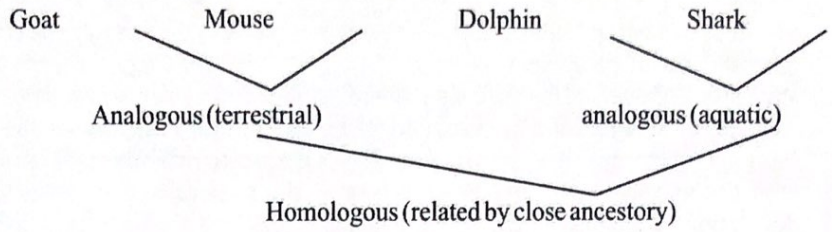
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INTRODUCTION

The study of plant and animals many years ago were done by the description of the general form of plants and animals referred to as **morphology** (analysis of structure). This marked the beginning of the discipline now known as anatomy. Anatomy meaning cut apart, has come to refer to that science which deals with the form and structure of all organisms. **Anatomy is the branch of science that deals with the external and internal structure of the organism**. It involves the description of the basic architectural patterns of cells, tissues, organs and the organisms. The science of anatomy is often subdivided into sub-disciplines based on the nature of the various component and the methods by which they are studied. For example, **gross (macroscopic) anatomy** includes all those structural features that are studied by direct visual inspection, palpation and/ or dissection, while histology involve the study of structures that are not visible to the unaided eyes. Hence, the terms **histology** and **microscopic anatomy** are synonymous since both are concerned with tissue structure and function from broad and multidisciplinary approaches.

The study in a typical gross anatomy laboratory is based primarily on dissection of animals that have been preserved by embalming, and one or more parts of the vascular system have been injected with a coloured material to facilitate identification of the vessels. Careful dissection with close observation gives the concept of the shape, texture, location, and relation of the structures that are visible to the unaided eye. The use of microscope with properly prepared tissue sections on slides is equally essential for an understanding of structures that are so small they cannot be seen without optical assistance. The anatomist applies the principles of physics to the different microscopes he uses, and applies knowledge of chemistry in the staining of various parts of cells/ tissues. There is an interrelationship of anatomy (structure) and physiology (function) and this is most clearly observed in comparative study of animal development. The development of individual animals from a fertilized egg to adulthood (maturity) is called **ontogeny** while the development of specific types or species of animals from their prehistoric origin to the present time is called **phylogeny.** The sequence of event in ontogeny in individuals often parallel that of phylogeny in the species. The knowledge of ontogeny and phylogeny could be of help in understanding the functions of structures in living organisms. In comparative anatomy, it is a known fact that two organisms can be ontogenically different but phylogenically the same, e.g. apes and human. In ontogeny or phylogeny, two individuals with similar developmental origin are referred to as **homologous** (corresponding in origin/ structure; opposite - heterologous) while those that are similar in function but not in origin are said to be **analogous.**



Physiology is the study of the integrated functions of the body, and the functions of all of its parts (systems, organs, tissues, cells, and cell components), including the biophysical and biochemical processes involved. It is the area of science that deals with the co-ordinated processes and activities taking place in living systems. Physiologists performs experiments usually with living animals probably anesthesized to avoid discomfort to gain a better understanding of normal function of the body and the effects of changes in environment (both internal and external) on the normal animal. This includes the use of drugs, changes in temperature or altitude, surgical modification and monitoring of changes such as blood pressure, heart rate, breathing, electrical changes in the heart, muscle contractions and nerve impulse velocities with the use of electronic equipment.

One of the special attributes of life processes is the limitation of body cells to operate within very narrow physiological confines. Body cells must be able to recognize sudden changes in conditions and put in place mechanisms for appropriate adjustments (adaptation). Even very small fluctuations in osmotic pressure, temperature or disruptions in the equilibrium of the internal environment can disrupt biochemical activities and in extreme cases result in termination of cellular functions. The body operates within a narrow limit, exceeding the limit will result in death. The field of physiology is an all encompassing discipline that has frontiers with biochemistry, anatomy, genetics and pharmacology. It is inadequate to discuss physiology without mentioning biochemistry. Biochemistry is concerned mainly with molecular events in the cell and it is therefore the study of nature and its interaction with biological materials. Biochemistry is also concerned with the elucidation of various metabolic pathways by which energy transformation occurs in cells. It inevitably involves the characterization of DNA and RNA, which are regarded as the basis of life. The most important aspects of body physiology are the integrity of the environment. The environment of an animal may be the chemical constituents (internal environment) or the external environment characterized by factors such as climate, disease, feed and management system.

The internal environment is the immediate surroundings of the cells and defines the nature of extra-cellular activities. Mammalian tissue cells are usually encompassed by tiny channels and numerous spaces filled with fluid. This fluid is called **intercellular** or **interstitial** or **tissue fluid** and provides the cells with the medium in which they have to live. **Intracellular fluid** exists within the cells. The need for a very efficient co-ordinatory mechanism for the body functions is based on the fact that the conditions in which cells can function properly are very narrow and small changes in osmotic pressure, temperature or the concentration of chemical agents such as cations, enzymes, drugs etc can affect biochemical activities and in extreme cases put an end to life. To survive, an animal must positively react to changes in its environment. This necessitates mechanisms for detecting such changes and putting the appropriate responses into operation. The science of physiology cannot be limited to the study of body systems functions alone. It also involves a thorough knowledge of structure, which throws considerable light on its function. Before the development of the light microscope, most animal study was based on gross anatomy. However scientists soon discovered that structure and function are inseparable because a mere description of structure without a good account of function is of very little practical value and as it is also impossible to acquire a sound understanding of function without a working knowledge of the type of structures involved, either at organ or molecular level. The major differences between anatomy and physiology are listed below:

Table 1: Differences between anatomy and physiology

Anatomy	Physiology
1. Involves the use of dead (fixed) matter	Living or functional matter is used
2. Record observations at a fixed time	Record changes occurring with time
3. Studies are static	Studies are active

SOME DESCRIPTIVE TERMS IN ANATOMY OF ANIMALS

1. **Morphology** is the analysis of structure, involves the use of dead (fixed) static matter.
2. **Gross (macroscopic) anatomy** is the study of form and relations (relative positions) of the structures of the body that can be seen with the unaided eye.
3. **Comparative anatomy** is a study of the structures of various species of animals, with particular emphasis on those characteristics that aid in classification.
4. **Embryology** is the study of developmental anatomy, covering the period from conception (fertilization of egg) to birth.
5. **Histology (microscopic anatomy)** is the study of tissues and cells that can be seen only with the aid of a microscope.

6. **Histochemistry** is the study of tissues and cells with the use of chemical substances to know the fluid state.
7. **Cytology** is the study of cells with the aid of the electron microscope
8. **Physiology** is the study of body functions; involves the use of active, living or functional matter.

Studyof----------**cells**----------**tissues**----------**organs**----------**system**

↓ ↓ ↓

Cytology **Histology** **Anatomy**

9. **Comparative physiology** describes the different mechanisms by which different species of animals meet their physiological needs. The emphases are on the special mechanisms that aid specie classification and normally provide the clue to the relationship between the organism and its place in the evolution table. It is deep rooted in phylogenetics.
10. **General physiology** is concerned with attempts to understand the basic principles underlying the biological activities in all living systems.

DIRECTIONAL TERMS describe the position of one organ relative to the other

11. **Cranial** or **anterior** - this means toward the head e.g. the shoulder is cranial to the hip i.e. it is closer to the head than is the hip.
12. **Caudal** or **posterior** this means toward the tail e.g. the rump is caudal to the loin.
13. **Dorsal** this means toward the back or beyond the backbone or vertebral column e.g. the kidneys are dorsal to the intestine i.e. the kidney are closer to the backbone than the intestine).
14. **Ventral** this means towards the underside of the body i.e. the surface facing the ground or away from the vertebral column e.g. the udder is the most ventral part of the body of a cow.
15. **Rostral or caudal** these are directional terms used within the head to mean toward the nose (rostral) and toward the tail (caudal).

PLANE OF REFERENCES (imaginary lines across the body)

16. **Median plane** - is an imaginary plane passing through the body craniocaudally, dividing the body into two equal halves i.e with every part represented in the halve (bilateral symmetry). An animal is split into two halves on the median plane.
17. **Sagittal plane** is any plane parallel to the median plane. It divides the body like the median plane but not into two equal halves. The median plane is sometimes called the **midsagittal plane.**
18. **Transverse plane** - this is a plane at right angle or perpendicular to the median plane and divides the body into cranial (anterior) and caudal (posterior)

Plane and divides the body into cranial (anterior) and caudal (posterior) segments. A cross section of the body would be made on a transverse plane.

19. **Frontal plane** this is a plane at right angle or perpendicular to both the median plane and transverse planes dividing the body into dorsal (upper) and ventral (lower) segments.

TERMS USED TO LOCATE ARE AS OF STUDY IN AN ANIMAL

20 **Medial** (adj.) mean close to or toward the median plane e.g. the heart is medial to the lungs i.e. it is closer to the median plane than are the lungs.

21. **Lateral** (the antonym of medial) mean towards the sides or away from the median plane e.g. ribs are lateral to the lungs i.e. they are farther from the median plane.

22 **Deep** or **internal** this refer to closeness to the center of gravity or the center of an extremity e.g. the humerus (arm bone) is deep to all other structures in the arm.

23. **Superficial** or **external** this refer to proximity to the skin or surface of the body or surface of an extremity e.g. hair is superficial to all other structures of the body.

24. **Proximal** means relatively close to a given part, usually the vertebral column, body, or center of gravity. Proximal is generally used in reference to portions of an extremity or limb e.g. carpus or knee is proximal to the foot.

25. **Distal** means relatively farther from the vertebral column e.g. hoof is distal to the carpus or knee. The suffix **ad** is used to indicate movement in the direction of, or toward, as in **dorsad, ventrad, caudad, craniad**.

26 **Palmar (volar)** refers to the flexion or caudal surface of the forelimb distal to (below) the elbow. Dorsal, when used in reference to forelimbs, refers to the opposite (cranial) side.

27 **Plantar** refers to the caudal surface of the hind-limb below the hock, and dorsal refers to the side directly opposite (the cranial side).

28. **Prone** refers to a position in which the dorsal aspect or dorsum of the body or any extremity is uppermost. **Pronation** refers to the act of turning toward a prone position.

29. **Supine** refers to the position in which the ventral aspect of the body or volar or plantar aspect of an extremity is uppermost. **Supination** refers to the act of turning toward a supine position.

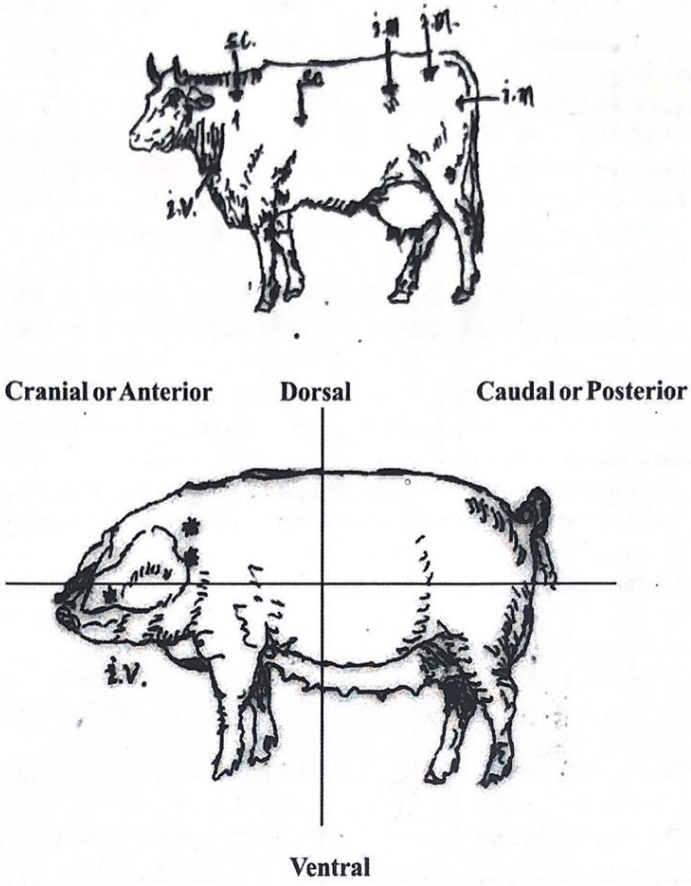


Figure 1.0: *Structure of an animal showing locational description of its body parts and spots where injections could be applied intravenous (i.v.), intramuscular (i.m.) and subcutaneous (s.c.)*

SYSTEMIC APPROACH TO ANATOMY

Systemic anatomy is the study of anatomy mainly by systems. In order to name the study, the suffix "ology" (branch of knowledge or science) is added to the root word referring to the system.

Table 2: The main structure and name of study of the body system

System	Name of Study	Chief or main Structure
Skeletal system	Osteology	Bones
Articular system	Arthrology	Joints
Muscular system	Myology	Muscles
Digestive system	Splanchnology	Stomach/ intestine
Respiratory system	Splanchnology	Lungs/ air passage
Urinary system	Splanchnology	Kidney/ bladder
Reproductive system	Splanchnology	Ovaries/ testes
Endocrine system	Endocrinology	Ductless glands
Nervous system	Neurology	Brain, nerves, Spinal cord
Circulatory system	Angiology	Heart/ vessels
Integumentary system	Dermatology	Skin
Sensory system	Esthesiology	Eye, ear

SYSTEMATIC APPROACH TO PHYSIOLOGY

- Cell physiology
- Neurophysiology
- Gastrointestinal physiology
- Cardiovascular physiology
- Renal physiology
- Metabolic physiology
- Respiratory or pulmonary physiology
- Endocrine physiology
- Reproductive physiology

These systematic approaches are parts of an overall study such as

- Applied physiology
- Comparative physiology
- Pathologic physiology
- Medical physiology
- Mammalian physiology

CHAPTER ONE
HISTOLOGY

Histology also called **microanatomy** is the study of the anatomy of cells and tissues of plants and animals using microscopy. It is commonly studied using a light microscope or electron microscope, the specimen having been sectioned, stained, and mounted on a microscope slide. Histological studies may be conducted using tissue culture, where live animal cells are isolated and maintained in an artificial environment for various research projects. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of staining. Histology is an essential tool of biology and medicine.

Histopathology (the microscopic study of diseased tissue) is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. Trained physicians, frequently licensed pathologists, are the personnel who perform histopathological examination and provide diagnostic information based on their observations. The trained personnel who prepare histological specimens for examination are **histotechnicians, histotechnologists, histology technicians (HT), histology technologists (HTL), medical scientists**, medical laboratory technicians, or biomedical scientists and their support workers. Their field of study is called **histotechnology**.

Cells are arranged in organized groups called tissues that carry out specific functions. Tissues are organized together to form Organs, which carry out more complex functions. Organs work together in groups to form Systems which in turn carry out a higher order function. There are only four basic tissues in the body, although each category can be subdivided. In turn, the four basic tissues are organized into the various organs of the body, and these generally exist as interrelated functional units termed organ systems. The four basic tissues of the body are Epithelium, Connective tissue, Muscular tissue and Nervous tissue. In emphasize, all of the organs of the body are composed of varying proportions of the four basic tissues, and each of the four basic tissues consists of cells and extracellular matrices. This simple concept is fundamental to the study of histology.

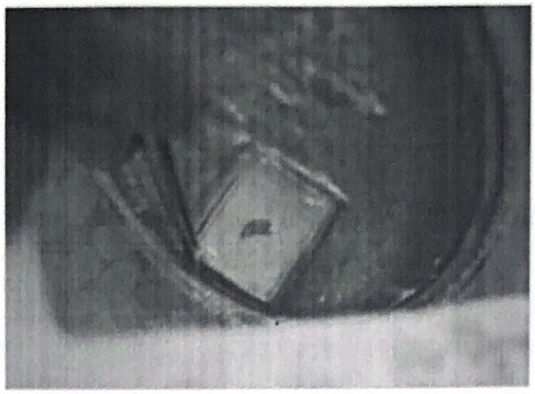
Cells are usually studied after undergoing specific manipulation so that what is actually seen with the microscope bears little resemblance to the living cells. Tissue treatments for histological studies include the following steps:

1. Fixation.
2. Embedding.
3. Sectioning.
4. Staining.
5. Examination (microscopic).

- **Fixation**: This involves fixing with some agent such as formalin that will coagulate the problem and prevent further changes in the tissue, such as autolysis and bacterial action. Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria). The most common fixative for light microscopy is 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). For electron microscopy, the most commonly used fixative is glutaraldehyde, usually as a 2.5% solution in phosphate buffered saline. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of methylene bridges ($-CH_2-$), in the case of formaldehyde, or by C_5H_{10} cross-links in the case of glutaraldehyde. This process, while preserving the structural integrity of the cells and tissue can damage the biological functionality of proteins, particularly enzymes, and can also denature them to a certain extent. This can be detrimental to certain histological techniques. Further fixatives are often used for electron microscopy such as osmium tetroxide or uranyl acetate. Formalin fixation leads to degradation of mRNA and DNA as well as denaturation and modification of proteins in tissues. However, extraction and analysis of nucleic acids and proteins from formalin-fixed, paraffin-embedded tissues is possible using appropriate protocols.

- **Embedding**: This involves treating the tissue with a substance that will permit cutting very thin sections such as paraffin, nitrocellulose, etc. Since most embedding media are not water-soluble, the fixed tissue must be dehydrated and then infiltrated with some material such as xylene, which is miscible with the embedding medium. The aim of Tissue Processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5 μm (micrometres; 1000 micrometres = 1 mm) thick for light microscopy and 80-100 nm (nanometre; 1,000,000 nanometres = 1 mm) thick for electron microscopy. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene. Paraffin wax does not provide a sufficiently hard matrix for cutting very thin sections for electron microscopy. Instead, resins are used. Epoxy resins are the most commonly employed embedding media, but acrylic resins are also used, particularly where immunohistochemistry is required.

Thicker sections (0.35μm to 5μm) of resin-embedded tissue can also be cut for light microscopy. Again, the immiscibility of most epoxy and acrylic resins with water necessitates the use of dehydration, usually with ethanol.



Optimal Cutting Temperature (OCT) embedding compound

After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatine, or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax and heating (curing) in the case of the epoxy resins. The acrylic resins are polymerized by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned. Because Formalin-fixed, paraffin-embedded (FFPE) tissues may be stored indefinitely at room temperature, and nucleic acids (both DNA and RNA) may be recovered from them decades after fixation, FFPE tissues are an important resource for historical studies in medicine. Embedding can also be accomplished using frozen, non-fixed tissue in a water-based medium. Pre-frozen tissues are placed into molds with the liquid embedding material, usually a water-based glycol, OCT, Cryogel, or resin, which is then frozen to form hardened blocks. Plastic embedding is commonly used in the preparation of material for electron microscopy. Tissues are embedded in epoxy resin. Very thin sections (less than 0.1 micrometer) are cut using diamond or glass knives. The sections are stained with electron dense stains (uranium and lead) so that they can be seen with the electron microscope.

3. **Sectioning**: This involve cutting the tissue into very thin slices (generally between 2 and 20μ in thickness), so that the sections may be placed on a glass slide. An instrument called a microtome is used for this purpose. It consists of a sharp knife blade and a mechanism for moving the tissue past the blade and then advancing it a definite distance after each cutting. For light microscopy, a steel knife mounted in a microtome is used to cut 4 micrometer-thick tissue sections which are mounted on a glass microscope slide.

for transmission electron microscopy, a diamond knife mounted in an ultra-microtome is used to cut 50 nanometer-thick tissue sections which are mounted on a 3 millimeter-diameter copper grid. Then the mounted sections are treated with the appropriate stain. Sections can be cut through the tissue in a number of directions. For pathological evaluation of tissues, vertical sectioning, (cut perpendicular to the surface of the tissue to produce a cross section) is the usual method. Horizontal (also known as transverse or longitudinal) sectioning, cut along the long axis of the tissue, is often used in the evaluation of the hair follicles and pilosebaceous units. Cryosectioning (Frozen section procedure) - Fixed or unfixed tissue may be frozen and sliced using a microtome mounted in a refrigeration device known as a cryostat. The frozen sections are mounted on a glass slide and may be stained to enhance the contrast between different tissues. Unfixed frozen sections can also be used for studies requiring enzyme localization in tissues and cells. It is necessary to fix tissue for certain procedures such as antibody linked immunofluorescence staining. Frozen sectioning can also be used to determine if a tumour is malignant when it is found incidentally during surgery on a patient.

4. **Staining**: This involve placing the tissue section in dyes to give a contrasting colour so that the different cells or different parts of cells can be differentiated according to colour. Hematoxylin/eosin are stains commonly used together, designated by **"H & E stain".** The hematoxylin tends to stain acid portions (basophilic area) of a cell **dark-blue** or **purple** while eosin stain the basic portions (**acidophilic areas**) of a cell **pink** to **red**. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Wright's stain is used to stain blood cells, and it stains the basophilic areas blue with methylene blue and the acidophilic areas red with eosin. A variety of stains can be applied to a tissue to determine how different portions react with various chemicals. This type of study is known as histochemistry. Biological tissue has little inherent contrast in either the light or electron microscope, Staining is employed to give both contrasts to the tissue as well as highlighting particular features of interest. Uranyl acetate and lead citrate are commonly used to impart contrast to tissue in the electron microscope. Numerous dyes originating from the textile industry have been used to selectively stain cells and cellular components. These dyes include safranin, Oil Red O, Congo red, silver salts, and numerous natural and artificial dyes. One of these techniques involves marking peripheral tumors or surgical margins, in which a certain color of dye is applied to the posterior border of a sample, another to the anterior, etc., so that one can identify the location of a tumor or other pathology within a specimen. Digital cameras are increasingly used to capture histological and histopathological image.
5. **Microscopic examination of stained tissue**: Examination of the stained section of tissue on the slide by means of a microscope and light transmitted through the section.

The relationship of the tissue sections to the actual tissue is about the same as that of a sack of potato chips to a growing potato. Both the selective and the potato chips have been processed so that actual resemblance to the original structure is practically nil. Both are seen in two dimensions, length and width, with thickness being of little importance as far as visualization is concerned. A technique known as wax plate reconstruction is sometimes used to visualize microscopic structures seen on a slide are magnified and projected onto a wax plate, and the unwanted portions are then cut away. This process is repeated with the next section that was cut from the block of tissue, and the corresponding areas of this wax plate are matched with the preceding wax plate. After many repetitions, a reconstruction has been produced that resembles the original tissue, except that it is much larger and consists of wax rather than tissue. This is a time consuming and meticulous technique, but the results are gratifying because it is almost the only way to gain a good, three-dimensional concept of microscopic structures.

Common laboratory stains

Stain	**Common use**	**Nucleus**	**Cytoplasms**	**Red blood cell (RBC)**	**Collagen fibers**	**Specifically stains**
Haematoxylin	General staining when paired with eosin (i.e. H&E)	Orange, Cyan Blue or Green	Blue/Brown/Black	N/A	N/A	Nucleic acids—blue ER (endoplasmic reticulum)—blue
Eosin	General staining when paired with haematoxylin (i.e. H&E)	N/A	Pink	Orange/red	Pink	Elastic fibers—pink Collagen fibers—pink Reticular fibers—pink
Toluidine blue	General staining	Blue	Blue	Blue	Blue	Mast cells granules—purple

Masson's trichrome stain	Connective tissue	Black	Red/pink	Red	Blue/green	Cartilage—blue/green Muscle fibers—red Keratin—orange
Mallory's trichrome stain	Connective tissue	Red	Pale red	Orange	Deep blue	Cartilage—blue Bone matrix—deep blue Muscle fibers—red
Weigert's elastic stain	Elastic fibers	Blue/black	N/A	N/A	N/A	Elastic fibers—blue/black
Heidenhain's AZAN trichrome stain	Distinguishing cells from extracellular components	Red/purple	Pink	Red	Blue	Muscle fibers—red Cartilage—blue Bone matrix—blue
Silver stain	Reticular fibers, nerve fibers, fungi	N/A	N/A	N/A	N/A	Reticular fibers—brown/black Nerve fibers—brown/black Fungi—black

Wright's stain	Blood cells	Bluish/purple	Bluish/gray	Red/pink	N/A	Neutrophil granules—purple/pink Eosinophil granules—bright red/orange Basophil granules—deep purple/violet Platelet granules—red/purple
Orcein stain	Elastic fibres	Deep blue	N/A	Bright red	Pink	Elastic fibres—dark brown Mast cells granules—purple Smooth muscle—light blue
Periodic acid-Schiff stain (PAS)	Basement membrane, localizing carbohydrates	Blue	N/A	N/A	Pink	Glycogen and other carbohydrates—magenta

Source: *Michael H. Ross and Wojciech Pawlina (2006). Histology: A Text and Atlas. Hagerstown, MD: Lippincott Williams & Wilkins.*

The Nissl method and Golgi's method are useful in identifying neurons.

MICROSCOPY

The microscope is an indispensable tool for the study of cells. The first development of microscope used in the laboratory is the all light microscopes.

LIGHT MICROSCOPE: In a light microscope **(LM),** visible light is passed through the specimen and then through glass lenses. The lenses refract (bend) the light in such a way that the image of the specimen is magnified as it is projected into the eye, onto photographic film or a digital sensor, or onto a video screen. The two important parameters in microscopy are magnification and resolving power (resolution). Magnification is the ratio of an object's image size to its real size. Resolution is a measure of the clarity of the image; it is the minimum distance two points can be separated and still be distinguished as two points e.g. what appears to the unaided eye as one star in the sky may be resolved as twin stars with a telescope. In the same way that the resolving power of the human eye is limited, the light microscope cannot resolve specimen that are smaller than about 0.2 micrometer (μm), or 200 nanometers (nm), the size of a small bacterium, regardless of the magnification factor (Figure 2A). This resolution is limited by the shortest wavelength of light used to illuminate the specimen. Light microscopes can magnify effectively to about 1,000 times the actual size of the specimen; but at higher magnifications additional details cannot be seen clearly. Most subcellular structures including organelles are simply too small to be resolved by the light microscope. The light microscope is advantageous in the study of living cells which is a disadvantage in electron microscopy as a result of the methods of specimen preparation that kills the cells. In addition, specimen preparation can introduce artifacts (structural features observed in micrographs that do not exists in living cell). Contrast is the third important parameter in microscopy which accentuates the differences in the various parts of the specimen. This has been improved by various methods that enhance contrast such as staining or labeling cell components to stand out visually (Figure 2B).

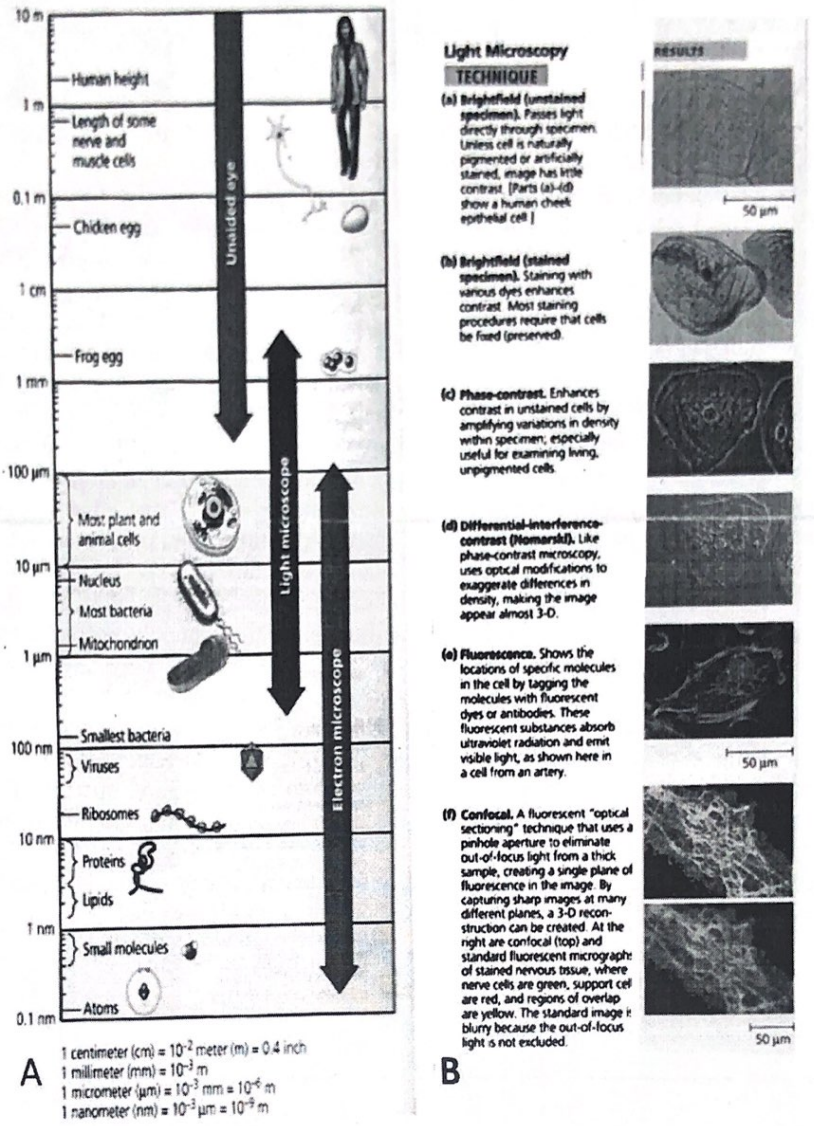


Figure 2.0: (A) Specimen size determination of types of eye aids; (B) Light microscopy technique showing phase contrast

ELECTRON MICROSCOPE: The development of the electron microscope has helped to solve the disadvantages of the light microscope. In electron

microscopy (EM), a beam of electrons (top of the microscope) is used instead of light, and electromagnets are used instead of glass lenses. The electron beam is focused on the specimen by a condenser lens; the image is magnified by an objective lens and a projector lens for projection on a digital detector, fluorescent screen, or photographic film. Resolution is inversely related to the wavelength of the radiation a microscope uses for imaging, and electron beams have shorter wavelengths than visible light. Modern electron microscopes can theoretically achieve a resolution of about 0.002nm, although for practical purposes they usually cannot resolve biological structures smaller than about 2nm, which is a hundredfold improvement over the light microscope. The term "cell ultrastructure" refers to the cellular anatomy revealed by an electron microscope. There are two types of electron microscope

A. Scanning electron microscope (SEM): SEM is used in detailed study of the surface of a specimen. The electron beam scans the surface of the specimen which is usually coated with a thin film of gold. The beam excites electrons on the surface, and these secondary electrons are detected by a device that translates the pattern of electrons into an electronic signal to a video screen. The result is an image of the specimens' topography. SEM has great depth of field, resulting in an image that appears three-dimensional.

B. Transmission electron microscope (TEM): TEM on the other hand is used to study the internal ultrastructure of cells. It aims an electron beam through a very thin section of the specimen, similar to the way a light microscope transmits light through a slide. The specimen is first stained with atoms of heavy metals which attach to certain cellular structures, thus enhancing the electron density of some parts of the cell more than others. The electrons passing through the specimen are scattered more in the denser regions, so that few are transmitted with the image displaying the pattern of electrons transmitted. TEM uses electromagnets as lenses (instead of glass lenses) to bend the paths of the electrons and focus the image on a screen or photographic film. Some microscopes have digital camera to photograph the image on the screen while others have digital detector instead Of both screen and camera.

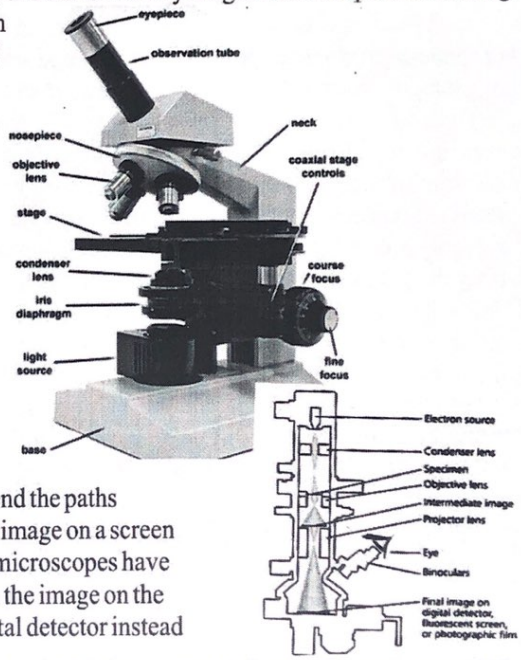


Figure 3.0: A Light microscope (LM) and an Electon microscope (EM)

CHAPTER TWO
PHYSIOLOGICAL BASIS FOR GROWTH

Growth and development is an ongoing process that begins at conception and continues throughout life. There is a broad spectrum of physical and psychological changes that are part of the maturation and life of the individual. Growth is a physical change that can be weighed. Development is the psychological and social changes to the individual such as behaviours and thinking/perception patterns. Growth and development are two complimentary processes that together make up the individual. Growth is a process by which an organism increases in size. Body growth is the irreversible changes that take place in all parts of the body (intercalary growth). It is definite except for certain fishes and lizards that grow throughout their life. Growth is controlled by hormones. Growth is one of the characteristics of living things. It has been used to describe many biological phenomenons. Population growth involves the reproduction of animals while body growth involves the multiplication of cells (hyperplasia) i.e. single cell develops into many different types of cells in many different tissues or increase in cell size. The increase in cell size or organ beyond normal is called hypertrophy while a decrease in size of cell from normal is called atrophy. Failure of a tissue or organ to develop is called aplasia while incomplete or defective development of a tissue or organ is called hypoplasia.

Growth generally is the increase in size or weight resulting from the predominance of anabolic processes over catabolic processes. Anabolic process is the building up of storage, structural and functional materials from simple foodstuffs or its intermediates. Catabolic process on the other hand is used to describe the total breakdown of foodstuffs to its final end-products with the liberation of energy. Catabolic processes liberate energy because the energy content of a complex feedstuff is greater than that of its simple degradation products. Anabolic process will only proceed if energy from elsewhere is pumped into them. A mere increase in body weight or size may not always be considered as growth because it could be due to abnormal water or feed intake or reduction or deposition of fat. For growth to occur, the organism must transfer essential nutrient to the living protoplasm. When the animal grows, development also occurs. Growth is an increment in space while growth rate is an increment with time. Growth rate and feed efficiency have large influence on economic results of animal production and are positively correlated. Growth rate of neonates are particularly rapid during the first month of life. Regular monitoring of weight gain can thus be a practical aid in determining healthy growth and development. Growth curve is an empirical model of the evolution of a quantity over time. Growth curves are widely used in biology for quantities such as population size or biomass (in population ecology and demography, for population growth analysis), individual body height or biomass (in physiology, for growth analysis of individuals). Values for the measured property can be plotted on a graph as a function of time.

Development is a gradual progression from lower to a higher stage of complexity as well as a gradual expansion in size. Development includes tissue differentiation and morphogenesis resulting in the organization of various cells into special tissues and organs of definite forms and function, and ultimately into the body as a whole. Differentiation, however, could be defined as the process by which cells and organs acquire complete individual characteristics. This occurs with the progressive diversification of cells and the embryo into muscle cells, nerve cells, brain cells, etc.

Hormonal Control of Growth: The process of growth and differentiation is under the control of hormones. The early part of embryonic development is controlled by genetic properties, while the late embryonic and postembryonic developments are largely regulated by hormonal influences and neural regulatory mechanisms. The process of metamorphosis, typical of most invertebrates and insects are controlled by hormones. Metamorphosis is the period of postembryonic development when the larval features undergo gradual modifications until an adult is developed. In amphibians, hormones from the thyroid gland trigger the metamorphic changes and are continuously required as stimulants for the developmental processes. Thyroid hormones are amino acids containing three or four atoms of iodine (tri-iodothyronine and thyroxine). If the thyroid gland of a tadpole is removed, the tadpole continues to grow but never metamorphoses. If thyroxine is administered to a young tadpole, it would show precocious metamorphosis. Sensitivity of tadpoles to thyroxines has been extensively studied and it has been found that extremely low concentrations of about 1 part per billion (10-9 molar concentration) are effective in inducing metamorphosis. The hormone acts directly on the transforming tissues. The thyroid function is controlled by the anterior pituitary gland which secretes a trophic hormone (TSH). TSH serves to stimulate the thyroid gland and regulates the hormone production thereby controlling metamorphosis.

THE FUNDAMENTAL UNITS OF LIFE

Cells are the smallest unit of a living organism. The word cell comes from the Latin "cella" meaning "a small chamber". Cells are the individual units of living structure found in groups in which individual cells are restrained mainly by adjacent cells. A typical cell consists of three main parts

- the cytoplasm
- the nucleus
- and the cell membrane

The protoplasm is a living material that is jelly-like in nature and bounded by a membrane which is flexible and semi-permeable. Cell can exist as a single free-living organism e.g. Amoeba; as filament e.g. spirogyra; as colony e.g. volvox; and as part of living organism. Living organisms may be unicellular or multicellular. When the number of cells increases in animals, certain cells become specialists in one or more of the functions of the animal body. Specialized cells

grouped together are called tissues e.g. cells specialized in conducting impulses make-up **nervous tissue** while cells specialize in holding structures together makeup **connective tissue**.

Various tissues are associated in functional groups called **Organs** e.g. the stomach is an organ that functions in digestion of food. A group of organs that are involved in a common enterprise make up a **System** e.g. the stomach, liver, pancreas and intestines are all part of the digestive system. Most cells range in diameter from about 10 to 100μ (micra); cells that are multiplying range from about 20 - 30μ in diameter.

$1\mu = 1/1000mm$

Since there are about 25mm in 1inch, $1\mu = 1/25000inch$

NB: In the International System of Units, it is recommended that the term **micron** (μ) be replaced by the term **micrometer** (μm), and the **angstrom** (Ä) be conyerted to the measurements of **nanometer** or **picometer** (1Ä = 0.1nm = 100pm).

Table 3: The Unit in cells and its relationships

Unit/ Abbreviation	Relationships
Centimeter, cm	$1cm = 10^{1}mm, 10^{4}\mu m, 10^{7}nm, 10^{8}Å$
Millimeter, mm	$1mm = 10^{-1}cm, 10^{3}\mu m, 10^{6}nm, 10^{7}Å$
Micrometer (micron), μm	$1\mu m = 10^{-4}cm, 10^{-3}mm, 10^{3}nm, 10^{4}Å$
Nanometer (millimicron), nm	$1nm = 10^{-7}cm, 10^{-6}mm, 10^{-3}\mu m, 10^{1}Å$
Angstron, Å	$1Å = 10^{-8}cm, 10^{-7}mm, 10^{-4}\mu m, 10^{-1}nm$

Generally a cell under the light microscope consists of a nucleus and cytoplasm surrounded by the plasma membrane. The nucleus contains nucleolus and chromatin material which forms into chromosomes during cell division and it is surrounded by a nuclear membrane. The cytoplasm contains a number of different structures, including Endoplasmic reticulum, Golgi bodies, Mitochondria, Lysosomes, Peroxisomes, Micro filaments, Centrioles, Microtubules, Nucleus and Nucleolus.

Basically, there are two types of cells- Eukaryotic and prokaryotic cell.

1. **Eukaryotic** cells are cells with defined or the true nucleus. It is the typical cell of the metazoan multicellular animals clearly defined into nucleus and cytoplasm with well defined cell membranes.
2. **Prokaryotic** cells are cells that do not have true nuclei e.g. bacteria, blue green algae. The DNA is present in not well defined areas called nucleoids and the inclusions are without membranes it is more primitive. The cell as revealed by the microscope is made up of two major parts the nucleus surrounded by the cytoplasm both of which form the protoplasm.

Table 4: Differences between eukaryotic and prokaryotic cells

Eukaryotic cells	Prokaryotic cells
1. Cells with defined or true nucleus	Cells without true nuclei
2. Typical of metazoan	Typical of protozoa
3. Well defined cell membrane	Inclusions are without membranes
4. Cells made up of three major parts nucleus, cytoplasm and cell membrane.	Cells made up of two major parts - nucleus and cytoplasm.
5. More advanced	It is more primitive.

Characteristics of living organisms: Cell can be defined as the "smallest basic unit of life that carries out all life processes summed-up in the characteristics of living organism" such as movement, respiration, nutrition, irritability, growth, excretion, reproduction and has a definite life span (death). Therefore the properties of the cell are equated with those of life. These properties include growth, reproduction, absorption, metabolism, excretion, secretion, irritability, conductivity and contractibility. The last two are primarily characteristics of nerve and muscle cells respectively.

Growth is a process by which organism increase in size and weight, usually by increase in the amount of protoplasm. Body growth is irreversible and takes place in all parts of the body (intercalary growth) and it is definite except in some fishes and lizards that grow throughout life. Growth is controlled by hormones.

It is one of the characteristics of all living things to increase in cells (hyperplasia). Increase in size of a structure because of increased number of cells is called **hyperplasia. Hypertrophy** is an increase in size of a cell or organ beyond normal while a decrease in size from normal is called **atrophy**. Failure of a tissue or organ to develop is called **aplasia**, while incomplete development or defective development of a tissue or organ is called **hypoplasia**. Growth is accomplished through the process of taking dissolved materials into the substance of cell (absorption). Absorption can be a passive process dependent on the forces of diffusion and osmosis, or it can be an active process requiring the expenditure of energy from adenosine triphosphate (ATP), or it may occur by electrochemical ionic forces and affinities that require no direct expenditure of energy. All three can be occurring at the same time across the same membrane. Extracellular materials can also be gotten into the cell by phagocytosis and pinocytosis.

Phagocytosis involves the engulfing of materials by an amoeboid-like action of the cell. Cells with such ability are called **phagocytes**. The cell flows around the material until it is completely surrounded and thereby engulfed e.g. White blood cells (WBC) which engulf particulate matter, tissue debris, degenerated red blood cells (RBC) or bacteria.

Pinocytosis is the process by which cells take in fluid and molecules too large to be carried across the plasma membrane by active transport, particularly small proteins. The substance first becomes adsorbed to (in physical contact with) the plasma membrane. This changes the surface tension and electrical properties of the membrane, causing it to invaginate into the cell and taking in with it the substance and some extracellular fluid. The invagination forms a membrane-bound pocket or tear-drop-like pinocytotic vesicle which blebs off, and the plasma membrane closes over the invagination. The vesicle, which is now within the cytoplasm, contains extracellular particles and fluid which are finally released into the cytoplasm proper when its membrane is digested away by lysosomes (pockets of enzymes in the cytoplasm). Pinocytosis has been observed in mammalian cells equipped with microvilli such as epithelial cells of the intestinal tract and renal proximal tubules.

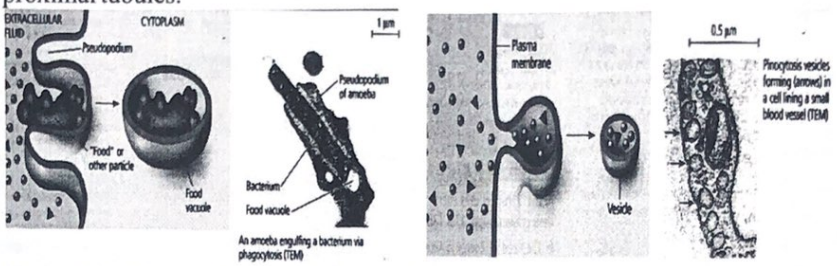


Figure 4.0: Phagocytosis and pinocytosis

Growth is generally through **metabolism (internal respiration)** which is the utilization of nutrients by the cell, consisting essentially of oxidation of carbon compounds to carbon dioxide and water, with the release of energy. It is the sum total of the biochemical reactions occurring in each cell of the entire animal. It involves anabolic and catabolic processes - those reactions that build/ maintain the body through energy formation are called **anabolic**, and those reactions that release energy by the breakdown of substances are called **catabolic**. Excretion and Secretion which is a characteristic of living organism is the loss of material from the cell. If waste products are lost, the process is called **excretion**; if useful products are released, the process is called **secretion**. For effective functioning, all parts of the cell protoplasm must be reasonably close to the plasma membrane for absorption of nutrients, excretion of wastes, and secretion of synthesized products, hence the limited size of the cells. Secretion of products synthesized by the cell into the extracellular fluid that bathes the cells, occurs by a process called **exocytosis**, **emiocytosis**, or **reverse pinocytosis**. Membrane-bound vesicles containing secretory substances that have been "packaged" by the Golgi apparatus migrate in the cytoplasm to the plasma membrane. Here they become adsorbed to the membrane, causing the membrane at those points to rupture, releasing the contents of the vesicles into the extra-cellular fluid space. This expulsion of vesicular contents is also a means of excreting some metabolic waste products from the cell.

Irritability (or excitability) is the ability to react to a stimulus. It consists of the properties of the protoplasm such as conduction or contraction. **Conductivity** is the property of transmitting an impulse from one point in the cell to another. Nerve cells and muscle cells are specialized for the functions of conductivity and irritability. **Contractility** is the ability of shortening in one direction. Muscle cells are specialized for contractions, although some other cells and cell organelles also contain contractile protein and exhibit limited movement (e.g. Cilia).\

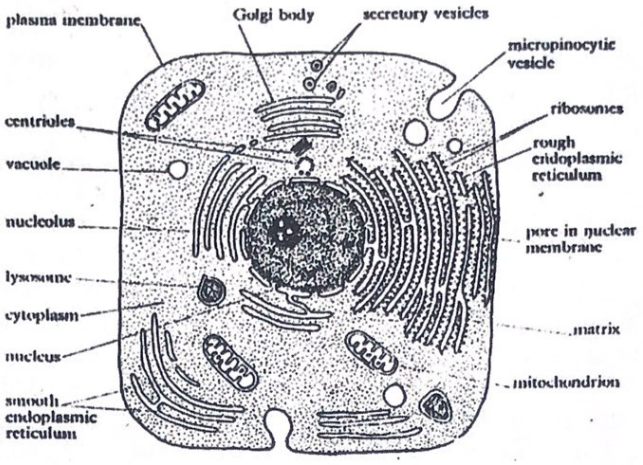


Figure 5.0: Structure of an animal cell

1. Cell membrane: This is the outermost component of the cytoplasm, which maintains its cellular integrity and separates it from its extracellular environment, is the plasma membrane. The plasma membrane consists of 3 layers composed two layer of proteins with a middle bimolecular layer of phospholipids sandwiched between them. The proteins serve as stabilizer or a protective cover for the membrane. The total thickness of the plasma membrane is between 70Å to 100Å. This type of structure is normally is normally referred to as a unit membrane and it also occurs in the cells surrounding the mitochondria, and other cell organelles. Evidence of a cell membrane has been demonstrated experimentally through the principles of osmosis whereby cells in a hypertonic solution lose water to the surrounding medium resulting in their shrinkage whereas cells in a hypotonic medium take in water from the surrounding medium. This passage of water without the escape of materials to and from the cells suggests a selective ability of the cell membrane to preferentially allow passage of solvent over the solute.
The existence of pores in the plasma membrane has been predicted for a long time. The plasma membrane has been known to be a major factor in homeostasis and it is known that certain materials, which are not soluble in lipids, can still penetrate the membrane.

The pores are believed to be lined with hydrophilic (polar group of protein) molecules and therefore offers no resistance to water-soluble substances. Experimental evidence shows that these pores are less than 10Å in width and are too small to be seen with the electron microscope.

of the various features associated with plasma membrane, one is the invagination of the cell wall (**caveoli**). These caveoli usually present a flask-like appearances and the neck of the flask eventually seals off and form small membrane bound vesicles called pinocytotic vesicles or **vacuole**. By this process of pinocytosis, numerous small vacuoles are formed in the cell. By a reverse mechanism, these pinocytotic vesicles may also discharge their contents to the exterior by attaching to the cell membrane and then opening to the exterior.

The plasma membrane of the epithelial cells of some specialized organs of the body like the lining of the convoluted tubules of the kidney and the epithelial lining of the small intestine and trachea are folded to form numerous minute, hair like projections called **microvilli**. These projections increase the cell surface and function in absorption or secretion. When viewed with the electron microscope, each microvillus is just about 1.0μm long and 0.07 0.08μm wide, but under the light microscope appear as a brush border or striated border. The microvilli are lined with plasma membrane and are continuous with the cytoplasm of the cell.

Function: It protects the cell and as well controls selectively the passage of substances in and out of cell.

2. Cytoplasm: They are grey granular jellies which make up the bulk of the cell, excluding the nucleus. The cytoplasm is composed of a matrix in which are contained several structures viz: organelles, inclusions and other components. The organelles are present in all eukaryotic cells and are enclosed in a membrane. They contain enzymes, which participate in metabolic activity such as endoplasmic reticulum, the mitochondria, the Golgi apparatus and the lysosomes. The inclusions generally are temporary components of certain cells and usually contain or may not be enclosed with a membrane. The other components are the centriole, microtubules and microfilaments.

Function: The cytoplasm performs most cell activities with nucleus e.g. diffusion, translocation, excretion, etc.

3. Nucleus: It is spherical, dense mass part of the protoplasm. Enveloping it is a permeable membrane called the nuclear membrane. It contains thread-like nuclear materials called chromosomes and nucleolus. The nucleus is mainly concerned with the genetic or hereditary continuity and control of the cell. The continued life of the cytoplasm depends on the presence of the nucleus. The constituents of the nucleus can be identified by chemical analysis usually by histochemistry. The nucleus has profound affinity for basic dyes due to the presence of chromatin materials. The nucleus of cells contains DNA (deoxyribonucleic acid), RNA (ribonucleic acid), proteins, lipids and inorganic compounds. Most of the RNA is located in the nucleus and a small amount of DNA is present around the nucleolus. Also present in the nucleus are proteins and enzymes.

Techniques have been determined for staining nuclear materials particularly DNA. Two kinds of chromatin materials are distinguishable:

i. Euchromatin
ii.Heterochromatin

Euchromatins stain lightly and are not sharply defined whereas Heterochromatins are darkly stained. The difference between these two materials is the state of aggregation of DNA. Thus Euchromatin materials are loosely twisted and are in the metabolically active state. The Heterochromatin strands are tightly twisted and folded and relatively inactive.

Function: It controls the activities of the cell especially protein synthesis, cell division and the presence of chromosomes aid the transfer of genetic traits.

4. Nucleolus: A small round body inside the nucleus concerned with the manufacture of ribonucleic acid (RNA).

5. Centrioles: They are found only in the cells of all animal. It consists of a pair of short rods lying close to the nuclear membrane and give rise to spindle fibres which form an integral part of cell division. They are cylindrical structure located at outer part of the nucleus.

Function: They are centres for spindle formation during cell division.

6. Golgi body: It is a special development of endoplasmic membrane. It is formed by the fusion of vesicles which are pinched off from the cavities of the rough endoplasmic reticulum. The Golgi bodies are a series of flattened membranous cavities closely associated with the smooth endoplasmic reticulum. It is thought that the Golgi body is the site of glycoprotein synthesis formed by the movement of newly synthesized protein from the rough ER. These proteins then move to the Golgi bodies where they are conjugated with carbohydrate. The vesicles then move to the surface of the cell and eventually discharge their contents to the exterior through exopinocytosis. It contains large flattened cisternae with no ribosomes on the walls. It is discontinuous (discrete) in animal cells

Function: It is known to contribute in the transport of newly synthesized lipid and protein. It also plays a secretory role in the cell.

7. Mitochondria: This is a spherical, elliptical, double-layered organelle containing cristae, and is found in the cytoplasm. Each mitochondrion is about 0.2 to 1.0μm in diameter and 2.5 3.0μm in length. The wall of the mitochondrion consists of two thin membranes - an outer membrane and an inner highly folded membrane separated by a thin fluid filled space. The folds of the inner membrane are numerous and project inwards to form cristae. Attached to the cristae are numerous granules. The mitochondria in the organelles are responsible for the major chemical reactions of the cell particularly respiration by which means energy is generated for the needs of the cell. The mitochondrion contains many of the enzymes associated with oxidation occurring in the citric acid cycle.

The cristae have the effect of increasing the surface area for attachment of enzymes so that large amounts of energy can be expanded in a comparatively small space.

By centrifugation of the cellular homogenate, mitochondria are shown to be next to the nuclei in specific gravity. All the enzymes and coenzymes involved in the Krebs cycle are predominantly found in the mitochondria. This cycle is the mechanism by which carbohydrates, fatty acids and amino acids are broken down. The energy released by the oxidation of carbon and hydrogen to carbon dioxide and water is principally stored in the form of ATP.

It is interesting that cells whose function requires them to expend particularly large amounts of energy contain unusually large numbers of mitochondria. These are often packed close together in the part of the cell where the energy is required e.g. in spermatozoa where the mitochondria are tightly packed at the base of the motile tail. They are also found in great abundance alongside the contractile fibrils in muscle, particularly the flight muscle of insects, and at the surface of cells in which active transport occurs. The mitochondria content of cells of organs actively involved in energy metabolism is very high e.g. cardiac muscle, liver cells and spermatozoa.

Function: It is the centre for cell respiration. Due to its role in generation of energy, it is commonly called the 'power house' of the cell.

8. Endoplasmic reticulum: It occupies most of the cytoplasm of the mature cell. In some parts it is continuous from the nucleus. In other parts they are contiguous (i.e. very close) to the plasma membrane. It contains cytoplasmic matrix, some colloidal material containing soluble protein on one side, while on the other a large number of spaces or cisternae are enclosed. The parallel flattened cavities lined with thin membrane of about 4nm thick occupying the cytoplasm matrix is the **endoplasmic reticulum** (ER). It is made up of cavities that are interconnected and the lining membranes are continuous with the nuclear membrane. Attached to the matrix side of the membranes are numerous granules which, because they are rich in ribonucleic acid, are called ribosomes. Manufactured in the nucleolus, these are the sites where proteins are synthesized in the cell. The bulk of the ER in most cells is encrusted with ribosomes and accordingly is known as **rough endoplasmic reticulum**. Its general function is to isolate and transport the proteins which have been synthesized by the ribosomes. Many of these proteins are not required by the cell in which they are made but are for "export", i.e. they are secreted by the cell. Such proteins include digestive enzymes and hormones. The ER is thus a kind of intracellular transport system facilitating movement of materials from one part of the cell to another. In this connection it is interesting that the nuclear membrane is pierced by tiny pores which are continuous with the ER, thus providing a route by which materials might move from nucleus to cytoplasm and vice versa.

In certain parts of the cell the ER is not encrusted with ribosomes and accordingly is known as **smooth endoplasmic reticulum**. This is not continuous with the rough ER and its cavities are tubular rather than flattened sacs. It is thought to be concerned with the synthesis and transport of lipids and steroids. Nearly all cell secretions are glycoproteins, i.e. proteins conjugated with a carbohydrate. Newly synthesized proteins are found in the channels of the rough ER.

From here they move to the Golgi body where the carbohydrate is added to them. Enveloped in part of the Golgi membrane, they then leave the cell. Thus the Golgi apparatus is an assembly point through which raw materials for secretion are funneled before being shed from the cell. How does this movement of materials take place? We are a long way from knowing the full answer to this question but certain facts are clear. In the first place the rough ER is not continuous with the Golgi body. The latter is formed by the fusion of vesicles which are pinched off from the cavities of the rough ER. Vesicles containing the secretory molecules then get pinched off from the channels of the Golgi body. These vesicles then move to the surface of the cell and discharge their contents to the exterior. Thus, to summarize, the function of the Golgi body is to add the carbohydrate component to the protein and package the finished product before it leaves the cell.

Function: They help in transport of substances due to their large surface area. They support, localize pH and potentials in different parts of the cytoplasm.

9. Ribosome: This is found in the walls of the rough endoplasmic reticulum. Some are suspended in the cytoplasmic matrix. It contains ribonucleic acid (RNA). There are thousands in a given cell.

Function: It helps in protein synthesis.

10. Lysosomes: They consist of a membrane enclosing fluid-filled vacuole. They are spherical, small membrane lined sacs, which contain certain proteolytic enzymes known as hydrolases responsible for splitting complex chemical compounds into simpler units. The obvious purpose of this organelle is to confine these potentially destructive hydrolytic enzymes so that they can perform their functions without injuring the cell the enzymes in the lysosomes are potent in digesting materials, which the cell has ingested. Another important function is that they destroy worn out organelles. The mechanism is that the organelles are enveloped in a membrane, which completely encloses them. Lysosomes also serve as organelles for clearing the cells of accumulated secretions, excessive membranes and ribosomes when they outlive their usefulness to them. The lysosomes then discharge their contents into these bags and the enzymes so liberated digest the organelles and their products absorbed into cytoplasmic matrix to form new organelles. On occasions, lysosomes may rupture their membranes and thus destroy the entire cell particularly when the cell is worn out and due for replacement. Lysosomes are also of importance in anti inflammatory actions. Function: They digest foreign bodies like virus, bacteria by secreting enzyme hydrolase (lysozyme) which causes lysis. It can also cause ageing of cells due to hydrolytic enzyme produced which affects cells.

11. Peroxisomes: Like Lysosomes, peroxisomes are enzymes containing sacs. The peroxisomes are globular and often smaller than lysosomes. Peroxisomes are found in the liver. They are rich in aerobic dehydrogenases and catalase. Peroxisomes also seem to be involved in purine metabolism i.e. the breakdown of Nucleic acid and also in the conversion of fat to glucose by the glyoxylate cycle particularly in the plant.

12. Microfilaments: These refer to numerous fibrils in the cell e.g. those in the microvilli. Microfilaments especially microvilli increase the surface area of the plasma membrane many times over and accelerate the exchange of materials across it. They occur as a result of the ability of certain specialized cells to form tiny hair like extrusions filled with cytoplasm, which is continuous with the main body of the cell. They may show up as ciliated border, or when very densely packed as striated or brush border. They are numerous in the convoluted tubules of the kidney, the lining of the small intestine and the seminiferous tubule.

13. Microtubules: These are small tubules in the cytoplasm of many cells. They are between 200 - 300Å in diameter and are of indefinite but considerable length. They are generally associated with centrioles or basal bodies.

They are concerned with maintenance of shape and rigidity of cell and are made of protein. They are also believed to be involved with the transport of cellular materials within the cells and their growth and dissolution may account for the changes which occur within the cell.

14. Lipid droplets: Lipid droplets appear to be very important in the secretion of steroid hormones. They sometimes originate from either the Golgi body or the smooth ER. The droplet finally fuses with the plasma membrane, and the contents usually adrenocorticosteroids are discharged from the cell surface through endoplasmocrine secretion.

Separation of cellular Constituents:

The structure and functions of cell organelles can be determined to some extent by separating the various cellular constituents (Figure 7.0), analyzing them chemically, and determining what biochemical reactions they can perform. First, an appropriate sample of cells is homogenized (finely ground) and then subjected to centrifugation (rapid spinning) at different speeds to separate the homogenate into different fractions. The heaviest structures, the nuclei, settle out first, then the mitochondria, and finally the microsomes, which are fragments of rough Endoplasmic reticulum.

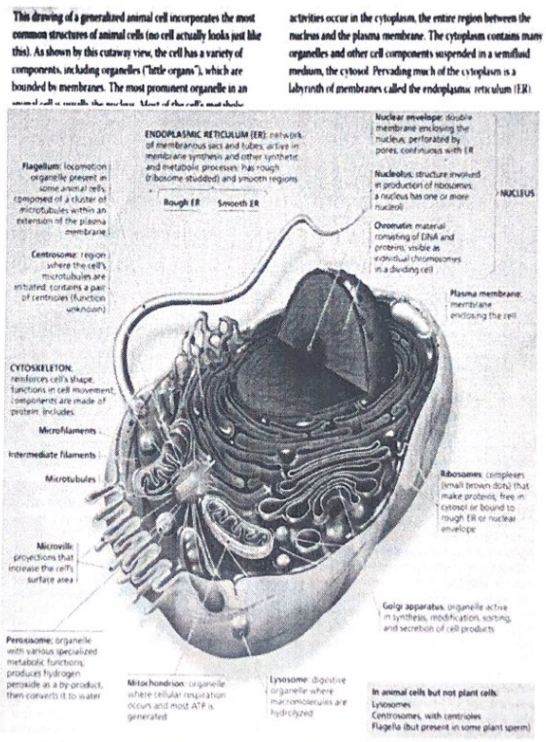


Figure 6.0: Components of an animal cell

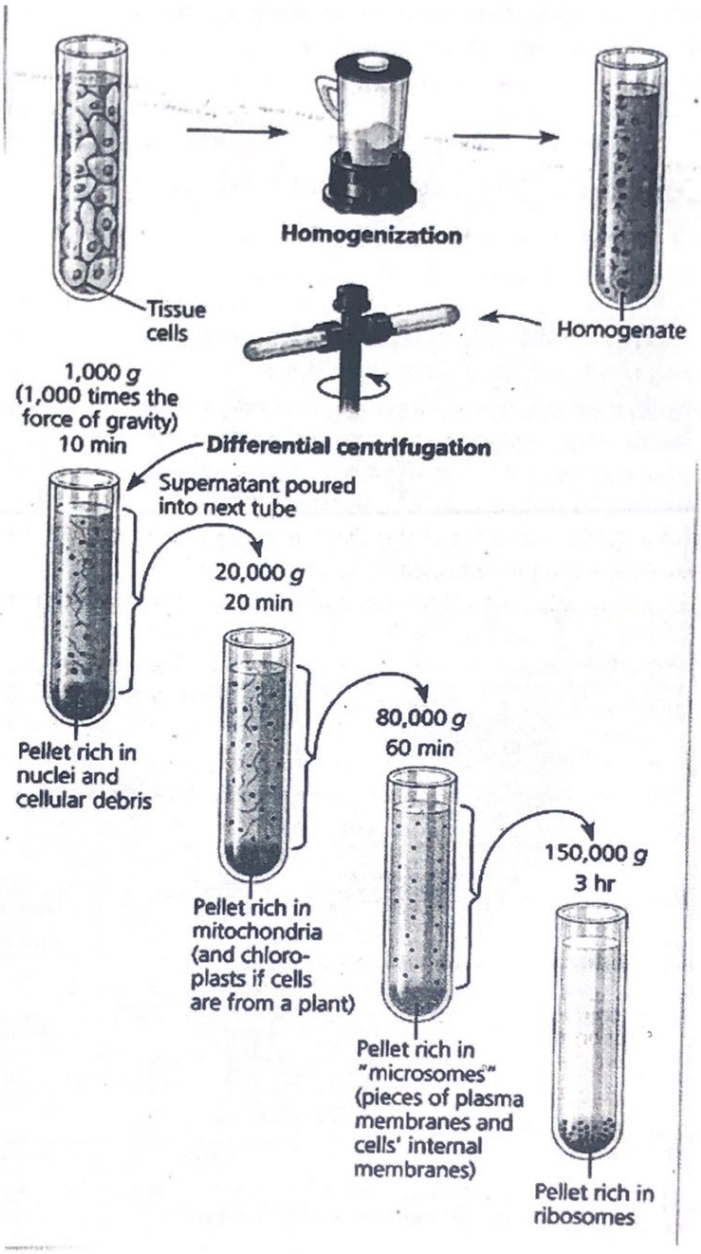


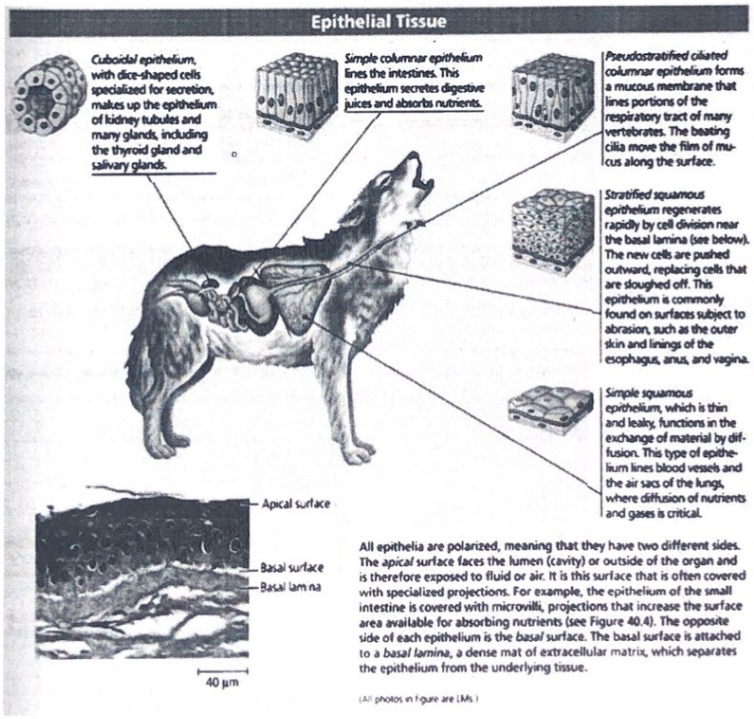
Figure 7.0: Cell fractionation technique

THE TISSUE

These are specialized cells that perform similar or the same function e.g. cells that specialize in holding structures together are called connective tissues while those that help in conducting impulses makes up the nervous tissue. There are only four basic tissues in the body, although each category can be subdivided. In turn, the four basic tissues are organized into the various organs of the body, and these generally exist as interrelated functional units termed organ systems. The four basic tissues of the body are:

- Epithelial tissue
- Connective tissue
- Muscular tissue
- Nervous tissue

In emphasize, all of the organs of the body are composed of varying proportions of the four basic tissues, and each of the four basic tissues consists of cells and extracellular matrices. All tissue types are subtypes of these four basic tissue types (for example, blood is classified as connective tissue, since the blood cells are suspended in an extracellular matrix, the plasma). This simple concept is fundamental to the study of histology.



EPITHELIAL TISSUE (epithelium -singular): Epithelial tissues are made up of cells that are tightly packed to form a continuous sheet, almost with no intercellular spaces and are separated from the underlying tissues by an extracellular fibrous basement membrane. They are derived from all of the embryological germ layers such as ectoderm (e.g., the epidermis); endoderm (e.g., the lining of the gastrointestinal tract); and mesoderm (e.g., the inner linings of body cavities). Epithelial are tissues consisting of closely apposed cells without intervening intercellular substances. Epithelial are avascular, but all epithelia "grow" on an underlying layer of vascular connective tissue. The connective tissue and the epithelium are separated by a basement membrane i.e. all epithelial tissue adheres to a connective tissue membrane layer referred to as the basement membrane which attaches the epithelium to the underlining connective tissue. **Basement membrane** is the foundation on which epithelial tissues rest and acts as a scaffolding on which epithelium can grow and regenerate after injuries. The basement membrane acts as a selectively permeable membrane that determines which substances will be able to enter the epithelium. Epithelial tissues have no blood supply but nerve supply and are nourished by substances that diffuse from the blood vessels in the underlying tissue. The contact points between plasma membrane and tissue cells are called **Cell junctions** and are especially abundant in epithelial tissues. The cell junctions consist of protein complexes and provide contact between neighbouring cells, between a cell and the extracellular matrix, or they build up the paracellular barrier of epithelia and control the paracellular transport. The five main types of cell junctions are - tight junctions, adherens junctions, desmosomes, hemidesmosomes, and gap junctions. Tight junctions are a pair of trans-membrane protein fused on outer plasma membrane. Adherens junctions are a plaque (protein layer on the inside plasma membrane) which attaches both cells' microfilaments. Desmosomes attach to the microfilaments of cytoskeleton made up of keratin protein. Hemidesmosomes resemble desmosomes on a section. They are made up of the integrin (a transmembrane protein) instead of cadherin. They attach the epithelial cell to the basement membrane. Gap junctions connect the cytoplasm of two cells and are made up of proteins called connexins (six of which come together to make a connexion). Epithelial tissues line the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs. They lines both the outside (skin - epidermis) and the inside cavities and lumina of bodies. The outermost layer of human skin is composed of dead stratified squamous, keratinized epithelial cells. The lining of the mouth, lung alveoli and kidney tubules are all made of epithelial tissue. The lining of the blood and lymphatic vessels are of a specialised form of epithelium called endothelium. Tissues that line the inside of the mouth, the oesophagus, the vagina, and part of the rectum are composed of nonkeratinized stratified squamous epithelium. Other surfaces that separate body cavities from the outside environment are lined by simple squamous, columnar, or pseudostratified epithelial cells. Other epithelial cells line the insides of the lungs, the gastrointestinal tract, the reproductive and urinary tracts, and make up the exocrine and endocrine glands.

The outer surface of the cornea is covered with fast-growing, easily regenerated epithelial cells. A specialized form of epithelium (endothelium) forms the inner lining of blood vessels and the heart, and is known as vascular endothelium, and those lining lymphatic vessels as lymphatic endothelium. Epithelium also lines the large internal body cavities, where it is termed mesothelium, which makes up the walls of the pericardium, pleurae, and peritoneum.

There are three principal shapes of epithelial cell: squamous, columnar, and cuboidal. These can be arranged in a single layer of cells as simple epithelium, either squamous, columnar, cuboidal, pseudo-stratified columnar or in layers of two or more cells deep as stratified (layered), either squamous, columnar or cuboidal. All glands are made up of epithelial cells. Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport, and sensing.

However, it is important to note that pathologists do not consider endothelium and mesothelium (both derived from mesoderm) to be true epithelium. This is because such tissues present very different pathology. For that reason, pathologists label cancers in endothelium and mesothelium sarcomas, whereas true epithelial cancers are called carcinomas. Also, the filaments that support these mesoderm-derived tissues are very distinct. Outside of the field of pathology, it is, in general, accepted that the epithelium arises from all three germ layers. Carcinomas develop in epithelial tissues. Sarcomas develop in connective tissue. When epithelial cells or tissues are damaged from cystic fibrosis, sweat glands are also damaged, causing a frosty coating of the skin.

There are two basic form of epithelial tissue:

a. Covering and lining epithelium forms the outer covering of external body surfaces, - some internal organs, - lines body cavities, - lines interiors of respiratory tract, gastrointestinal tract, blood vessels, ducts, hollow reproductive organs and vessels.

b. Glandular epithelium makes up the secreting portion of glands

Type of Tissue	Function	Location
Pseudostratified columnar	Removing dust and particles from airways, has cilia	lines the respiratory passageways
Simple Columna	Absorption	lines the uterus and most organs of the digestive tract

Simple Cuboidal	Secretion and Absorption	glands, kidney tubules, ovaries
Simple Squamous	Diffusion and Filtration	lungs, walls of capillaries and vessels
Stratified Squamous	Protects underlying cells	Skin (keratinized) and the throat, vagina, mouth (soft)
Stratified Cuboidal	Protection	lines ducts of the mammary glands, sweat glands, pancreas
Stratified Columnar	Protection, secretion	male urethra/vas deferens, parts of the Pharynx
Transitional (unstretched)	Specialized to become distended	urinary tract

Classification of Epithelial tissues: In general, epithelial tissues are classified by the number of their layers and by the shape and function of the cells.

By layer, epithelium is classed as either simple epithelium, only one cell thick (unilayered) or stratified epithelium that are two or more cells thick (multi-layered) (stratified squamous epithelium, stratified cuboidal epithelium, and stratified columnar epithelium), and both types of layering can be made up of any of the cell shapes. However, when taller simple columnar epithelial cells are viewed in cross section showing several nuclei appearing at different heights, they can be confused with stratified epithelia. This kind of epithelium is therefore described as pseudostratified columnar epithelium.

The three principal shapes associated with epithelial cells are squamous, cuboidal and columnar.

- **Squamous epithelium** has cells that are wider than their height (flat and scale-like) i.e scale-like or plate-like.
- **Cuboidal epithelium** has cells whose height and width are approximately the same (cube shaped).
- **Columnar epithelium** has cells taller than they are wide (column-shaped).

Cell types: The basic cell types are squamous, cuboidal, and columnar classed by their shape.

Type	Description
Squamous	Squamous cells have the appearance of thin, flat plates that can look polygonal when viewed from above. Their name comes from *squâma*, Latin for scale as on fish or snake skin. The cells fit closely together in tissues; providing a smooth, low-friction surface over which fluids can move easily. The shape of the nucleus usually corresponds to the cell form and helps to identify the type of epithelium. Squamous cells tend to have horizontally flattened, nearly oval shaped nuclei because of the thin flattened form of the cell. Squamous epithelium is found lining surfaces such as the skin, and alveoli in the lung, enabling simple passive diffusion as also found in the alveolar epithelium in the lungs. Specialized squamous epithelium also forms the lining of cavities such as in blood vessels, as endothelium and in the pericardium, as mesothelium and in other body cavities.
Cuboidal	Cuboidal epithelial cells have a cube-like shape and appear square in cross-section. The cell nucleus is large, spherical and is in the centre of the cell. Cuboidal epithelium is commonly found in secretive tissue such as the exocrine glands, or in absorptive tissue such as the pancreas, the lining of the kidney tubules as well as in the ducts of the glands. The germinal epithelium that covers the female ovary, and the germinal epithelium that lines the walls of the seminferous tubules in the testes are also of the cuboidal type. Cuboidal cells provide protection and may be active in pumping material in or out of the lumen, or passive depending on their location and specialisation. Simple cuboidal epithelium commonly differentiates to form the secretory and duct portions of glands. Stratified cuboidal epithelium protects areas such as the ducts of sweat glands, mammary glands, and salivary glands.
Columnar	Columnar epithelial cells are elongated and column-shaped and have a height of at least four times their width. Their nuclei are elongated and are usually located near the base of the cells. Columnar epithelium forms the lining of the stomach and intestines. The cells here may possess microvilli for maximising the surface area for absorption and these microvilli may form a brush border. Other cells may be ciliated to move mucus in the function of mucociliary clearance. Other ciliated cells are found in the fallopian tubes, the uterus and central canal of the spinal cord. Some columnar cells are specialized for sensory reception such as in the nose, ears and the taste buds. Hair cells in the inner ears have stereocilia which are similar to microvilli. Goblet cells are modified columnar cells and are found between the columnar epithelial cells of the duodenum. They secrete mucus, which acts as a lubricant. Single-layered non-ciliated columnar epithelium tends to indicate an

absorptive function.

Stratified columnar epithelium is rare but is found in lobar ducts in the salivary glands, the eye, pharynx and sex organs. This consists of a layer of cells resting on at least one other layer of epithelial cells which can be squamous, cuboidal, or columnar. These are simple columnar epithelial cells whose nuclei appear at different heights, giving the misleading (hence "pseudo") impression that the epithelium is stratified when the cells are viewed in cross section. Ciliated pseudostratified epithelial cells have cilia. Cilia are capable of energy dependent pulsatile beating in a certain direction through interaction of cytoskeletal microtubules and connecting structural proteins and enzymes. In the respiratory tract the wafting effect produced causes mucus secreted locally by the goblet cells (to lubricate and to trap pathogens and particles) to flow in that direction (typically out of the body). Ciliated epithelium is found in the airways (nose, bronchi), but is also found in the uterus and Fallopian tubes, where the cilia propel the ovum to the uterus.

Pseudostratified

Transitional epithelium has cells that can change from squamous to cuboidal, depending on the amount of tension on the epithelium.

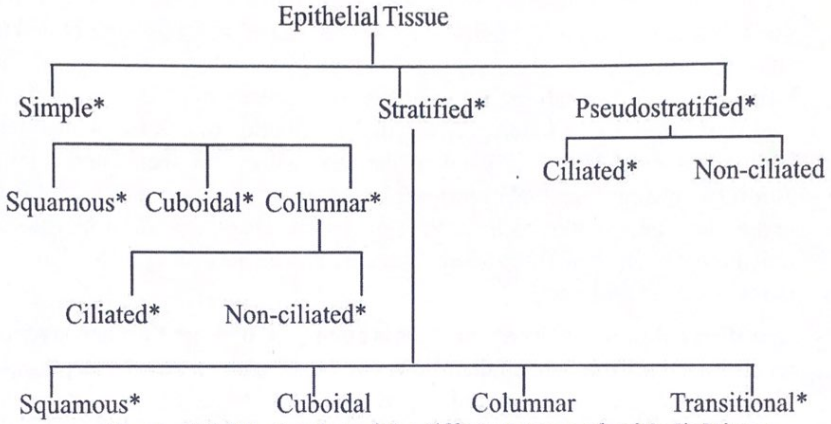


Figure 8.0: Illustration of the different types of epithelial tissue

*Most observed in laboratories

Simple or single layered epithelial tissue

- **Simple squamous epithelial** this consists of thin plate-like cells that are much expanded in two direction but have little thickness, little tensile strength and are only found as covering layers for stronger tisssues. It is also found where a smooth surface is required to reduce friction e.g. the visceral (GIT), the lining of body cavities and blood vessels.

- **Cuboidal epithelium** this consists of cells that are approximately equal in all directions. They are found in some ducts/ passage ways and active tissues of many glands.
- **Columnar epithelium** - this consists of cells that are arranged like the cells of honeycomb with whip-like projection called cilia. They are found in cells lining the trachea (wind pipe). The cilia move in such a way to carry foreign materials in the trachea towards the mouth where it can be coughed out or swallowed.
- **Pseudo-stratified columnar epithelium** these are made up of columnar cells with varying length giving the appearance of more than one (stratum) cell. They are found in the upper respiratory tract where the lining cells are ciliated.

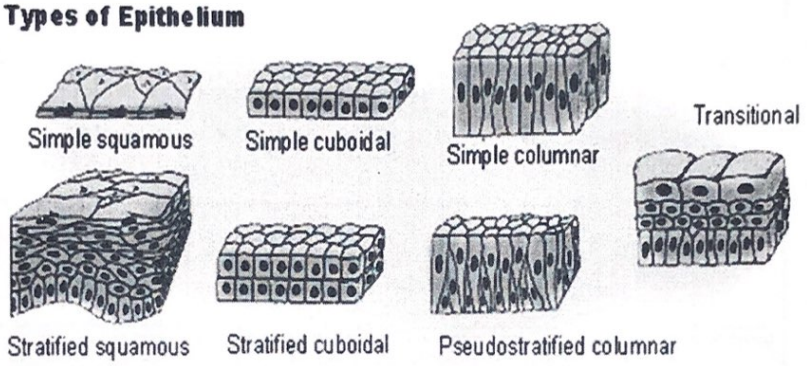
Stratified or many layered epithelial tissue

- **Stratified squamous epithelium: Stratified (arranged in layers)** this is the thickest and toughest of the epithelial, the deepest layer is known as stratum granulosum germinativum (basal layer) which contains the actively growing and multiplying cells. The stratified squamous epithelium is somewhat cuboidal in shape but become flat, tough and lifeless and in constant process of peeling off as it is pushed away from the source of nutrient. **Cornified (keratinized)** variety is located on the outer surface of the epidermis of the skin while the non-keratinized variety lines the mouth, oesophagus and vagina. The layer of cornified (keratinized) dead cells become very thick in areas that are subjected to constant friction resulting in the formation of **calluses** sometimes (calluses is what is on the hand of farmers that makes their hand thick). Stratified squamous epithelial forms the outer layer of skin, lining of the first part of the digestive tract as far as the stomach (ruminant fore stomach rumen, reticulum and omasum). Stratified tissues are named according to the shape of the cells next to the lumen.
- **Stratified columnar epithelium:** This is composed of more than one layer of columnar cells. Columnar epithelia have cells with relatively large cytoplasmic volumes and are often located where secretion or active absorption of substances is an important function. Stratified columnar epithelium are found lining part of the pharynx and salivary duct.
- **Pseudostratified columnar epithelium (ciliated):** This tissue looks like a stratified tissue but it is not (hence the prefix pseudo). This tissue also has numerous goblet cells and looks a lot like Simple Columnar Epithelium. However, unlike Simple Columnar Epithelium this tissue is not found lining villi. The presence of cilia is an important identification mark. Each cell extends from the basement membrane to the lumen.

It functions in secretion and movement of mucous by ciliary action. It is found lining the upper respiratory tract, the epididymis and part of the male urethra.

- **Transitional epithelium:** This tissue has features common to stratified cuboidal and stratified squamous epithelium (hence the name transitional). It can be identified easily from two distinguishing characteristics: 1. the surface cells are scalloped; 2. in the cells next to the lumen the nucleolus can be clearly seen in the nucleus. The appearance of this tissue can vary greatly, that is why it is called transitional epithelium. It permits distention and so forms the lining of the portion of the urinary system that is subjected to stretching. The area it is found include the urinary bladder and ureter. It has the ability of piling up many cells making it thick when the bladder is relaxed yet stretched out to a single layer when completely filled. When it is relaxed looks like a stratified epithelium but when stretched it shows its true nature as a single epithelial cell.
- **Glandular epithelium:** This is often cuboidal or columnar and has the ability to secrete various products. The cells may be arranged as single goblet cells which produce mucus or may consists of aggregation of many cells such as those found in most glands.

Types of Epithelium



- Simple
- Stratified

Columnar epithelial cell

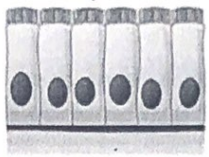
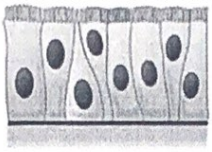
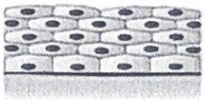
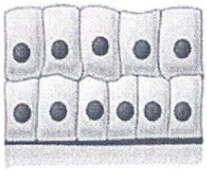
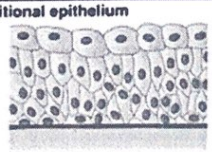
- Simple
- Stratified
- Pseudostratified

Cuboidal epithelial cell

- Simple
- Stratified

Specialised epithelia

- Olfactory
- Respiratory
- Intestinal
- Transitional
- Vaginal
- Germinal
  - female
  - male

Cells	Location	Function
Simple squamous epithelium 	Air sacs of lungs and the lining of the heart, blood vessels, and lymphatic vessels	Allows materials to pass through by diffusion and filtration, and secretes lubricating substance
Simple cuboidal epithelium 	In ducts and secretory portions of small glands and in kidney tubules	Secretes and absorbs
Simple columnar epithelium 	Ciliated tissues are in bronchi, uterine tubes, and uterus; smooth (nonciliated tissues) are in the digestive tract, bladder	Absorbs; it also secretes mucous and enzymes
Pseudostratified columnar epithelium 	Ciliated tissue lines the trachea and much of the upper respiratory tract	Secretes mucus; ciliated tissue moves mucus
Stratified squamous epithelium 	Lines the esophagus, mouth, and vagina	Protects against abrasion
Stratified cuboidal epithelium 	Sweat glands, salivary glands, and the mammary glands	Protective tissue
Stratified columnar epithelium 	The male urethra and the ducts of some glands	Secretes and protects
Transitional epithelium 	Lines the bladder, uretha, and the ureters	Allows the urinary organs to expand and stretch

Simple epithelium: Simple epithelium is a single layer of cells with every cell in direct contact with the basement membrane that separates it from the underlying connective tissue. In general, it is found where absorption and filtration occur. The thinness of the epithelial barrier facilitates these processes.

In general, simple epithelial tissues are classified by the shape of their cells. The four major classes of simple epithelium are: (1) simple squamous; (2) simple cuboidal; (3) simple columnar; (4) pseudostratified.

(1) simple squamous; which is found lining areas where passive diffusion of gases occur. e.g. skin, walls of capillaries, linings of the pericardial, pleural, and peritoneal cavities, as well as the linings of the alveoli of the lungs.

(2) simple cuboidal: these cells may have secretory, absorptive, or excretory functions. Examples include small collecting ducts of kidney, pancreas, and salivary gland.

(3) simple columnar; cells can be secretory, absorptive, or excretory; Simple columnar epithelium can be ciliated or non-ciliated; ciliated columnar is found in the female reproductive tract and uterus. Non-ciliated epithelium can also possess microvilli.

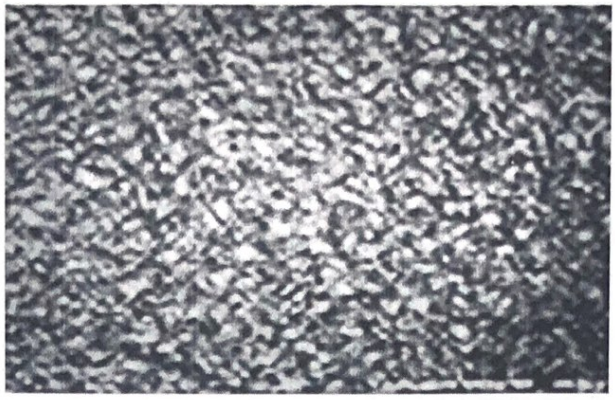
(4) pseudostratified columnar epithelium; can be ciliated or non-ciliated. The ciliated type is also called respiratory epithelium as it is almost exclusively confined to the larger respiratory airways of the nasal cavity, trachea and bronchi.

Stratified epithelium: Stratified epithelium differs from simple epithelium in that it is multilayered. It is therefore found where body linings have to withstand mechanical or chemical insult such that layers can be abraded and lost without exposing subepithelial layers. Cells flatten as the layers become more apical, though in their most basal layers the cells can be squamous, cuboidal or columnar. Stratified epithelia (of columnar, cuboidal or squamous type) can have the following specializations:

Specialization	Description
Keratinized	In this particular case, the most apical layers (exterior) of cells are dead and lose their nucleus and cytoplasm, instead contain a tough, resistant protein called keratin. This specialization makes the epithelium waterproof, so is found in the mammalian skin. The lining of the esophagus is an example of a non-keratinized or "moist" stratified epithelium.
Parakeratinized	In this case, the most apical layers of cells are filled with keratin, but they still retain their nuclei. These nuclei are pyknotic, meaning that they are highly condensed. Parakeratinized epithelium is sometimes found in the oral mucosa and in the upper regions of the esophagus.

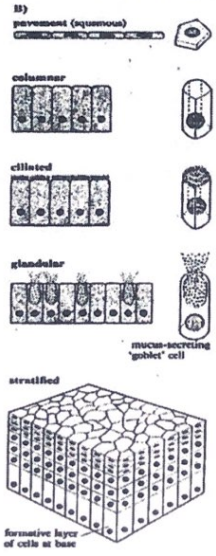
Transitional

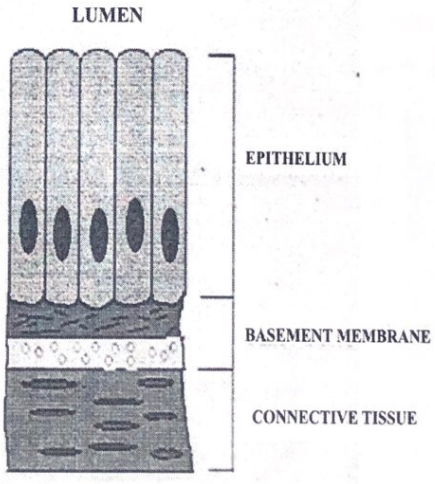
Transitional epithelia are found in tissues that stretch and it can appear to be stratified cuboidal when the tissue is relaxed, or stratified squamous when the organ is distended and the tissue stretches. It is sometimes called urothelium since it is almost exclusively found in the bladder, ureters and urethra.



Squamous Epithelium 100x

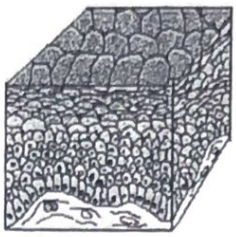
Sensing the extracellular environment: "Some epithelial cells are ciliated, especially in respiratory epithelium, and they commonly exist as a sheet of polarised cells forming a tube or tubule with cilia projecting into the lumen." Primary cilia on epithelial cells provide chemosensation, thermoception, and mechanosensation of the extracellular environment by playing "a sensory role mediating specific signalling cues, including soluble factors in the external cell environment, a secretory role in which a soluble protein is released to have an effect downstream of the fluid flow, and mediation of fluid flow if the cilia are motile."





Simple columnar epithelium

Drawing of Stratified Squamous Epithelium – Superior and lateral aspect



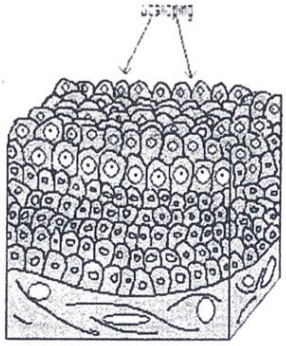
Notice that the cells near the basement membrane are columnar to Cuboidal in shape, and the cells near the surface (lumen) are squamous in shape. This tissue is named according to the shape of the cells near the lumen, so this is Stratified Squamous Epithelium

Photomicrograph of Stratified Squamous Epithelium – non-keratinized (40X)



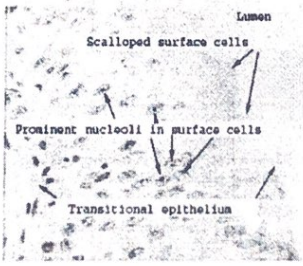
At this magnification the Stratified Squamous Epithelium appears as a darkly stained band next to the lumen. This is a slide of the epidermal layer of the vagina.

Drawing of Transitional Epithelium – Superior and lateral aspects



This drawing illustrates the stratified nature of this tissue. The cells next to the lumen can be squamous, Cuboidal, and/or columnar in shape, hence the name transitional

Photomicrograph of Transitional Epithelium (400X)

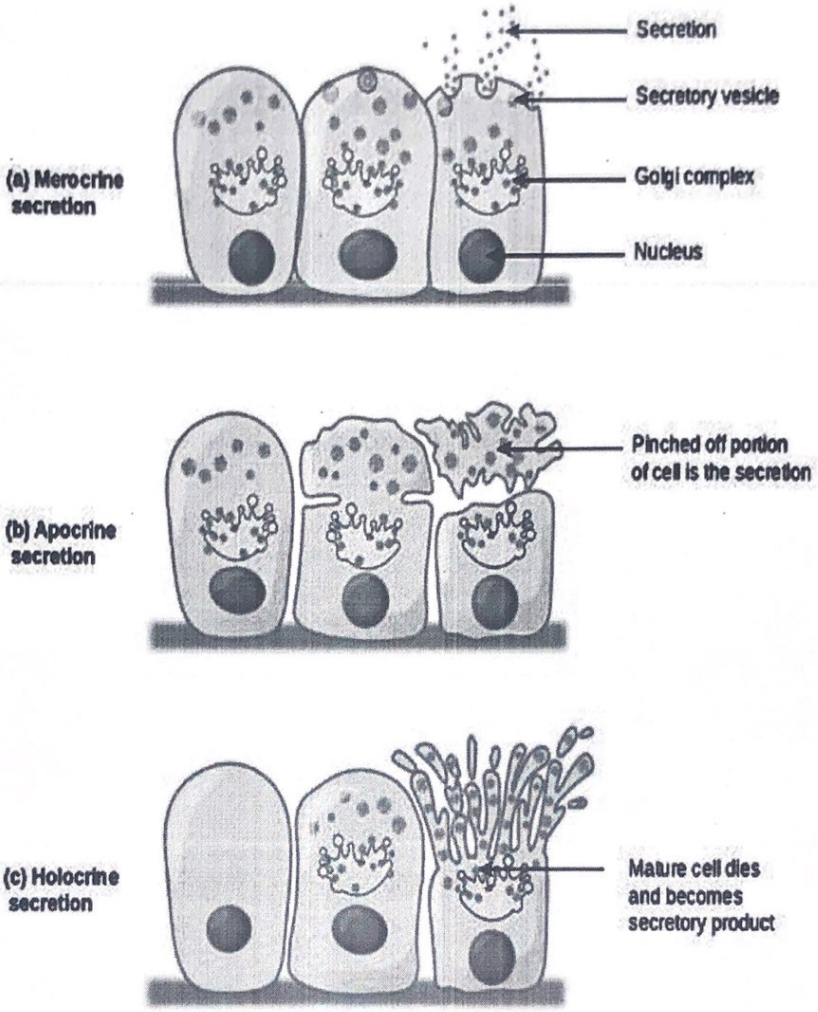


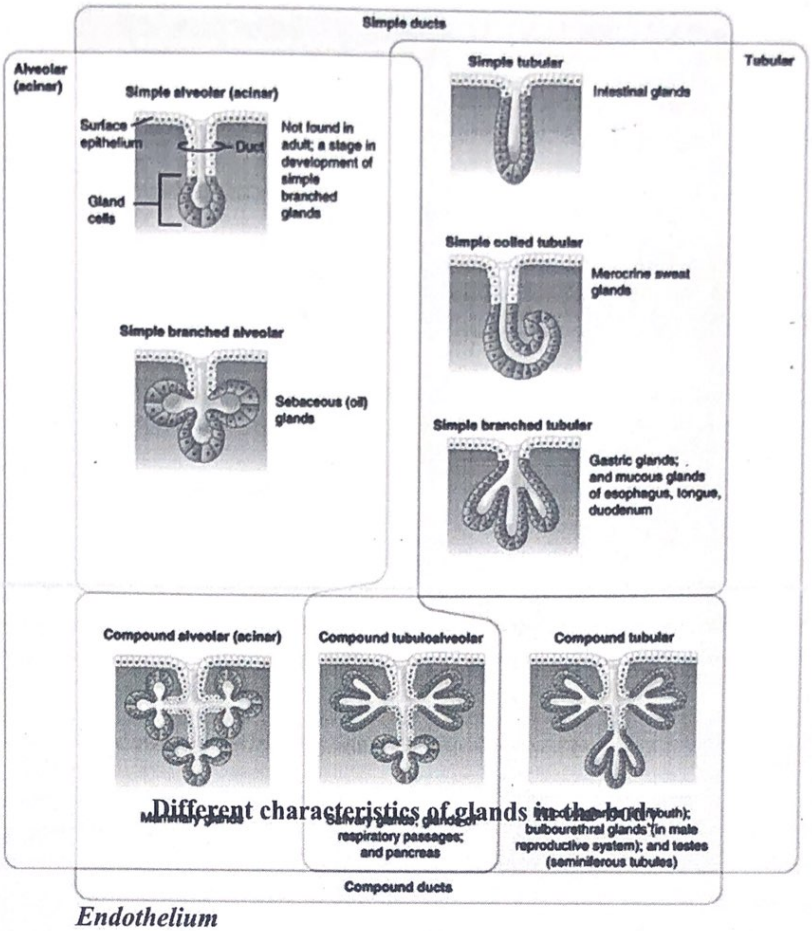
The shape of the cells next to the lumen, the presence of scalloping, and the fact that in the nucleus of the cells next to the lumen the nucleolus can be seen, are all identification clues

Figure 9.0: Structure of the different types of epithelial tissues in animals

Glandular tissue is the type of epithelium that forms the glands from the infolding of epithelium and subsequent growth in the underlying connective tissue. There are two major classifications of glands: endocrine glands and exocrine glands:

- Endocrine glands secrete their product into the extracellular space where it is rapidly taken up by the circulatory system.
- Exocrine glands secrete their products into a duct that then delivers the product to the lumen of an organ or onto the free surface of the epithelium.





Different characteristics of glands in the body

Endothelium

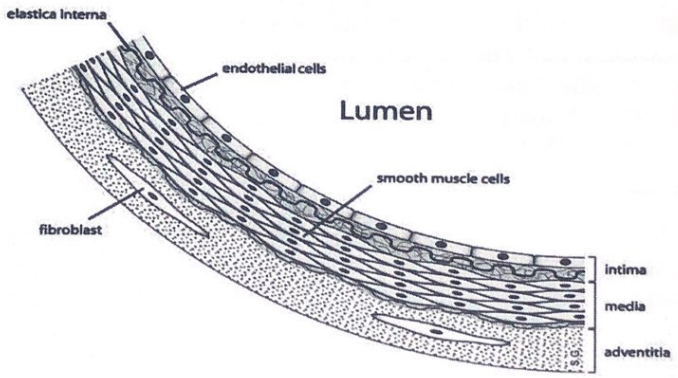
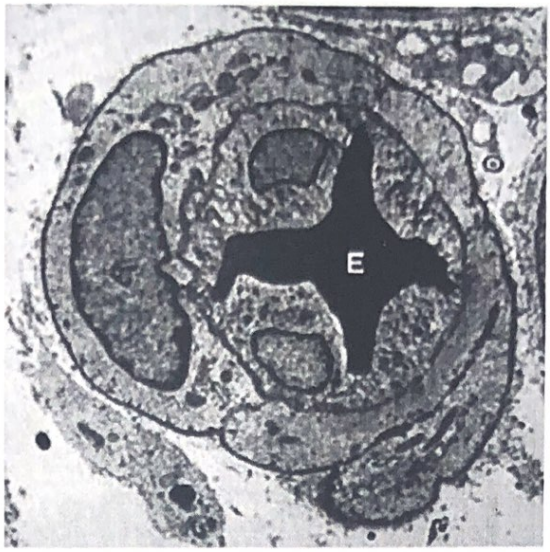


Diagram showing the location of endothelial cells

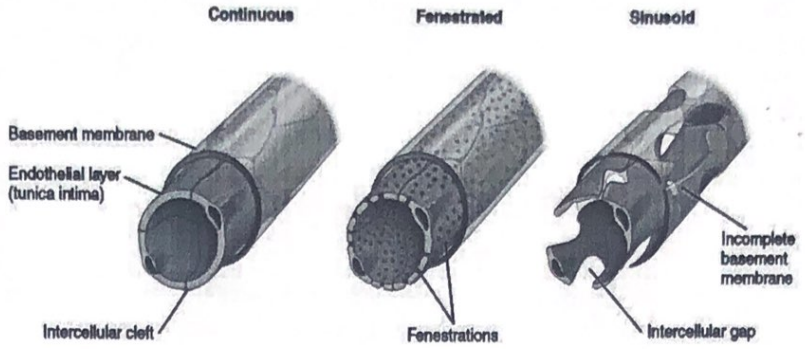


Transmission electron micrograph of a microvessel showing endothelial cells, which encircle an erythrocyte (E), forming the innermost layer of the vessel, the tunica intima

Endothelium refers to cells that line the interior surface of blood vessels and lymphatic vessels, forming an interface between circulating blood or lymph in the lumen and the rest of the vessel wall. It is a thin layer of simple, or single-layered, squamous cells called **endothelial cells**. Endothelial cells in direct contact with blood are called vascular endothelial cells, whereas those in direct contact with lymph are known as lymphatic endothelial cells.

Vascular endothelial cells line the entire circulatory system, from the heart to the smallest capillaries. These cells have unique functions in vascular biology. These functions include fluid filtration, such as in the glomerulus of the kidney, blood vessel tone, hemostasis, neutrophil recruitment, and hormone trafficking. Endothelium of the interior surfaces of the heart chambers is called endocardium.

Endothelium is mesodermal in origin. Both blood and lymphatic capillaries are composed of a single layer of endothelial cells called a monolayer. In straight sections of a blood vessel, vascular endothelial cells typically align and elongate in the direction of fluid flow. The distinctive difference between endothelial cells and epithelial cells are on the basis of the tissues they develop from, as well as the presence of vimentin rather than keratin filaments as in epithelial cells. Many considered the endothelium a specialized epithelial tissue.



Endothelium lines the inner wall of vessels

Endothelial cells are involved in many aspects of vascular biology, including:

- Barrier function - the endothelium acts as a semi-selective barrier between the vessel lumen and surrounding tissue, controlling the passage of materials and the transit of white blood cells into and out of the bloodstream. Excessive or prolonged increases in permeability of the endothelial monolayer, as in cases of chronic inflammation, may lead to tissue edema/swelling.
- Blood clotting (thrombosis & fibrinolysis). The endothelium normally provides a non-thrombogenic surface because it contains, for example, heparan sulfate which acts as a cofactor for activating antithrombin, a protease that inactivates several factors in the coagulation cascade.
- Inflammation
- Formation of new blood vessels (angiogenesis)
- Vasoconstriction and vasodilation, and hence the control of blood pressure
- Repair of damaged or diseased organs via an injection of blood vessel cells
- Angiopoietin-2 works with VEGF to facilitate cell proliferation and migration of endothelial cells

Endothelial dysfunction, or the loss of proper endothelial function, is a hallmark for vascular diseases, and is often regarded as a key early event in the development of atherosclerosis. Impaired endothelial function, causing hypertension and thrombosis, is often seen in patients with coronary artery disease, diabetes mellitus, hypertension, hypercholesterolemia, as well as in smokers. Endothelial dysfunction has also been shown to be predictive of future adverse cardiovascular events, and is also present in inflammatory disease such as rheumatoid arthritis and systemic lupus erythematosus. One of the main mechanisms of endothelial dysfunction is the diminishing of nitric oxide, often due to high levels of asymmetric dimethylarginine, which interfere with the normal L-arginine-stimulated nitric oxide synthesis and so leads to hypertension.

The most prevailing mechanism of endothelial dysfunction is an increase in reactive oxygen species, which can impair nitric oxide production and activity via several mechanisms. The signalling protein ERK5 is essential for maintaining normal endothelial cell function.[9] A further consequence of damage to the endothelium is the release of pathological quantities of von Willebrand factor, which promote platelet aggregation and adhesion to the subendothelium, and thus the formation of potentially fatal thrombi.

Functions of Epithelial tissues are:

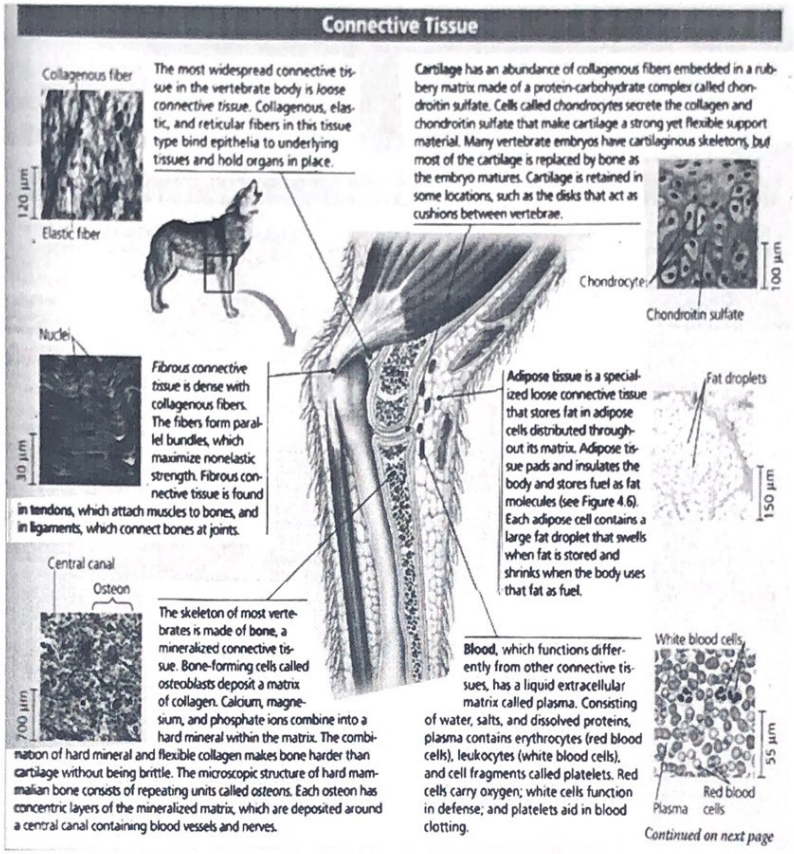
1. to protect the tissues that lie beneath from radiation, desiccation, toxins, invasion by pathogens, and physical trauma
2. the regulation and exchange of chemicals between the underlying tissues and a body cavity
3. the secretion of hormones into the circulatory system, as well as the secretion of sweat, mucus, enzymes, and other products that are delivered by ducts
4. to provide sensation

CONNECTIVE TISSUES (CT) is one of the four basic types of animal tissue, along with epithelial tissue, muscle tissue, and nervous tissue. It develops from the mesoderm. Connective tissue is found in between other tissues everywhere in the body, including the nervous system. In the central nervous system, the three outer membranes (the meninges) that envelop the brain and spinal cord are composed of connective tissue. They support and protect the body. All connective tissue consists of three main components: fibers (elastic and collagenous fibers), ground substance and cells. Not all authorities include blood or lymph as connective tissue because they lack the fiber component. All are immersed in the body water. The cells of connective tissue include fibroblasts, adipocytes, macrophages, mast cells and leucocytes. It is the most abundant tissue type in the body. Most connective tissue has a rich blood supply (highly vascular) except the hyaline cartilage that is avascular i.e. all connective tissue (except cartilage) has an extensive nerve and blood supply. Connective tissue is characterized by widely scattered cells found in an intercellular matrix. They are tissues that support and bind other tissues together. It gives form and strength to many organs and often serves for protection and leverage. They include yellow elastic tissue, collagenous tissue (white fibrous), reticular tissues (net-like), adipose tissue (fat), cartilage (gristol) and bone. The connective tissue is made up of three basic elements:

1. **Fibers (such as collagen, reticular, and elastic).**
2. **Cells (such as fibroblasts, macrophages, and adipose).**
3. **Ground substances (matrix).**

The matrix (ground substance) of the connective tissue may be:

1. Fluid as in Vascular Connective Tissue (blood).
2. Semi fluid as in Loose Connective Tissue (Areolar).
3. Gelatinous as in Mucous Connective Tissue.
4. Fibrous as in Dense Regular Connective Tissue.
5. Calcified as Osseous Connective Tissue (bone).



Classification of the Connective Tissue

Classification of the connective tissue may be based on the consistency of the matrix, the physical characteristics or types of cells. Connective tissue can be broadly subdivided into **connective tissue proper** and **special connective tissue**. **Connective tissue proper** consists of loose connective tissue and dense connective tissue (which is further subdivided into dense regular and dense irregular connective tissues).

Loose and dense connective tissues are distinguished by the ratio of ground substance to fibrous tissue. Loose connective tissue has much more ground substance and a relative lack of fibrous tissue, while the reverse is true of dense connective tissue. Dense regular connective tissue (found in structures such as tendons and ligaments) is characterized by collagen fibers arranged in an orderly parallel fashion, giving it tensile strength in one direction. Dense irregular connective tissue provides strength in multiple directions by its dense bundles of fibers arranged in all directions.

Special connective tissue consists of reticular connective tissue, adipose tissue, cartilage, bone, and blood. Other kinds of connective tissues include fibrous, elastic, and lymphoid connective tissues. New vascularised connective tissue that forms in the process of wound healing is termed granulation tissue. Fibroblasts are the cells responsible for the production of some CT.

Connective tissues could therefore be majorly classified into:

- Proper Connective Tissues
- Supportive Connective Tissue

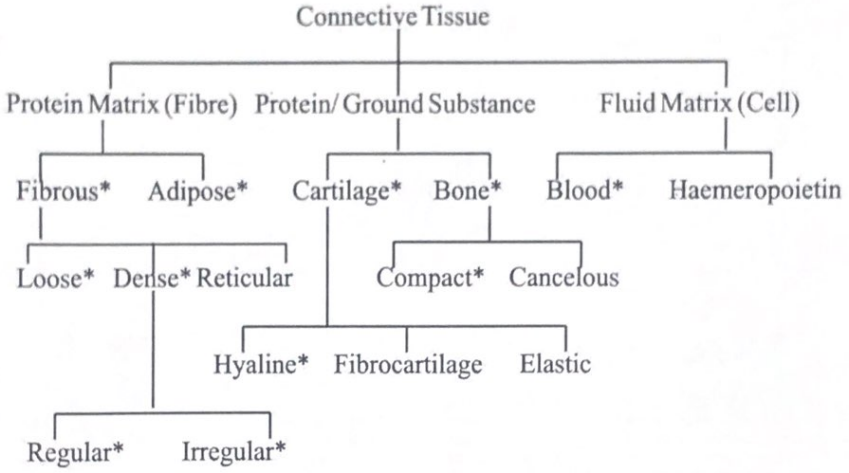
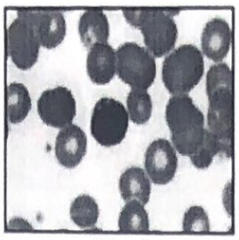
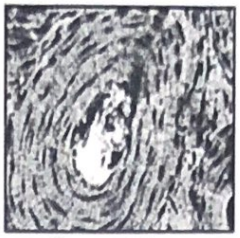


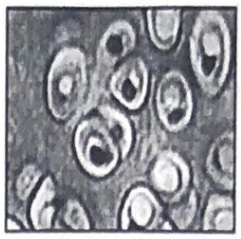
Figure 10.0: Flow chart of the various types of connective tissue

*Most identified in laboratory

Type I collagen is present in many forms of connective tissue, and makes up about 25% of the total protein content of the mammalian body. Laboratory


Blood


Osseous tissue


Hyaline cartilage

Characteristics of CT:

- Cells are spread through an extracellular fluid.
- Ground substance - A clear, colourless, and viscous fluid containing glycosaminoglycans and proteoglycans to fix the body water and the collagen fibers in the intercellular spaces. Ground substance slows the spread of pathogens.
- Fibers. Not all types of CT are fibrous. Examples of non-fibrous CT include adipose tissue and blood. Adipose tissue gives "mechanical cushioning" to the body, among other functions. Although there is no dense collagen network in adipose tissue, groups of adipose cells are kept together by collagen fibers and collagen sheets in order to keep fat tissue under compression in place (for example, the sole of the foot). The matrix of blood is plasma.
- Both the ground substance and proteins (fibers) create the matrix for CT. Connective tissues are derived from the mesenchyme.

Types of fibers:

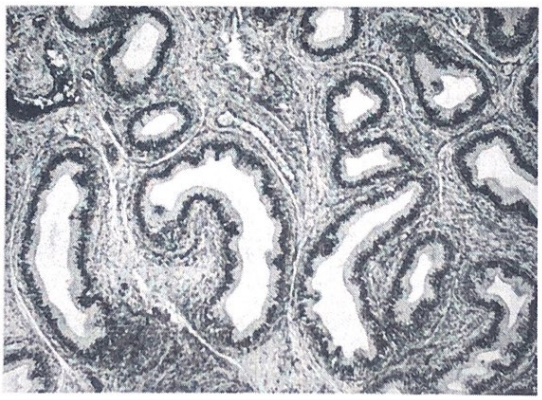
Tissue	Purpose	Components	Location
Collagenous Fibers	Bind bones and other tissues to each other	Alpha polypeptide chains blood	tendon, ligament, skin, cornea, cartilage, bone, vessels, gut, and intervertebral disc.
Elastic fibers	Allow organs like arteries and lungs to recoil	Elast microfibril Elast and elastin	extracellular matrix
Reticular Fibers	Form a scaffolding for other cells	Type III collagen	liver, bone marrow, and lymphatic organs

Connective tissue has a wide variety of functions that depend on the types of cells and the different classes of fibers involved. Loose and dense irregular connective tissue, formed mainly by fibroblasts and collagen fibers, have an important role in providing a medium for oxygen and nutrients to diffuse from capillaries to cells, and carbon dioxide and waste substances to diffuse from cells back into circulation. They also allow organs to resist stretching and tearing forces. Dense regular connective tissue, which forms organized structures, is a major functional component of tendons, ligaments and aponeuroses, and is also found in highly specialized organs such as the cornea. Elastic fibers, made from elastin and fibrillin, also provide resistance to stretch forces. They are found in the walls of large blood vessels and in certain ligaments, particularly in the ligamenta flava.

In hematopoietic and lymphatic tissues, reticular fibers made by reticular cells provide the stroma or structural support for the parenchyma or functional part of the organ.

Mesenchyme is a type of connective tissue found in developing organs of embryos that is capable of differentiation into all types of mature connective tissue. Another type of relatively undifferentiated connective tissue is mucous connective tissue, found inside the umbilical cord.

Various types of specialized tissues and cells are classified under the spectrum of connective tissue, and are as diverse as brown and white adipose tissue, blood, cartilage and bone. Cells of the immune system, such as macrophages, mast cells, plasma cells and eosinophils are found scattered in loose connective tissue, providing the ground for starting inflammatory and immune responses upon the detection of antigens. Most staining-techniques, colour tissue fibers in contrasting shades. Collagen may be differentially stained by any of the following: Van Gieson's stain, Masson's trichrome stain, Mallory's trichrome stain, Aniline blue stain, Eosin, and Reticulin stain.



Section of epididymis. Connective tissue (blue) is seen supporting the epithelium (purple)

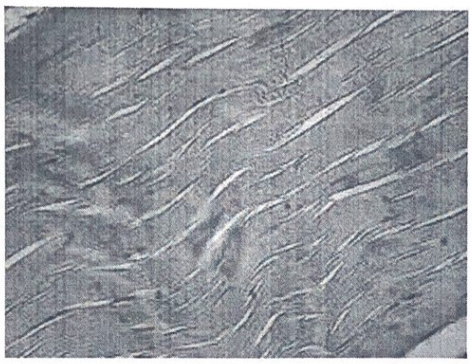
Proper Connective Tissue

FIBRE There are three different types of fibre, namely:

Dense Regular Fibrous Connective Tissue (white collagenous fibre): This tissue contains numerous densely packed collagen fibres oriented in the same direction i.e. arranged orderly in parallel fashion. This produces great strength in the direction the fibres run, but can be relatively weaker in a direction at right angle to the fibre direction. The fibres are slightly wrinkled, allowing them to stretch to some degree in the direction of the fibre. It has less flexibility than the Areolar connective tissue but is more resistant to stress. It appears as fine white glittering strands that occur in interlacing bundles. They are seen as many collagen fibres running in the same direction with individual fibres appearing to be "wavy". Fibroblasts can be seen between the fibres. They are found all through the body in various forms, specifically where strength is required. They make up tendons and ligaments. It functions in attaching muscles to bones (tendons) and bone to bone (ligaments).

- Dense Irregular Connective Tissue this is made up of primary white collagenous fibre that runs in all directions. The dermis of the skin which may be tanned to make leather is made up of this tissue. The tissue forms a strong covering that resists tearing and yet is flexible enough to move with the surface of the body.

Photomicrograph of Dense regular connective tissue (100x)

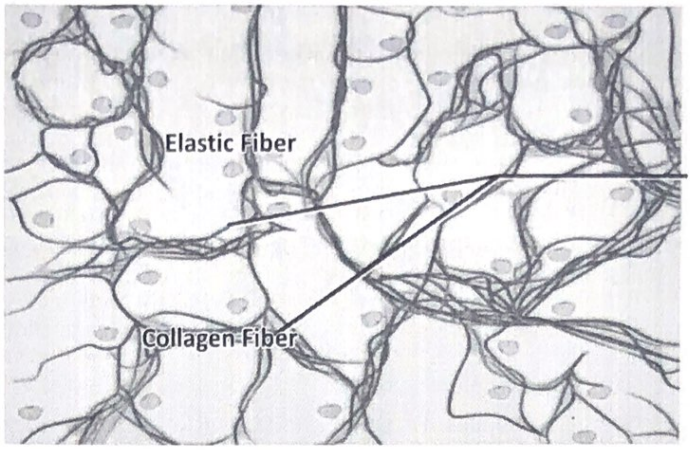


Note that the collagen fibers all run in the same direction (on this view from left to right). The "crinkly" or "wavy" appearance of the fibers is an identifiable characteristic of this tissue.

Loose (Areolar) Connective Tissue (Yellow Elastic Fibre): It has many collagen and elastic fibres. It is made up of linked fibre that tends to regain its original shape after being stretched. They are coarse and occur in separate branched strands. It is the most common and most typical because it contains practically all of the cells and fibres. The nuclei of many cells associated with the connective tissue can be seen. It consists of collagen, elastic and reticular fibres embedded in a semifluid matrix, together with fibroblasts, mast cells, plasma cells and macrophages. This type of tissue is found where protective cushioning and flexibility are required such as in blood vessel and beneath the dermis where it is attached to the skin. They are found in ligamental nuchae (a strong band that supports the head) in the horse and cattle. They are located in the hypodermis (subcutaneous layer of skin) and around most body organs. It functions by loosely binding organs together.

- Dense Elastic Connective Tissue the major fibre in this tissue is the yellow

Photomicrograph of Loose (Areolar) Connective Tissue (100x)

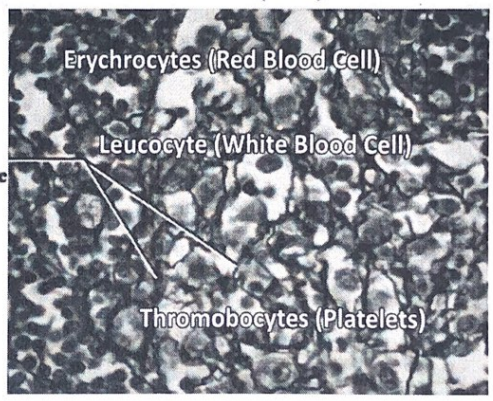


Reticular Fibre: These consist of fine fibrils and cells that make up part of the frame work of endocrine and lymphatic organs.

CELLS: Vascular Connective Tissue. Blood cell (haematocyte) is produced by haematopoiesis in the bone marrow. Blood is a connective tissue with a fluid matrix called plasma. Three basic types of cells are found suspended in the plasma: Erythrocytes (red blood cells), Leukocytes (white blood cells) and thrombocytes (platelets). These three blood cell types' makes up 45% of the total blood tissue with the remaining 55% composed of plasma (the liquid component of blood).

Photomicrograph of Vascular Connective Tissue (400x)

The erythrocytes (red blood cells) are pink with a light center (where the cell is thinner and the light shines through). The Leukocytes (white blood cells) are stained dark pink to purple and have an obvious darkly stained nucleus. The Thrombocytes appear as small purple specks.



Erythrocytes or red blood cells Red blood cells are small cells that lack nuclei when mature. They are circular, non-nucleated biconcave disc that contain oxygen carrying protein pigment called haemoglobin which gives blood its red colour. Haemoglobin (the main component of RBC) is an iron containing protein that facilitates transportation of oxygen from the lungs to tissues of the body and carbondioxide from the tissues to the lungs.

The lack of nuclei makes more room for haemoglobin. RBC shape gives a larger surface area to volume ratio than that of a sphere and therefore increases the area which can be use for gaseous exchange. It also enhances the flexibility and allows it to squeeze through capillaries. It stains pink with the centre of the cell lighter than the rim. It functions in transport of respiratory gases (oxygen and carbon dioxide). Mature RBC has a relatively short life span of about 3 months with approximately five million red blood cells per cubic millimeter of blood. A condition known as anaemia results from low levels of RBC, insufficient haemoglobin, deficiency of folic acid or vitamin B_{12}, premature degradation of RBC or sickled cells. Anaemia can also be referred to as a condition of reduced concentration of functional red cells in the blood.

Leukocytes or white blood cells- these are nucleated cells capable of independent movement and with varying life span. They are larger than the RBC and are present in much smaller number. There are about 7000 WBC per cubic millimeter of blood. Though they have nuclei, their lifespan in the blood stream is normally only a few days. They are capable of growing (amoeboid) movement which allows them to move or squeeze through pores in capillary walls to reach tissues/ sites of infection. They function as defence mechanism against infections in the body. They are stained dark pink to purple with obviously darkly stained (usually multi-lobed) nucleus. Found in blood plasma and lymphatic tissues, they are involved in immunity. There are five basic types of white blood cells: neutrophils (phagocytes), eosinophils, basophils, monocytes and lymphocytes. These basic types of WBC are categorized into two groups depending on the presence of granules in their cytoplasm: Granulocytes and Agranulocytes

Granulocytes - these have granules in their cytoplasm and include the following:

- Neutrophils (phagocytes) these are actively phagocytic, engulf and digest disease causing organisms.
- Eosinophils - they possess cytoplasmic granules which stains red with eosin dye. More profound in people with allergic conditions such as asthma or hayfever. It possesses anti-histamine properties.
- Basophils - this produces heparin, an anti-clotting protein and histamine, a chemical found in damaged tissues which are involved in inflammation.

Agranulocytes these possess non granular cytoplasm and are sub divided into:

- Monocytes - they become macrophages which are phagocytic and engulf bacteria and other large particles.
- Lymphocytes-they are involved in immune reactions (such as antibody production, graft rejection and in killing tumour cells).

Thrombocytes (Platelets) they are irregularly shaped membrane bound cell fragments usually lacking nuclei and are formed from special bone marrow cells. They are responsible for starting the process of blood clotting. They survive for about 5-9 days before being destroyed by the spleen and liver.

They are darkly stained structures much smaller than the erythrocytes or appear as small purple specks. They are suspended in the blood plasma and are involved in blood clotting.

Fibroblast - these are a thin star-shaped cell that manufactures fibres and collagens.

Macrophages - these are large amoeboid cells that wandering around the body systems. They function by engulfing foreign bodies in the tissue.

Mast cells they are found in the capillaries and help to prevent coagulation of blood and that is why they secrete **heparin**.

Plasma cells - these are round connective tissues that secrete antibodies that are used to fight specific types of infections. They are mostly found in the milk, colostrums or blood. Plasma refers to the fluid part of unclotted blood. It is particularly useful as a substance for blood in transfusion because the protein in plasma gives the same osmotic pressure as the blood. Plasma therefore does not escape from blood vessels as readily as blood solution.

Serum - is the yellow supernatant fluid that is expressed out when blood clots and retracts. It is plasma but without most of the clotting factors. Serum is used for prevention and treatment of diseases because it contains antibody fraction of the blood

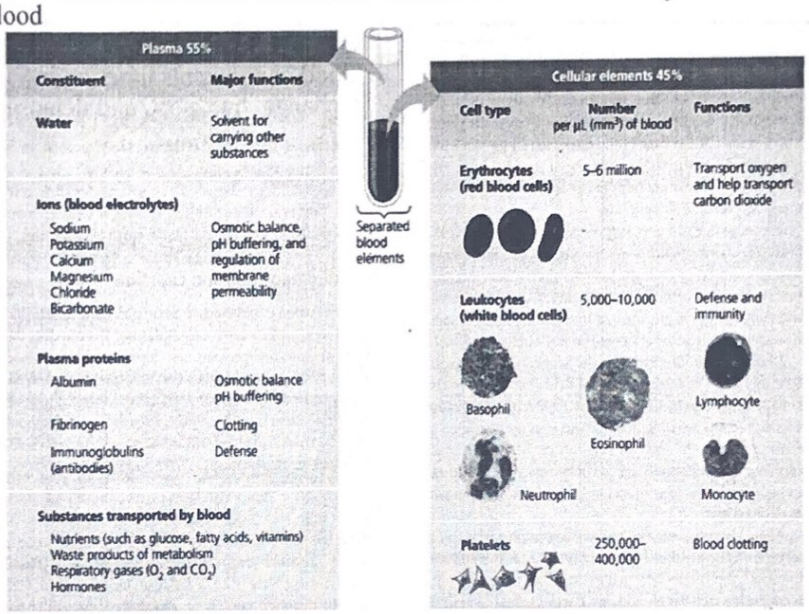
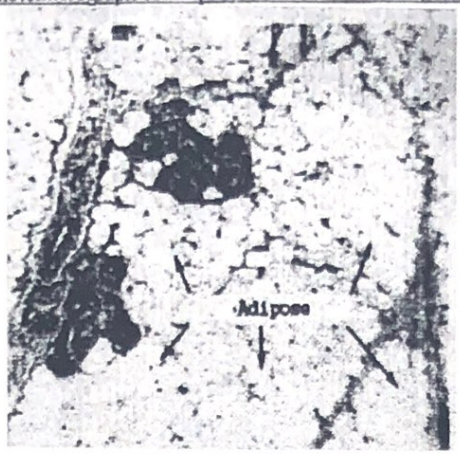


Figure 11.0: Composition of blood

Adipose Connective Tissue (Fat) - this tissue consists of adipocytes that wrap themselves around lipid droplets. It resembles a chicken-wire fence. The individual adipocytes have a large central storage area where fats and oils are stored. They are found in the subcutaneous layer of the skin, the fatty-capsule of the kidney, the yellow bone marrow and around the heart. It functions in energy storage, insulation and protection.

Photomicrograph of Adipose Connective Tissue (100X)



This is a slide of the hypodermis. Connective tissue fibers, blood vessels, and sweat glands can be seen embedded in the adipose tissue

GROUND SUBSTANCES: These are the various gel-like materials that are soluble in water. The ground substances and the fibre both form the matrix of the connective tissue, serving as the foundation on which other layers are placed. The matrix depends on the proportion of the two substances. The matrix may be fluid as observed in blood, gel-like as in adipose tissues, plastic as in cartilage or very hard and rigid as in bones.

Supportive Connective Tissue: This is subdivided into cartilage and bone.

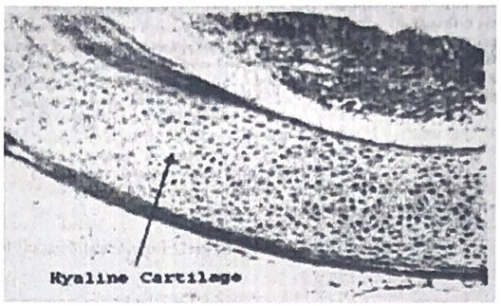
CARTILAGE CONNECTIVE TISSUE: This is a special type of connective tissue commonly called **gristle**. It looks like an opaque bluish tissue with the made up of hard rubber board. The nature of cartilage is due to the structure of the intercellular material found between cartilage cells (chondrocytes). The ground substance of cartilage is **plastic.** There are three types of cartilages namely:

- **Hyaline**
- **Elastic**
- **Fibrous**

Hyaline Cartilage Connective Tissue - This is a glass-like tissue covering the bones within joints. It forms a smooth surface that reduces friction so that one bone easily glides over another. This tissue has a matrix that is firm enough to bear a lot of pressure without permanent distortion. Hence, it is found in joints where it functions as a shock-absorber and friction reducing surface for bone articulation. It consists of **chondrocytes** (cartilage cells) located in spaces called **lacuna**, surrounded by a bluish matrix interlaced with collagen fibres. The actively growing areas at the end of long bones also contain hyaline cartilage. Bones in the embryo start off as hyaline cartilage templates for the later developing bone.

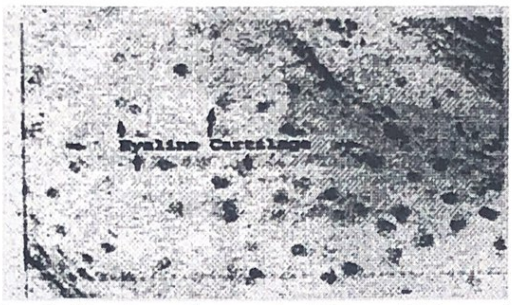
It is found at the ends of long bones, between the ribs and the sternum, tracheal rings, parts of the larynx, and embryonic skeleton. It functions to reduce friction in joints and give support with flexibility. The "crinkly" or "wavy" appearance of the collagen fibres which all runs in the same direction (left to right) is an identifiable characteristic of this tissue.

Photomicrograph of Hyaline Cartilage Connective Tissue (100X)



At this magnification to ring of Hyaline Cartilage can be seen occupying the center of the wall of the trachea. The individual cells of the cartilage (chondrocytes) are just visible.

Photomicrograph of Hyaline Cartilage Connective Tissue (400X)



The chondrocytes in the lacuna are obvious at this higher magnification. Notice that the chondrocytes often are found in pairs inside the lacuna. The matrix is blue to bluish-white.

Elastic Cartilage Connective Tissue - This consists of a mixture of cartilaginous substances and fibre. It forms the basis of the external ear.

Fibrous Cartilage Connective Tissue (Fibrocartilage) - These consist of a mixture of cartilage and collagenous fibre which forms a semi elastic cushion of great strength. Found in the intervertebral column.

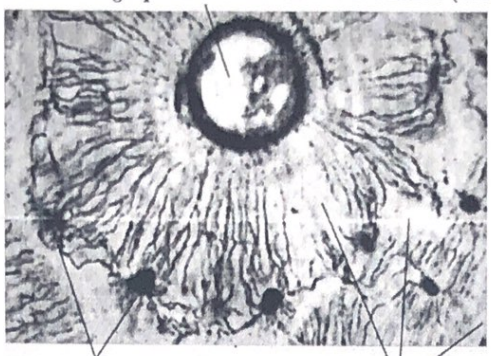
OSSEOUS CONNECTIVE TISSUE (Bone): This is formed from osteoblasts and osteocyte (bone cells). The structural unit of compact bone is the osteon or Haversian system. The bone cells produce osteoid tissues which later calcify to form bone. Calcification is the addition of calcium to osteoid tissue to form bone.

Osteocytes → Osteoid Tissue → Bone

Bone has ground substance that is harder than that of cartilage (plastic). The bones may be arranged in the form of spicules (small spikes) and flat plate forming a sponge-like network called **cancelous or spongy bone** as found in the extremities of long bones where resistance to compression without excessive weight is needed.

Bone tissue has a hard matrix containing ions such as calcium and phosphorus which is laid down around a dense network of collagen fibres in a layer called lamellae. System of canals containing blood vessels, nerves and lymphatic vessels can be identified. They resemble tree stumps and are known as Haversian systems or Osteons. The Haversian canal is located in the center of the osteon. It contains nerves, lymphatics and blood vessels that run the length of the bone. Bone cells (Osteocytes) can be seen inside their spaces (lacuna). Networks of osteons make ground bone resemble a field of tree stumps. It is found in the bones of the body and functions by providing protection, support, mineral and fat storage (marrow). Together with skeletal muscle it is responsible for movement of the body.

Photomicrograph of Osseous Connective Tissue (100X)



The Haversian canal is located in the center of the osteon. It contains nerves, lymphatics and blood vessels that run the length of the bone. .The wide opening on the left is a Volkmann's canal that transports the same structure from the outside of the bone to the inside of the bone (marrow cavity) and back.

MUSCLE TISSUE: **Muscle tissue** is a soft tissue that makes up the muscles in animal bodies, and gives rise to muscles' ability to contract. It is different from other components or tissues in muscle such as tendons or perimysium. Muscle tissues are formed during embryonic development through a process known as myogenesis. Muscle tissue varies with function and location in the body. In mammals the three types are: skeletal or striated muscle; smooth or non-striated muscle; and cardiac muscle, which is sometimes known as semi-striated. Smooth and cardiac muscle contracts involuntarily, without conscious intervention. These muscle types may be activated both through interaction of the central nervous system as well as by receiving innervation from peripheral plexus or endocrine (hormonal) activation. Striated or skeletal muscle only contracts voluntarily, upon influence of the central nervous system. Reflexes are a form of non-conscious activation of skeletal muscles, but nonetheless arise through activation of the central nervous system, albeit not engaging cortical structures until after the contraction has occurred. The different muscle types vary in their response to neurotransmitters and endocrine substances such as acetylcholine, noradrenaline, adrenaline, nitric oxide and among others depending on muscle type and the exact location of the muscle. Sub-categorization of muscle tissue is also possible, depending on among other things the content of myoglobin, mitochondria, myosin ATPase etc.

Muscle cells (myocytes) are elongated cells ranging from several millimetres to about 10 centimetres in length and from 10 to 100 micrometres in width. These cells are joined together in tissues that may be either striated or smooth, depending on the presence or absence, respectively, of organized, regularly repeated arrangements of myofibrillar contractile proteins called myofilaments. Striated muscle is further classified as either skeletal or cardiac muscle.[3] Striated muscle is typically subject to conscious control, while smooth muscle is not. Muscle tissue is composed of fibres that function to contract and short the muscle for contraction. There are three distinct types of muscle tissue based on location, structural and/ or functional characteristics.

- **Skeletal Muscle Tissue**
- **Smooth Muscle Tissue**
- **Cardiac Muscle Tissue**

- Skeletal muscle, striated in structure and under voluntary control, is anchored by tendons (or by aponeuroses at a few places) to bone and is used to effect skeletal movement such as locomotion and to maintain posture. (Though postural control is generally maintained as an unconscious reflexsees proprioceptionthe muscles responsible also react to conscious control like non-postural muscles.) An average adult male is made up of 42% of skeletal muscle and an average adult female is made up of 36% (as a percentage of body mass). It also has striations unlike smooth muscle.
- Smooth muscle, are non-striated in structure and under involuntary control, and is found within the walls of organs/ structures such as the esophagus, stomach, intestines, bronchi, uterus, urethra, bladder, blood vessels, and the erector pili in the skin (in which it controls erection of body hair).
- In vertebrates, there is a third muscle tissue, Cardiac muscle (myocardium), found only in the heart, is a striated muscle similar in structure to skeletal muscle but under involuntary control.

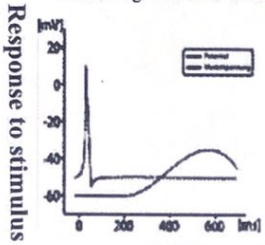
Cardiac and skeletal muscles are "striated" in that they contain sarcomeres and are packed into highly regular arrangements of bundles; smooth muscle has neither. While skeletal muscles are arranged in regular, parallel bundles, cardiac muscle connects at branching, irregular angles (called intercalated discs). Striated muscle contracts and relaxes in short, intense bursts, whereas smooth muscle sustains longer or even near-permanent contractions.

. **Comparison of types**

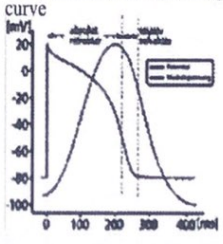
Anatomy	smooth muscle	cardiac muscle	skeletal muscle
Neuromuscular junction	none	none	present
Fibers	fusiform, short (<0.4 mm)	branching	cylindrical, long (<15 cm)
Mitochondria	few	numerous	many to few (by type)
Nuclei	1	1	>1
Sarcomeres	none	present, max. length 2.6 µm	present, max. length 3.7 µm
Syncytium	none (independent cells)	none (but functional as such)	present
Sarcoplasmic Reticulum	little elaborated	moderately elaborated	highly elaborated
ATPase	little	moderate	abundant
Physiology			
Self-regulation	spontaneous action (slow)	yes (rapid)	none (requires nerve stimulus)
Response to Stimulus	unresponsive	"all-or-nothing"	"all-or-nothing"
Action Potential	yes	yes	yes

Workspace

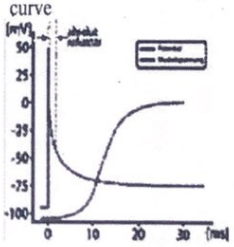
Force/length curve is variable



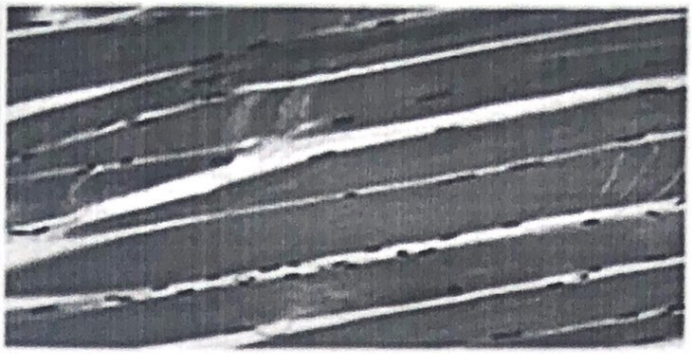
the increase in the force/length at the peak of the force/length curve



curve



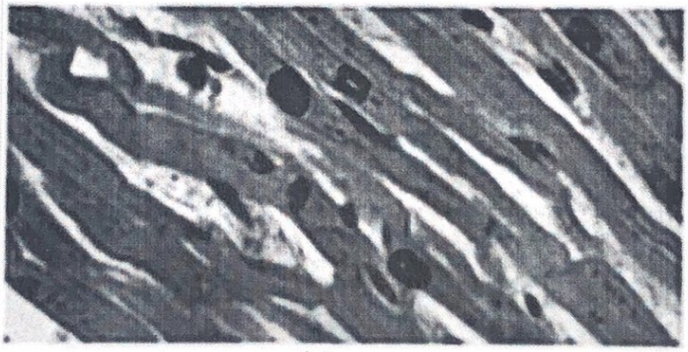
Muscle tissue



(a)

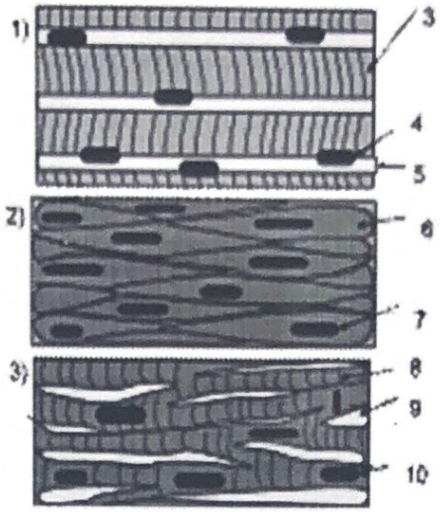


(b)



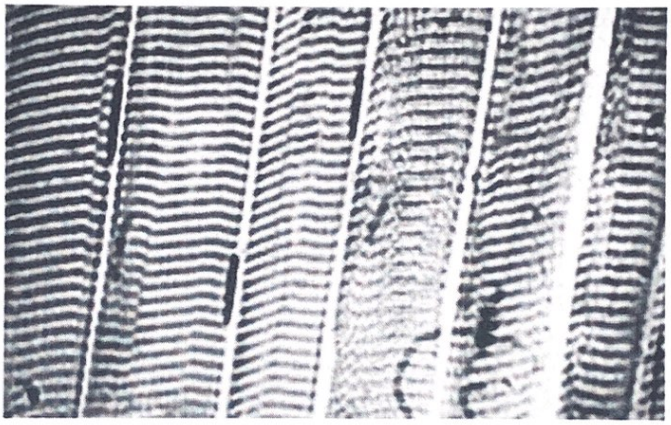
(c)

The body contains three types of muscle tissue: (a) skeletal muscle, (b) smooth muscle, and (c) cardiac muscle. (Same magnification)



A schematic diagram of the different types of muscle cells (same order as above).

Skeletal muscle



Striated skeletal muscle cells in microscopic view. The myofibers are the straight vertical bands; the horizontal striations (lighter and darker bands) that are visible result from differences in composition and density along the fibrils within the cells. The cigar-like dark patches beside the myofibers are muscle-cell nuclei.

Skeletal muscle is further divided into several subtypes:

- Type I, slow oxidative, slow twitch, or "red" muscle is dense with capillaries and is rich in mitochondria and myoglobin, giving the muscle tissue its characteristic red color. It can carry more oxygen and sustain aerobic activity.

- Type I muscle fiber are sometimes broken down into Type I and Type Ic categories, as a result of recent research.
- Type II, fast twitch muscle, has three major kinds that are, in order of increasing contractile speed:
  - Type IIa, which, like slow muscle, is aerobic, rich in mitochondria and capillaries and appears red when deoxygenated.
  - Type IIx (also known as type IId), which is less dense in mitochondria and myoglobin. This is the fastest muscle type in humans. It can contract more quickly and with a greater amount of force than oxidative muscle, but can sustain only short, anaerobic bursts of activity before muscle contraction becomes painful (often incorrectly attributed to a build-up of lactic acid). N.B. in some books and articles this muscle in humans was, confusingly, called type IIB.
  - Type IIb, which is anaerobic, glycolytic, "white" muscle that is even less dense in mitochondria and myoglobin. In small animals like rodents this is the major fast muscle type, explaining the pale color of their flesh.

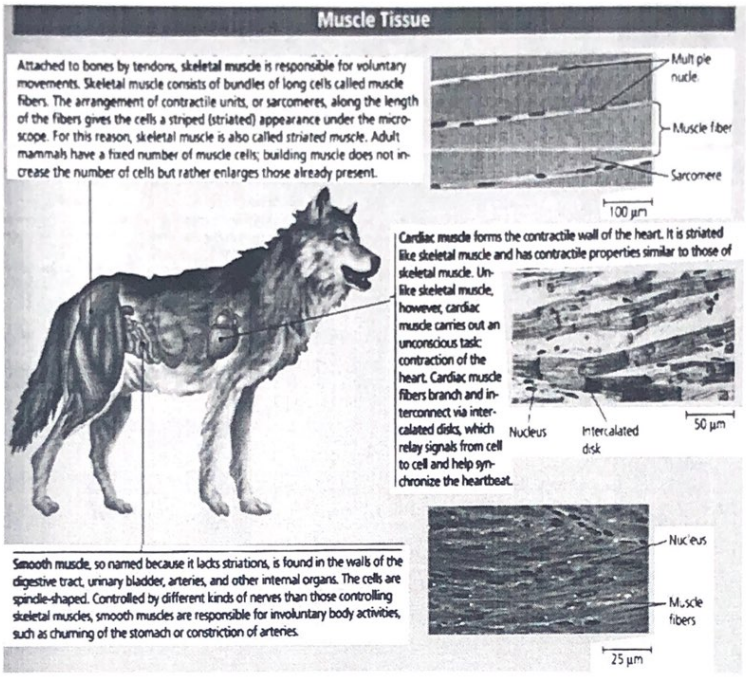
Skeletal muscle function in carrying out movements of the body, support the body and maintains the posture of the body.

Smooth muscle: Smooth muscle is an involuntary non-striated muscle. It is divided into two subgroups: the single-unit (unitary) and multiunit smooth muscle. Within single-unit cells, the whole bundle or sheet contracts as a syncytium (i.e. a multinucleate mass of cytoplasm that is not separated into cells). Multiunit smooth muscle tissues innervate individual cells; as such, they allow for fine control and gradual responses, much like motor unit recruitment in skeletal muscle.

Smooth muscle is found within the walls of blood vessels (such smooth muscle specifically being termed vascular smooth muscle) such as in the tunica media layer of large (aorta) and small arteries, arterioles and veins. Smooth muscle is also found in lymphatic vessels, the urinary bladder, uterus (termed uterine smooth muscle), male and female reproductive tracts, gastrointestinal tract, respiratory tract, arrector pili of skin, the ciliary muscle, and iris of the eye. The structure and function is basically the same in smooth muscle cells in different organs, but the inducing stimuli differ substantially, in order to perform individual effects in the body at individual times. In addition, the glomeruli of the kidneys contain smooth muscle-like cells called mesangial cells. **Smooth muscle** is responsible for the contractility of hollow organs, such as blood vessels, gastrointestinal tract and the bladder.

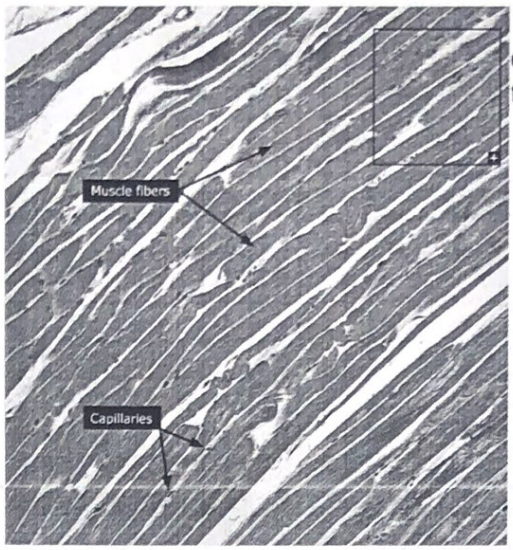
Cardiac muscle: Cardiac muscle is involuntary, striated muscle that is found in the walls and histological foundation of the heart, specifically the myocardium. Cardiac muscle is one of three major types of muscle, the others being skeletal and smooth muscle. These three types of muscle all form in the process of myogenesis.

The cells that constitute cardiac muscle, called cardiomyocytes or myocardiocytes, predominantly contain only one nucleus, although populations with two to four nuclei do exist. The myocardium is the muscle tissue of the heart, and forms a thick middle layer between the outer epicardium layer and the inner endocardium layer. Coordinated contractions of cardiac muscle cells in the heart propel blood out of the atria and ventricles to the blood vessels of the left/body/systemic and right/lungs/pulmonary circulatory systems. This complex mechanism illustrates systole of the heart. Cardiac muscle cells, unlike most other tissues in the body, rely on an available blood and electrical supply to deliver oxygen and nutrients and remove waste products such as carbon dioxide. The coronary arteries help fulfill this function. Cardiac muscle is the muscle of the heart. It is self-contracting, autonomically regulated and must continue to contract in rhythmic fashion for the whole life of the organism. Hence it has special features



Skeletal Muscle Tissue Skeletal muscle tissue is so- called because it attaches to bones and is involved in the movement of bones. It is also referred to as voluntary muscle because of the voluntary control of its contraction, and/or striated muscle because of its striated appearance when you look at it through a light microscope at high magnification. It is a long striated fibre with the nuclei located at the edges of the fibres. It is located attached to bones by tendons. It functions in movement of bones, posture and heat production.

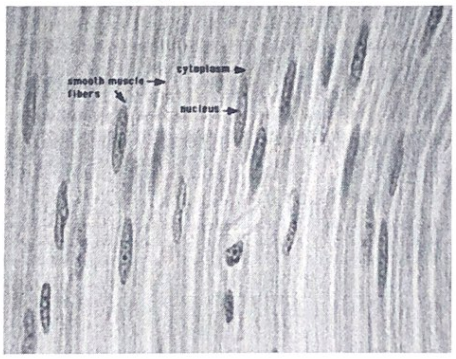
Photomicrograph of Skeletal Muscle Tissue (400X)



Notice the striated appearance of this tissue and the location of the nuclei at the edge of the fiber

Smooth Muscle Tissue Smooth muscle is composed of elongated cells that are not striated. Each smooth muscle cell is largest at its midpoint and tapered towards its ends. Each cell has a single nucleus located in the middle (or broadest) part of the cell. It's a long tapered fibre with a centrally located nucleus. It makes up the walls of hollow internal organs such as the urinary bladder, stomach, intestines, uterus, gall bladder, and blood vessels. It functions in the movement of the substances inside the hollow organ e.g. food in the intestines, blood in the heart, etc. The observeable features include the position of the single nucleus in the center, broadest portion of the fibre. The fibres are non-striated.

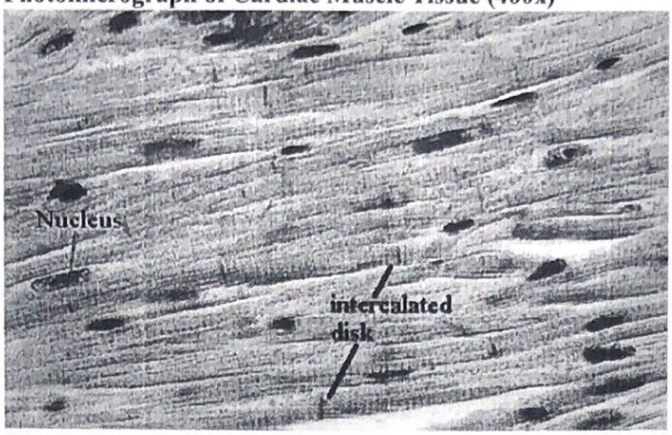
Photomicrograph of Smooth Muscle Tissue (400X)



Notice the position of the single nucleus in the center, broadest, portion of the fiber. Notice also that the fibers are nonstriated.

Cardiac Muscle Tissue Cardiac muscle is composed of striated fibres that are bifurcated or branched. The nuclei are centrally located. The presence of darkly stained intercalated discs is a good identification characteristic. Its features include the branched striated fibres with a centrally located nucleus. Found in the walls of the heart where it functions to pump blood to all parts of the body.

Photomicrograph of Cardiac Muscle Tissue (400x)



NERVOUS TISSUE: This is the main tissue component of the two parts of the nervous system; the **brain** and **spinal cord** of the **central nervous system** (CNS), and the branching peripheral nerves of the **peripheral nervous system** (PNS), which regulates and controls bodily functions and activity. It is composed of **neurons** (or nerve cells), which receive and transmit impulses, and **neuroglia** (or glial cells or *glia* -from the Greek, meaning glue), which assist the propagation of the nerve impulse as well as providing nutrients to the neuron. Nervous tissue is made up of different types of nerve cells, all of which have an **axon** (the long stem-like part of the cell that sends action potential signals to the next cell). Bundles of axons make up the nerves.

Neuron consists of a large cell body (soma), with cell projections called dendrites and an axon. Dendrites are thin, branching projections that receive electrochemical signaling (neurotransmitters) to create a change in voltage in the cell. Axons are long projections that carry the action potential away from the cell body toward the next neuron. The bulb-like end of the axon, called the axon terminal, is separated from the dendrite of the following neuron by a small gap called a synaptic cleft. When the action potential travels to the axon terminal, neurotransmitters are released across the synapse and bind to the post-synaptic receptors, continuing the nerve impulse. The nervous tissue consists of two basic types of cells neurons and neuroglia. Neurons are large cells that function to conduct impulses from one part of the nervous system to another (e.g. from the spinal cord to the brain). Neuroglias are small cells that "glue" the neurons together, though with many other functions as well. Large neuron cell bodies have obvious processes (axon and dendrite) extending from them. Many small nuclei of neuroglial cells (astrocytes) can be observed even though the neuroglial cells themselves cannot be seen. The nervous tissues are found in the nervous system where they function to conduct nerve impulses.

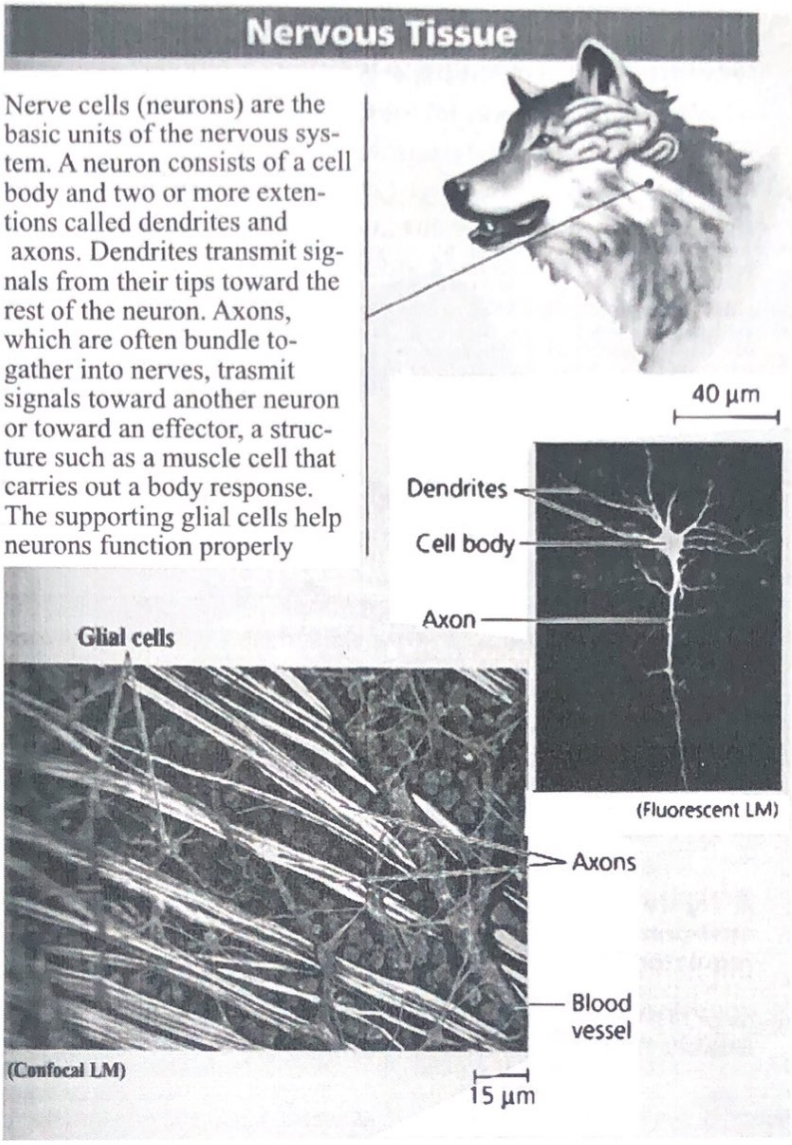
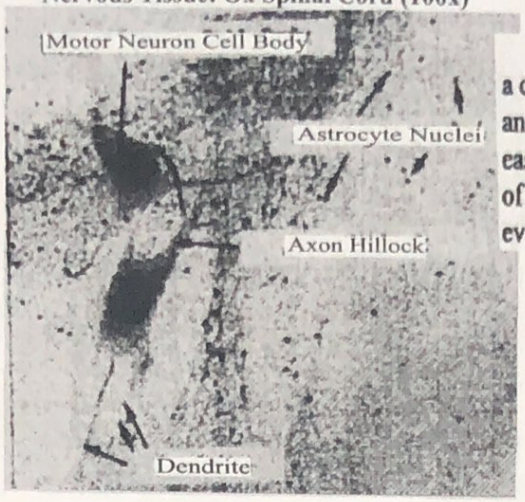


Figure 12.0: Structure and function of animal tissues

Nervous Tissue: Ox Spinal Cord (100x)



Notice the large cell body with a conspicuous nucleus. The axon and dendrite (cell processes) are easily identified. The small nuclei of neuroglial cells (astrocytes) are evident.

Nervous or **nerve tissue:**

Neurons are classified both functionally and structurally.

Functional classification:

- Sensory neurons (afferent): Relay sensory information in the form of an action potential (nerve impulse) from the PNS to the CNS
- Motor neurons (efferent): Relay an action potential out of the CNS to the proper effector (muscles, glands)
- Interneurons: Cells that form connections between neurons and whose processes are limited to a single local area in the brain or spinal cord

Structural classification:

- Multipolar neurons: Have 3 or more processes coming off the soma (cell body). They are the major neuron type in the CNS and include interneurons and motor neurons.
- Bipolar neurons: Sensory neurons that have two processes coming off the soma, one dendrite and one axon
- Pseudounipolar neurons: Sensory neurons that have one process that splits into two branches, forming the axon and dendrite
- Unipolar brush cells: Are excitatory glutamatergic interneurons that have a single short dendrite terminating in a brush-like tuft of dendrioles. These are found in the granular layer of the cerebellum.

Neuroglia encompasses the non-neural cells in nervous tissue that provide various crucial supportive functions for neurons. They are smaller than neurons, and vary in structure according to their function.

. Neuroglial cells are classified as follows:

- Microglial cells: Microglia are macrophage cells that make up the primary immune system for the CNS. They are the smallest neuroglial cell.
- Astrocytes: Star-shaped macroglial cells with many processes found in the CNS. They are the most abundant cell type in the brain, and are intrinsic to a healthy CNS.
- Oligodendrocytes: CNS cells with very few processes. They form myelin sheaths on the axons of a neuron, which are lipid-based insulation that increases the speed at which the action potential, can travel down the axon.
- NG2 glia: CNS cells that are distinct from astrocytes, oligodendrocytes, and microglia, and serve as the developmental precursors of oligodendrocytes
- Schwann cells: The PNS equivalent of oligodendrocytes, they help maintain axons and form myelin sheaths in the PNS.
- Satellite glial cell: Line the surface of neuron cell bodies in ganglia (groups of nerve body cells bundled or connected together in the PNS)
- Enteric glia: Found in the enteric nervous system, within the gastrointestinal tract.

The nervous tissue is categorized into four types of tissue based on its neuronal and neuroglial components. They are **grey matter** and **white matter** (found in the central nervous system), **nerves** and **ganglia** (found in the peripheral nervous system).

- Grey matter is composed of cell bodies, dendrites, unmyelinated axons, protoplasmic astrocytes (astrocyte subtype), satellite oligodendrocytes (non-myelinating oligodendrocyte subtype), microglia, and very few myelinated axons.
- White matter is composed of myelinated axons, fibrous astrocytes, myelinating oligodendrocytes, and microglia.
- Nerves are composed of myelinated and unmyelinated axons, Schwann cells surrounded by connective tissue.
- Ganglion tissue is composed of cell bodies, dendrites, and satellite glial cells.

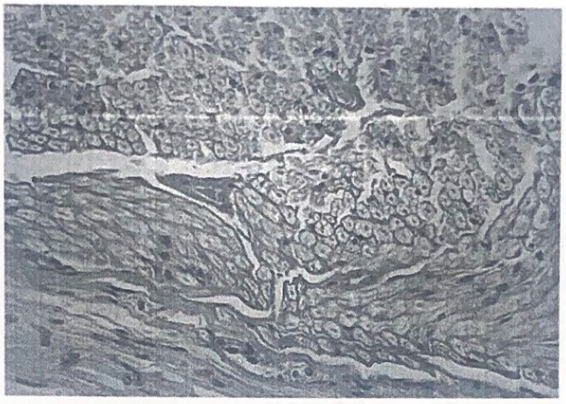
The three layers of connective tissue surrounding each nerve are:

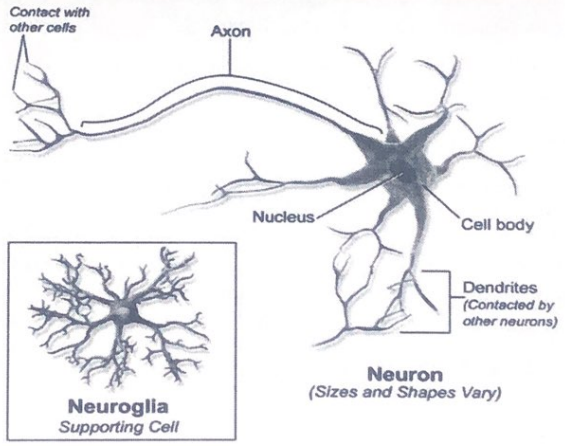
- Endoneurium. Each nerve axon, or fiber is surrounded by the endoneurium, which is also called the endoneurial tube, channel or sheath. This is a thin, delicate, protective layer of connective tissue.
- Perineurium. Each nerve fascicle containing one or more axons is enclosed by the perineurium, a connective tissue having a lamellar arrangement in seven or eight concentric layers.

This plays a very important role in the protection and support of the nerve fibers and also serves to prevent the passage of large molecules from the epineurium into a fascicle.

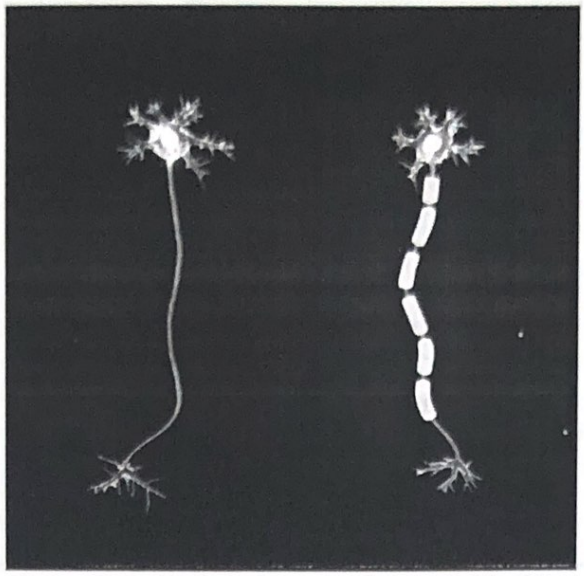
- Epineurium. The epineurium is the outermost layer of dense connective tissue enclosing the (peripheral) nerve.

Functions of the nervous system are sensory input, integration, control of muscles and glands, homeostasis, and mental activity. The nervous tissue forms the communication network of the nervous system by conducting electric signals across tissue. In the CNS, grey matter, which contains the synapses, is important for information processing. White matter, containing myelinated axons, connects and facilitates nerve impulse between grey matter areas in the CNS. In the PNS, the ganglion tissue, containing the cell bodies and dendrites, contain relay points for nerve tissue impulses. The nerve tissues, containing myelinated axons bundles, carry action potential/nerve impulses.





Neural Tissue



Myelinated axon conduct impulse faster than unmyelinated axon.

Terminology

- Epithelium: the lining of glands, bowel, skin, and some organs like the liver, lung, and kidney
- Endothelium: the lining of blood and lymphatic vessels
- Mesothelium: the lining of pleural and pericardial spaces
- Mesenchyme: the cells filling the spaces between the organs, including fat, muscle, bone, cartilage, and tendon cells
- Blood cells: the red and white blood cells, including those found in lymph nodes and spleen
- Neurons: any of the conducting cells of the nervous system
- Germ cells: reproductive cells (spermatozoa in male, oocytes in female)
- Placenta: an organ characteristic of true mammals during pregnancy, joining mother and offspring, providing endocrine secretion and selective exchange of soluble, but not particulate, blood-borne substances through an apposition of uterine and trophoblastic vascularised parts
- Stem cells: cells with the ability to develop into different cell types

CHAPTER THREE

BASIC LABORATORY TEST ON BLOOD

Haematology is the science that deals the formation, composition, functions and diseases of the blood, or **the study of blood, blood forming tissues, and blood disorders. Plasma** is the fluid portion of the blood when blood cells settles at the bottom of a container after treatment with an anti-clot factor, plasma cells are immune cell that produces antibodies derived from B- lymphocytes or cells. **Serum** is the clear supernatant yellow fluid that is expressed out after the solid red mass formed when blood clot contract i.e. serum is plasma minus fibrinogen and most clotting factors.

Biological factors capable of causing interpretation error

a. **Keeping to faulty reference range:** An animal whose WBC is 5000-6000mm^3 when healthy may shoot up to 8000mm^3 in ill-health, but a reference range of between 5000 - 10,000mm^3 may deceive the clinician into believing that the animal is healthy. In another case, if the animals WBC value increase from its individual baseline of 9000mm^3 to 10,300mm^3 which is of no clinical significance, but when compared with the reference value the clinician fall into error.

b. **Sex:** Erythrocyte sedimentation rate (ESR) is higher in female than males.

c. **Age:** Haematocrit values and Alkaline Phosphatase (ALP) are higher in the young than adult.

d. **Time of specimen collection:** serum iron levels increases as the day progresses. It is also of note that some blood parasites such as Filaria have nocturnal periodicity.

e. **Laboratory assay technique/ type of specimen collected:** relating two different techniques used in assay could introduce an error e.g. some laboratories obtain low density lipoprotein (LDL) cholesterol values by subtracting the high density lipoprotein (HDL) plus very low density lipoprotein (VLDL) values from total cholesterol while others perform the direct analysis of LDL. Values obtained from these two methods are usually not the same. It is also of note that variations exists in glucose values obtained using whole blood, as compared with methods using plasma. Technique used in assay is important for a more appropriate test interpretation to be achieved.

f. **Nutritional state:** Plasma cholesterol levels are usually affected by diet.

g. **Posture of animal during collection:** Blood specimens collected when animals are lying down have higher protein levels than when in upright position.

h. **Physical exercise:** WBC value increase in peripheral circulation following a strenuous exercise.

i. **Trauma:** blood required for analysis should be collected without tissue trauma that could introduce extraneous analytes into the serum meant for assay. Non-usage of appropriate specimen container/ exposure of specimen to heat, too narrow bore of syringe needle, too rapid release of blood from syringe into specimen bottle, traces of water in syringe, frothy blood collection, inappropriate blood volume to anticoagulant ratio (shrinkage of red blood cells occur when anticoagulant is in excess resulting in haemolysis and clotting occur when blood is more than anticoagulant); filling of specimen to the minimum level in the container is therefore necessary (full or differential blood count- 5mls in EDTA coated bottles, blood biochemistry 5ml in plain bottles without anticoagulant, urine/ faeces- in clean plain bottles). Plain bottles have greater tendency to cause haemolysis, hence the need for careful handling. Bottles containing specimen should be gently rolled along its side a few times (mixing and not shaking).

j. **Commencement time of laboratory work:** separation of serum/ plasma from whole blood should be carried out very quickly in the laboratory before deterioration sets in as it alters analyte concentration in blood.

k. **Use of specimen bottle:** The use of specimen bottles containing sodium or potassium anticoagulant salts (K_2 EDTA or Na_2 EDTA) may cause raised ion levels during electrolyte determination.

Other factors that may affect a laboratory report include patient's weight, geographical location, climate, genetic factors, emotional state, and phase of reproductive cycle in females.

1. **Packed cell volume (PCV): or Haematocrit :** is the volume of sedimented red cells found in 100ml of blood e.g. a value of 33% implies that there are 33mls of red blood cells in 100mls of blood. Low blood specimen volume to anticoagulant ratio may falsely shoot up the haematocrit value, especially if anticoagulant (usually EDTA) used is in solid form in the specimen bottle.
2. **Haemoglobin (Hb):** is the oxygen carrying pigment of the RBC. Cyanmethaemoglobin or haemoglobincyanide method reported in grams per 100ml is used in its determination. The interpretation is akin to that of PCV and may not be necessary once PCV has been carried out. PCV is generally preferred because it is faster, required less skill to perform and can be carried out with minimal facility. Accurate haemoglobin estimation requires competence on the part of the analyst, and reagents may be scarce. However, Hb and PCV determination may be required when the PCV value is consistently at variance with clinical findings.
3. **Red blood cell (RBC):** The red blood cell count represents the number of red cells in one milliliter (ml) of whole blood. It is important in the calculation of red cell indices like mean cell haemoglobin (MCH) and mean cell volume (MCV). It is now electronically determined rather than the previous tedious manual counting using haemocytometer method. The production of RBC in the bone marrow is stimulated by several condition such as anaemia and tissue

hypoxia (reduced tissue oxygen level), anaemic hypoxia (inadequate amounts of haemoglobin available to carry oxygen), histotoxic hypoxia (inability of cells to use oxygen due to poisoning), hypoxic hypoxia (low level oxygen tension in arterial blood) or stagnant hypoxia (insufficient blood flow to deliver oxygen). The initial stimulus for RBC production is initiated by the hormone, erythropoietin produced in the kidney; hence chronic kidney disease may lead to anaemia. Its production is inhibited by a rise in circulating red cell level. RBC count may decrease in red cell aplasia (incomplete development of tissues), and recent haemorrhage/ haemolytic episodes as in the use of some drugs. It should be noted that a normal RBC count does not imply the absence of haematological abnormality in the red cell portion of blood such as sickle shape etc. as it says nothing about the quality of the cells. Reticulocytes are immature RBC with remnants of ribonuclear protein which results from increased erythroid activity in bone marrow causing the appearance of reticulocytes in peripheral blood following acute haemolysis or haemorrhage, though depends on severity.

Red cell indices

Mean Cell Volume (MCV) - is the average size of the individual red blood cell, measured by dividing the packed cell volume value by the number of red blood cells and expressed in cubic microns or femtolitres, fl. (MCV = PCV/ RBC). Normal MCV ranges from 82 - 98fl.

Mean Cell Haemoglobin (MCH) is the average haemoglobin content of each red cell, measured by dividing the haemoglobin value by the red cell count and expressed in picograms, pg. (MCH = Hb/ RBC). Normal MCH ranges from 27-32pg.

Mean Cell Haemoglobin Concentration (MCHC) - is the concentration of haemoglobin in grams per 100ml of red blood cells, measured by dividing the haemoglobin value (in grams) by the packed cell volume multiplied by 100 and expressed in percentage (MCHC = Hb/ PCV x 100). Normal MCHC ranges from 32 - 36%.

Based on the red cell indices, a decreased MCV with decreased MCHC indicate hypochromic anaemia (deficient in colouring or pigmentation as a result of decreased haemoglobin) e.g. iron deficiency anaemia. An increased MCV with normal MCHC indicate normochromic anaemia e.g. folate or vitamin B_{12} deficiency. A normal MCV with normal MCHC indicate normocytic anaemia depending on reticulocyte response to treatment (a raised reticulocyte count indicate a regenerative type of anaemia while a low reticulocyte count indicates hyporegenerative or aplastic anaemia). A decreased MCV with normal MCHC indicates normochromic anaemia as in chronic disease or malignancy.

4. **White blood cell (WBC): or Leucocytes** is the combined population of the various white blood cell varieties - Granulocytes (neutrophils, eosinophils, basophils) and Agranulocytes (monocytes and lymphocytes) often referred to as total WBC count. The WBC is an indication of the body's cellular immune response to pathologic conditions. It is used in preliminary screening investigation to rule out infectious disease conditions/ malignancy.

Increased granulocytes are usually associated with bacterial infection while increased lymphocytes population accompanies viral infection, leukemias and even after surgical operation. Leucocytosis (increased WBC) is also caused by diurnal changes, physical exercise and stress. Presence of nucleated red cells will falsely shoot up WBC count which could be corrected using a given formula. The total WBC count has high susceptibility to interpretation error based on the concept of normal value or reference range which does not take cognizance of intra-normal range increases during disease in comparison with the individual baseline value in health. The comparative leucopenia (decreased WBC) is race based and is referred to as genetic neutropenia which may be as a result of nutrition. Leucopenia is indicative of immune deficiencies (natural or acquired), aplastic anaemia, recent transplant, subleukemic leukemia or disease infection. It may also be drug-induced as some toxic drugs such as chloramphenicol,phenylbutazone and antithyroid drugs are capable of depressing the bone marrow leading to low WBC counts. Decrease in WBC value often result from neutropenia (decreased neutrophil) since neutrophil is the most abundant WBC in circulation. Decreased neutrophils and other granulocytes have been known to be caused by steroids.

Differential WBC count - the differential count is necessary to identify the relative number of the different WBC types and to calculate the absolute numbers of each leucocyte type. The absolute number for each cell type is obtained as a product of the total WBC and the relative percentage of the cell type in question. The granular leucocytes include neutrophil, eosinophil and basophil while the non-granular (mononuclear) cells are lymphocytes and monocytes. Cell morphological examinations such as toxic granulation, number of lobulations, shift of granulocytes and reactive lymphocyte types can also be done. The use of differential WBC count alone for diagnostic decision alone can be misleading e.g. an increase in lymphocyte count without a corresponding increase in granulocytes (neutrophil, eosinophil or basophil) will invariably lead to neutropenia in the differential count but an increase in neutrophils would affect the proportion of lymphocytes relative to neutrophils causing a reduced lymphocyte differential count. **It should be noted that a decrease in neutrophil will always results in total WBC decrease because neutrophils are the most abundant WBC cell types in peripheral smear.** Absolute figures of WBC types give a better representation of cellular immune response than either the total WBC or differential count alone.

Neutrophilia - is an increase in absolute neutrophil counts. This is often due to bacterial infection, inflammatory disorders, tumors, certain medication, physical/ emotional stimuli, stress, pain/ anxiety, fear, extreme heat or cold, poisoning, etc.

Neutropenia - is a decrease in the number of neutrophil below an acceptable level. In acute viral infections the body produces more lymphocytes in relation to neutrophils, resulting in neutropenia. Factors that could induce neutropenia include drugs (chloramphenicol, phenylbutazone, antithyroid drugs, phenindione, sulphonamides, aminophylline, phenylthiazine, etc)

that acts directly on the bone marrow, excessive ingestion of alcohol, starvation (anorexia nervosa). Low level of neutrophil below acceptable threshold exposes the body tissues to microbial penetration, although it becomes more severe when the absolute count falls below 0.5 x 10^9/litre (500/mm^3).

Lymphocytosis - is an increase in the lymphocyte count, as seen in majority of viral infections. It should be noted that relative lymphocytosis (in which the total number of lymphocytes remain unchanged but the percentage lymphocytes is pushed up by neutropenia) may be found in conditions associated with granulocytopenia as found in very young animals. Lymphocyte count in young animals is generally higher than adults because that is the age of antibody formation. Mild or moderate lymphocytosis related to mild or moderate leucocytosis may be observed in bacterial infections e.g. tuberculosis, brucellosis, typhoid fever, etc., adrenal insufficiency Generally, marked lymphocytosis may be observed in infectious disease such as syphilis, toxoplasmosis, etc and are often accompanied by moderate increase of total WBC count.

Lymphopenia- is a decrease in absolute lymphocyte count below the normal range. It may be observed in congestive cardiac failure, following administration of adrenocorticosteroid hormones (though temporarily), late phase of HIV/ AID infection (selective T- lymphocyte depletion). It is one of the earliest manifestations of protein-calorie malnutrition.

Monocytosis - is an increase in monocyte count. Young animals tend to have higher monocyte count than adults. Moderate monocytosis is generally observed in chronic inflammatory disorders, tuberculosis, brucellosis, typhoid fever, bacterial endocarditis, malaria, trypanosomiasis, solid tumours, etc though occasionally in some cases.

Eosinophilia - is an increase in eosinophil count, which an indication of allergic disorder, parasitic (worm) infections, drug abuse/over-use, liver diseases/ accumulation of toxic waste products in the body. The prevailing allergic or parasitic diseases in the geographical environment must be taken into consideration when making a differential diagnosis of eosinophilia.

Basophilia- is an increase in basophil count, which is a rare condition. Basophilia indicates the presence of myeloproliferative disease or its onset such as basophilic leukemia, myelofibrosis, etc. other less common conditions associated with basophilia include liver cirrhosis, hypothyroidism, post infection/ post-splenetomy states, anaphylactic reactions, use of oestrogens/ antithyroid drugs.

5. **Platelet (thrombocyte):** is the cellular fragments that are concerned with blood coagulation. It plays a prominent role in the maintenance of haemostatic balance. Platelet plugs one of the four overlapping stages of haemostasis. Its physiologic processes involve adhesion, aggregation and secretion. Platelets aggregate and adhere to form a temporary plug at the site of blood vessel damage. Thrombocytopenia, the commonest platelet disorder may occur due to immunological disorder (causing an increased destruction of platelets), excessive platelet consumption (disseminated intravascular coagulation, DIC and thrombosis), increased platelet loss (haemorrhage) or sequestration in the

spleen, liver and bone-marrow. The platelet surface contains alloantigenic proteins, when antibodies are produced to those antigens possibly following transfusion of blood or blood products, or as a result of pregnancy, platelet destruction occurs. Tendency for bleeding increase as platelet counts approach 5000/ mm^3 and below. An absolute absence of platelets in peripheral blood smear is an indication of the incomplete ability of the bone-marrow to form new cells i.e. if there is no immunological disorder. Animals with normal or subnormal platelet counts i.e. with functional platelet defects may experience spontaneous bleeding. Thrombopenia may be observed in liver disease, DIC, myeloproliferative disorders, use of antithrombotic drugs like heparin, etc. Thrombocytosis may be seen in malignancy, splenectomy, polycythaemia, etc.

LIVER FUNCTION TESTS

Tests carried out to determine the normal function of the liver include the following:

1. **Serum bilirubin**
2. **Alkaline phosphatase (ALP)**
3. **Alanine aminotransferase (ALT) or Serum glutamic pyruvic transaminase (SGPT)**
4. **Aspartate aminotransferase(AST) or Serum glutamic oxaloacetic transaminase (SGOT)**
5. **Serum Total protein**
6. **Serum Albumin**
7. **Serum Globulins**

1. **Bilirubin:-**is the major bile pigment formed from the breakdown of haemoglobin after red cell destruction. It is normally bound to albumin immediately after release into the blood circulation, and as a waste-product, it must be excreted. The first step towards its excretion is its transport to the liver where it is conjugated with **glucuronic acid.** This process is aided by **uridine diphosphate** (UDP)-**glucuronyl transferase**, an enzyme which human neonates lack because it is yet to be formed in them, hence the physiological jaundice commonly seen in newborn infants. The conjugated bilirubin is more water soluble than the free (unconjugated) bilirubin. As such the conjugated bilirubin is more easily secreted into bile while the rest unconjugated bilirubin remain protein-bound. TOTAL BILIRUBIN = Conjugated + Unconjugated bilirubin, so UNCONJUGATED (protein- bound) = Total bilirubin Conjugated (direct-reacting). About 300mg of bilirubin is normally produced daily. Excess bilirubin in circulation diffuses into tissues of the skin, eyes/ mucous membrane making the tissues to turn yellow (jaundice). There are three (3) different types of pathological jaundice apart from the physiological jaundice of neonates haemolytic, hepatic and post-hepatic jaundice.

Table 5.0: Types of pathological jaundice

Haemolytic	Hepatic	Post-hepatic
Low RBC/ Hb.	Inability of liver to conjugate/ excrete bilirubin even when the rate of production is normal as a result of infection, drugs, alcohol/ neoplasms.	Obstruction of excretory ducts. Presence of bilirubin in urine giving it a dark colour.
High unconjugated bilirubin	High conjugated bilirubin in blood.	High conjugated bilirubin
Normal ALP, ALT and AST	High ALT and AST while ALP is moderate.	High ALP and total cholesterol/ moderate ALT/ AST

2. **Alkaline Phosphatase (ALP):-** is a non-specific enzyme since its common to many tissues. It is found majorly in liver/bones, but also in minute quantities in kidney/intestine. Elevation of the enzymes indicate inflammation or injury to the particular tissue in which they are found, so since it is found essentially in liver/bones, its elevation in serum indicates either a liver or bone disease which must tally with clinical symptoms. The determination of the serum levels of other enzymes common to the tissue in question may be of help in the differential diagnosis. Different levels indicate different clinical condition. Levels attaining the upper reference limit or slightly above it may not be significant as such is found in the elderly, pregnancy, rickets and growing young animals. A low ALP value may not be clinically significant as its been observed in kwashiorkor, vitamin C deficiency, cretinism etc while moderate elevation of ALP with liver disease denote liver cirrhosis, and cholangiolitic hepatitis. Moderate elevation of ALP without liver disease suggests osteomalacia because ALP is normal in osteoporosis.
3. **Alanine aminotransferase (ALT):-** is an enzyme that catalyzes the conversion of one amino acid to a keto-acid, while concurrently converting an existing keto-acid into an amino-acid. Such reactions occur principally in the liver, kidney, heart, red blood cell and skeletal muscle tissues. **ALT is found mainly in the liver**; hence **an increase indicates acute hepatocellular damage.** A normal ALT in the presence of raised levels of other enzymes such as AST and LDH (lactate dehydrogenase) rules out a liver disease. LDH is an enzyme that catalyses the interconversion of lactate and pyruvate in the myocardium, skeletal muscle/liver. The level of LDH in serum increases rapidly following necrosis of metabolically active tissue e.g. after myocardial infarction (death of a part of heart muscle). AST: ALT ratio greater than 2 is highly suggestive of alcoholic hepatitis or cirrhosis. Marked increase in ALT which exceeds the rise in ALP indicates viral or toxic hepatitis.

In post-hepatic (obstructive) jaundice only a marginal rise of serum ALT is seen with high serum ALP. Low ALT have been associated with alcoholic liver disease, diabetes mellitus, malnutrition, uraemia and pyridoxal phosphate deficiency. ALT reference values are race/ gender dependent.

4. **Aspartate aminotransferase (AST):-** is an enzyme released into the blood stream following injury or necrosis of physiologically active cells of the heart, muscle and liver, and to a limited extent kidney, RBC and skeletal muscle. Comparatively, AST is more widely distributed in the body's tissues than ALT. Raised levels of AST may be detected in serum following injury to affected cells within an interval of 24hours. Peak activity of AST occurs between 7 to 12days of infarction and gradually decreases thereafter to normal levels. Low AST values are rare but may accompany low ALT in alcoholic liver disease, malnutrition, pregnancy, etc.
5. **Serum Total Protein (TP):** This consists of the 52 to 69% albumin with the rest being essentially globulins. Albumin is synthesized by the liver while globulins consisting majorly of gammaglobulins (the antibodies responsible for the body's humoral immune defense) are produced by the kupffer cells. The main functions of serum proteins include: carrier of essential constituents of the blood such as hormones, vitamin, minerals (iron, Cu), enzymes and lipids; source of tissue protein replacement whenever there is tissue depletion; as buffer in the maintenance of the acid-base balance; plasma proteins exert an osmotic force across the capillary wall which represents about 80% of the colloid oncotic pressure in the serum. Hyperproteinaemia reflects an increase in one of the fractions (either albumin or globulin) or both while hypoproteinaemia reflects a decrease in one of the fractions or both. A decrease TP may be caused by loss of protein in urine, decreased synthesis in liver, malabsorption and low protein intake. A decrease in serum albumin with a slightly elevated serum globulin is usually a pointer to chronic liver disease, protein malnutrition and malabsorption syndrome due to decreased synthesis, as well as increased loss of protein (nephritic syndrome, thermal burns, protein-losing enteropathies) and increased catabolism (thyrotoxicosis, cancer chemotherapy, etc).
6. **Lipid profile:** The lipid profile is made up of blood total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) and cholesterol/ HDL ratio. It helps to assess the risk of artherosclerosis. Blood lipids are found bound to proteins in the circulation, hence the name **lipoproteins**. Total cholesterol has been found to correlate with total and cardiovascular mortality in the 30- 50age bracket in human and in adult broiler birds. It is a risk factor for coronary artery disease. Cardiovascular mortality increases by 9% for each 10mg/dL increase in total cholesterol over the baseline value of 180mg/dL. Essentially, genetics is a major determinant of a person's cholesterol level with diet/ medication serving as contributory factors.

Other risk factors are old age, gender, smoking, hypertension, diabetes and obesity. The guideline for interpreting lipid profile results usually links obtained values to existing risk factors in the patient e.g. a patient with a history of coronary heart disease (CHD) must institute an intervention when LDL values equals or greater than 100mg/dL while those without CHD but with other risk factors have a decision level of 130mg/dL.

(a) **Total Serum Cholesterol:** Cholesterol is vital in the synthesis of cholic acid required for bile salt formation in the liver. Bile salt is important in the digestion/ intestinal absorption of fats. Cholesterol exists free or bound to fatty acids (esterified). Excess cholesterol in circulation inhibits the formation of more cholesterol in the liver. Cholesterol is used by the body for the synthesis of hormones by the ovaries, testes and adrenal glands with large amount deposited in the skin to make it highly impermeable to water-soluble substances. The determination of total cholesterol alone is not sufficient for risk assessment for cardiovascular diseases; it must be done with its fractions. Determination of cholesterol fractions is also advisable in liver disease since liver damage causes esterification much more than the total cholesterol. Hypercholesterolaemia may be caused by hereditary, obstruction of biliary flow/ untreated diabetes mellitus (although there is a decrease cholesterol synthesis in this condition), chronic pancreatitis, pancreatectomy, hypothyroidism and nephritic stage of glomerulonephritis. Thyroid hormones and oestrogens are capable of lowering circulating blood cholesterol levels. It could also be lowered by dietary habits/ malnutrition as well as hyperthyroidism/ extensive hepatic disease.

(b) **High Density Lipoprotein (HDL) Cholesterol:**HDL is termed the 'good' cholesterol because it removes cholesterol from tissues and delivers them to the liver where it is metabolized into bile acids. Thus the higher the level of blood HDL, the lower the likelihood of cardiovascular disease.An HDL level lower than 35mg/dL (0.91mmol/L) is considered a coronary heart disease risk factor even if the total cholesterol falls within acceptable limits. Risk assessment may be carried out further by determining the ratio of total cholesterol to HDL- cholesterol. The lower the value, the less the risk. HDL cholesterol is assayed for while LDL cholesterol is mathematically derived, although a direct assay method of LDL now exists.

(c) **Low Density Lipoprotein (LDL) Cholesterol:**LDL is termed 'bad' cholesterol because it does not go to the liver to be metabolized into bile acids and so increases the cholesterol in the tissues. Total cholesterol is made up of about 60 to 70% LDL and high LDL is consistent with increased risk of coronary heart disease. Calculated LDL values are particularly prone to error since values obtained depend on the accuracy and precision of the three individual lipid assays required for the calculation

(Total Cholesterol, HDL-Cholesterol and Triglyceride). Moreover, a fasting specimen is required and the result is not considered valid in the presence of chylomicrons or when the triglyceride values exceed 400mg/dL. Chylomicrons are tiny particles formed from triglycerides, lipoproteins/ cholesterol and lipoproteins within the intestinal mucosa following the absorption of digested fat. It forms chyle (fatty, milky fluid) within the lacteals (lymphatic ducts in the intestinal villi where absorption of digested fats take place). The desired level for LDL is < 100mg/dL (< 2.6mmol/L).

(d) Triglycerides: is the principal lipid digested in the duodenum/ jejunum in the presence of bile salt/ lipase secreted by the pancreas. The triglyceride level in the blood is another risk factor in the assessment of CHD that is independent of cholesterol levels in serum. However its utility in the risk assessment of CHD becomes relevant only if triglycerides are not part of the chylomicron fraction. This could be tested by a simple visual inspection of an overnight refrigerated serum sample. If present the layer of chylomicron would be seen. The generally acceptable upper limit for triglyceride is 150mg/dL. Triglyceride levels above 200mg/dL are usually classified as undesirable for all patients. A markedly elevated triglyceride level in blood could mean that the specimen was from a non-fasting patient. However, hypertriglyceridaemia in fasting individuals may be caused by hyperlipoproteinaemia. Certain drugs such as oestrogen, corticosteroids and cholestyramine may cause raised triglyceridaemia. High blood triglyceride levels may also be caused by a high carbohydrate intake. Low serum triglyceride may be seen in malabsorption states, malnutrition, hyperthyroidism, chronic obstructive pulmonary disease, etc.

KIDNEY FUNCTION TESTS (Electolyte determinations)

The levels of electrolytes in each individual are the product of the body's homeostatic mechanism brought about by the functioning of organs such as kidney, lungs and endocrine system. The concurrent determination of serum urea (or blood urea nitrogen) and serum creatinine along with the electrolyte assays could be more beneficial than carrying out the electrolyte assay alone. The reason is because (a) the interpretation of electrolyte values in the presence of a raised urea level with the attendant metabolic acidosis would be quite different from a situation in which the serum urea is normal; (b) the urea levels in blood is a good pointer to the overall status of water metabolism in a patient because the hydration status in each individual exerts a great influence on the serum levels of the different electrolytes, and this is one of the body's physiological activities which the measurement of urea assesses e.g. raised serum sodium, chloride and urea in the presence of normal creatinine is an indication of dehydration; (c) serum creatinine or urea analysis is necessary to know the kidney's function where there is electrolyte imbalance and there is need to replace potassium. Tests necessary to assess the normal functioning of the kidney include the following:

1. Urea
2. Creatinine

3. Sodium
4. Potassium
5. Chloride
6. Bicarbonate
7. Anion gap
8. Uric acid
9. Phosphorus
10. Calcium

1. **Serum Urea:** Urea is a waste product of protein metabolism formed in the liver by the deamination of amino acids. Urea is the major means by which the body eliminates nitrogen (from protein metabolism) that is not used by the body. The urea formed is excreted in urine. Accumulation of urea in the system is highly toxic. Assay methodologies measure urea in form of blood urea nitrogen (BUN) while some others measure plain urea. Increased serum urea may be due to acute/ chronic intrinsic renal disease, reduced effective blood circulation with decreased renal perfusion resulting in reduced glomerular filtration, high protein intake and excessive catabolism of body protein in fever, sepsis/ gastrointestinal bleeding. Decreased serum urea may be seen in high carbohydrate/ low protein diet, high anabolic demand in pregnancy, infancy, malabsorption states and severe liver damage.
2. **Serum Creatinine:** Creatinine is a waste product produced from creatine that is present in skeletal muscles as creatine phosphate, a high-energy compound necessary for muscular activity. The determination of serum creatinine is used to assess renal function as it reflects the difference between creatinine formation and its filtration by the renal glomerulus. Diet, catabolic state and dehydration do not significantly affect creatinine like it affects urea. Increased creatinine is seen in renal impairment especially those that involve a damage of up to 50% or more of the nephrons such as those due to intrinsic renal lesions, decreased perfusion of kidney, or obstruction of lower urinary tract. Increased serum creatinine aside from the above factors is very rare. Deranged metabolic processes (such as in major surgery, stroke, severe infection, individuals with large muscle mass, hyperthyroidism, acromegaly, etc) may cause increase in serum creatinine. Decreased serum creatinine levels are found in conditions characterized by muscle wasting and pregnancy.
3. **Serum Sodium:** Sodium is a major cation of the extracellular fluid that plays an important role in acid-base balance by protecting the body against undue fluid loss. It also helps in normal functioning of muscle tissue. Hypernatreamia occurs as a result of inadequate water intake or excessive water loss. Raised sodium level is not common in normal individuals, but it is risky if serum sodium approaches 160mmol/L as could happen if the thirst mechanism is absent e.g. unconscious patients in emergency situation or infusion of patient under therapy with excess sodium. Increased serum sodium level may also be observed in inadequate water intake, hyperglucocorticoidism

or mineralocorticoidism, patients with water loss in excess of salt loss e.g. profuse sweating, severe diarrhea (common in young animals), vomiting, polyurial, etc and drug such as steroids (mineralocorticoids), carbenoxolone, methyldopa, sodium bicarbonate, oxyphenylbutazone, lithium, licorice, etc. There are four (4) classes of hyponatraemia Dilutional, K loss associated, Inappropriate ADH syndrome, and Absolute sodium loss. **Dilutionalhyponatraemia** is due to intake of free water or hypotonic solution e.g. fluid replacement therapy or Oral rehydration therapy (ORT) following diarrhoea, vomiting, sweating/ abuse of diuretic medication. It may also occur in cardiac failure, liver failure, malnutrition, diabetic acidosis and nephritic syndromes. **Potassium-loss associatedhyponatraemia** is due to loss of intracellular potassium as a result of a defective sodium-potassium pump mechanism with consequent intracellular sodium influx/ potassium efflux. Potassium lost thereby is excreted in urine causing the serum level of potassium to be normal while that of sodium becomes low. **Inappropriate antidiuretic hormone (ADH) syndromehyponatraemia** is characterized by continuous reabsorption of water at the distal tubules/ excretion of concentrated urine inspite of serum hypo-osmolarity e.g. pulmonary infections, metabolic diseases (porphyria), etc. **Absolute loss of sodiumhyponatraemia** is common in profuse sweating, vomiting, diarrhoea or with replacement of fluid with those that contains little or no electrolytes. Pseudohyponatraemia may be observed in hyperproteinaemia, hyperglycaemia, hyperlipidaemia, overdilution and in methods in which serum is diluted before analysis e.g. Flame photometry and Indirect ion-selective electrode methods.

4. **Serum Potassium:** Potassium is a major cation of the intracellular fluid involved in the regulation of acid-base balance in opposite direction to sodium; maintain osmotic pressure/ membrane potential (electric charge) of cells. Increased serum K^+ (hyperkalaemia) affects the functions of nervous/ myocardial tissues as seen in conditions characterized by excess destruction of cells (particularly muscle tissue) which cause efflux of K^+ from intracellular to extracellular part of the cells. The shift of K^+ from intracellular to extracellular space result in an increase in the resting membrane potential and this has a major impact on the heart which may suffer various degrees of arrhythmias (deviation from normal rhythm or heat-beat such as asystole, extrasystole, fibrillation, tachycardia increased frequency of heart-beats) e.g. in massive blood transfusion, massive haemolysis, crush injuries, hyperkinetic activity and malignant hyperpyrexia (high body temperature - fever), renal failure. Hyperkalaemia due to excess oral K^+ intake is rare as the excess ion would be excreted once the kidney is functioning normally. Many drugs are capable of increasing serum potassium levels e.g. marijuana, tetracycline, amphotericin B, heparin, spironolactone, etc. Hypokalaemia may occur as a result of excessive loss of potassium ion e.g. in chronic diarrhea, vomiting, malabsorption syndrome (GIT), in perspiration/ chronic pyrexia (skin), defects of renal tubules (renal K^+ loss is frequently associated with low serum magnesium, which is rectified when serum magnesium is normalized),

diuretic therapy that results in depletion of K^+ and Cl^-, supplementation of potassium without chloride, endocrine disorders (hyperaldosteronism, use of steroids/ sex hormones such as oestrogens, excess intake of licorice mimic aldosterone in activity), use of anti-inflammatory drugs, dietary habits (foods with inadequate K^+ content), in glucose-insulin therapy, chronic stress, chronic congestive heart failure, alkalosis (in which serum K^+ is lost to cells) and also through urine. Technical causes of false elevation of serum potassium include exercises before venipuncture, delayed separation of serum from cells, haemolysis during blood collection, marked thrombocytosis, and presence of myeloproliferative disease such as extreme leucocytosis, leukemias, etc.

5. **Serum Chloride:** Chloride is a major anionof the extracellular fluid which influences the body's acid-base balance and osmotic pressure. Increased serum chloride level (hyperchloraemia) may be observed in acute renal failure, renal tubular acidosis, dehydration, prolonged diarrhoea, hypokalaemia, respiratory alkalosis, salicylate toxicity and hypothalamic lesions. Low serum chloride level (hypochloraemia) is often associated with hypokalamia/ alkalosis and in such condition serum chloride/ potassium level is low while the serum bicarbonate level is high. In exceptional cases, serum chloride could be low while serum K^+ is normal e.g. giving of potassium replacement supplement which contains no chloride or diuretic therapy that is potassium saving. It is often advisable that potassium replacement therapy be accompanied with a concurrent chloride replacement in a one to one ratio (K^+: Cl^-). Hypochloraemia may also be found in excessive sweating, prolonged vomiting, adrenocortical deficiency, acid-base disturbances, salt-losing nephropathy, acute intermittent porphyria and conditions that involve the dilution of extracellular fluid. Metabolic alkalosis associated with GIT loss of chloride can be corrected with potassium chloride administration.
6. **Serum Bicarbonate:** Thedetermination of bicarbonate ions (HCO_3)helps to assess abnormalities in the acid-base balance of blood as it is not possible to do a direct determination of blood pH. The measurement of total Carbon dioxide (CO_2) is a better indicator of blood pH as it also measures the dissolved CO_2gasalong with the bicarbonate ion in the plasma.However,facilities for CO_2 determination are not usually available hence the determination for bicarbonate ions since the dissolved CO_2 content is only a small fraction. Measurements of increased serum CO_2 concentration mostly reflect an increase in serum bicarbonate concentration rather than dissolved CO_2 gas or PCO_2 only.Elevated HCO_3 level is often observed in chronic obstructive lung disease but if absent (lung disease) it indicate serum alkalosis/ intracellular acidosis due to hypokalaemia/ hypochloraemia, abuse of diuretics, corticosteroids/ laxatives, etc. Low blood bicarbonate level could be observed in metabolic acidosis e.g. diabetic ketoacidosis, renal tubular acidosis, uraemic acidosis and lactic acidosis. Substances that are capable of causing metabolic acidosis include ammonium chloride, ethylene glycol, paraldehyde

and methanol. Salicylate poisoning shows features of early respiratory alkalosis followed by metabolic acidosis with lowered bicarbonate concentration. Low blood bicarbonate suggests an high arterial blood pH (an indication of respiratory alkalosis observed in hyperventilation syndrome).

7. **Anion Gap:** Anion gap is calculated by subtracting serum chloride and bicarbonate concentration from sodium concentration. Ideally, the serum concentration of cations should almost be equal to that of anions for sustenance of neutral blood pH. Sodium, Chloride and bicarbonates are the only measurable electrolytes that contribute to electro- neutrality of blood. Potassium is not usually considered because it is essentially an intracellular ion and so contributes only minimally. The anion gap is approximately 12 in normal individuals. Anion gap is calculated to determine the level of metabolic acidosis in affected individuals, and it has helped to categorize metabolic acidosis into two (2) types normal anion gap and increased anion gap. The normal anion gap is usually caused by diarrhea with loss of bicarbonate, mild renal failure, renal tubular acidosis with hyperchloraemia, chronic nephritis/ ammonium chloride therapy. Metabolic acidosis with an increased anion gap is usually traceable to diabetic ketoacidosis, lactic acidosis, azotaemic renal failure, salicylate poisoning, methanol and paraldehyde abuse. Decreased serum anion gap is usually observed in dilutional states and hyperviscosity syndromes associated with paraproteinaemias, presence of bromide in blood, etc.
8. **Serum Uric acid:** Uric acid is a substance formed during purine metabolism; purine is present in nucleic acids and some food/ beverages. Uric acid is excreted in urine and may solidify to give rise to kidney stones which cause blockages of urinary tubes. High levels of uric acid in the blood may be due to faulty excretion of uric acid, excessive cell breakdown, or due to high purine intake. Hyperuricaemia is often associated with gout (a form of metabolic disorder in which blood levels of uric acid are raised, sometimes resulting in urate crystals in joints, big toes become acutely painful/ swollen, nodules in the ear, etc), although a normal serum uric acid level does not rule out gout because it's the balance of uric acid production and excretion that is reflected in the serum uric acid. Hyperuricaemia may also be caused by chronic renal failure but in such cases there will be a corresponding increase in serum urea/ creatinine levels indicating the failure of the kidney to excrete other waste products from the blood. Hyperuricaemia may also be in congestic heart failure, starvation, glycogen storage diseases, toxaemia of pregnancy, alpha lipoprotein deficiency, hyperparathyroidism, liver disease, ethanol consumption, primary hyperoxalouria, drug-induced (use of diuretics, ethambutol, pyrazinamide, salicylates/ catecholamines, etc.). Decreased serum uric acid (hypouricaemia) is of no clinical significance.
9. **Serum Phosphorus:** Phosphorus is an element whose absorption is determined by the activities of the parathyroid hormone, and its blood level is usually assayed in phosphate form.

The blood phosphate level is usually correlated with calcium level since the absorption of one depends on the other. The optimal ratio of both elements in the blood is ratio 1:1, provided there is adequate intake of vitamin D. Eighty-five percent (85%) of the available body phosphorus is combined with calcium in the skeleton. Phosphorus is present in almost all body metabolic activities, hence it is found in all body tissues. Rapid absorption of phosphorus (along with calcium) stimulates the release of parathyroid hormone which causes the lost of phosphate in the urine while absorbing calcium in the renal tubule.Howeverthe loss of phosphate is dependent on calcium to phosphorus ratio in the blood, since phosphate is a threshold substance. Excess of any of the two elements will lead to excretion of the other. Hyperphosphataemia may be observed in hyperthyroidism, increased growth hormone secretion, malignant hyperpyrexia, pseudohyperparathyroidism (in the healing stage of fractures), vitamin D overdose, neonates fed with high phosphate rich cow-milk, etc. Phosphate abnormalities in the absence of glomerular disease should point to endocrine system disorders or abnormalities of bone metabolism. In chronic glomerular disease, elevated phosphate levels may be observed with elevated urea/ creatinine levels. However, calcium determination should be carried out when phosphate studies are done and this is of great importance in hypoparathyroidism diagnosis since there is hyperphosphataemia/ hypocalcaemia with normal renal function in such cases. Hypophosphataemia may be observed in osteomalacia (in adults) or rickets (in the young) particularly if it is accompanied by increased ALP, chronic use of antacids having aluminium hydroxide which bind phosphate, rapid therapeutic correction of elevated blood glucose in diabetics, malabsorption syndromes, alcohol intoxication, hyperinsulinism, etc. In hyperparathyroidism, there is hypophosphataemia with hypercalcaemia although renal disease can also cause hypophosphataemia/ hypercalcaemia. False hypophophataemia could be caused by citrates, mannitol, oxalate, tartrate and phenothiazines which often interfere with assay.

10. **Serum Calcium:** Calcium is an element whose metabolism/ absorption are under the control of the parathyroid hormone. Parathyroid hormone acts in two ways in the metabolism of calcium (a) directly on the osteoclasts to cause the release of bone salts into the extracellular fluid resulting in an increased level of both calcium/ phosphate in the plasma; (b) by increasing the rate of calcium absorption from the intestines while at the same time acting on the renal tubular cells to save calcium with an attendant loss of phosphorus. Other factors affecting calcium metabolism include Vitamin D, which increases the rate of absorption of calcium in the intestine; and calcitonin which increases the rate of renal loss of calcium and acts in opposite direction to the parathyroid hormone. The ionized portion of calcium is vital in such physiological processes as heart, muscle and nerve function, as well as in blood coagulation. Incidentally, only about 50% of the total plasma calcium is ionized; and of the total body calcium, more than 98% resides in the skeleton/ teeth. Acidosis tends to increase the physiologically active ionized form

Of calcium for a given total body calcium. Hypercalcaemia may not indicate any disease condition unless it is related to a significant abnormality of some other blood parameters. Calcium determination alone is not of any significant use unless in relation to other blood parameters, its increase in the absence of any other abnormality should point to analytical error and the test should be repeated along with phosphorus estimation. Hypercalcaemia is associated with low blood phosphorus levels in hyperparathyroidism. Mild hypercalcaemia occur in pregnancy as a result of increased uptake of maternal calcium by the placenta (sporadic primary hyperparathyroidism), although it could also be seen post-partum. Hypercalcaemia may be found in vitamin D intoxication, thyrotoxicosis, polycythaemiavera, acute bone atrophy, acromegaly, diuretic phase of acute renal tubular necrosis. Hypocalcaemia should be interpreted only in relation to serum albumin concentration by determining serum protein in order to evaluate serum calcium binding by albumin. A significant decrease in albumin in the presence of hypocalcaemia (pseudohypocalcaemia) is caused by low level of carrier-albumin and so not a true reflection of serum calcium. Hypocalcaemia could be caused by the following conditions osteomalacia (in adults) or rickets (in the young), hypoparathyroidism, malabsorption syndrome, decreased absorption of calcium, pregnancy, magnesium deficiency, Vitamin D deficiency, respiratory alkalosis/ hyperventilation (alkalosis causes calcium ion to bind to protein thus reducing the availability of ionized calcium), diuretic intake, use of certain drugs like insulin/ oestrogens, low calcium intake, and feeding of cow-milk to neonates.

There is high possibility of an individual to have normal calcium and abnormal levels of certain biochemical analytes as could be found in the following:

(e) **Normal blood calcium in association with hyperuraemia** e.g. primary hyperparathyroidism in which there is an initial increase in serum calcium but the subsequent development of kidney disease/ uraemia results in lowering of the high calcium via phosphate retention thereby normalizing the calcium level. In secondary hyperparathyroidism, there is the existence of uraemia/ acidosis (which cause lowered calcium level) prior to stimulation of the parathyroid to raise the low calcium level to normal.

(f) **Normal blood calcium in association with a marked decrease in serum albumin** e.g. hypoalbuminaemia is capable of causing a falsely normal serum calcium level even when there is hypercalcaemia. Since free calcium ions are not measured directly, and close to 50% of the total calcium is protein-bound, it is to be expected that a low concentration of serum protein would result in a falsely lower level of ionized calcium in the blood. Some laboratories report only the corrected calcium which relates the calcium value to a normal albumin value.

(g) **Normal blood calcium in association with an abnormal blood phosphorus level** a normal calcium level in the presence of an abnormal blood phosphorus value is indicative of a disease condition. E.g. in children the product of serum

calcium and phosphorus (in mg/100ml) is expected to be about 50, whereas in children suffering from rickets, the product is less than 30.

PANCREAS FUNCTION TEST

The pancreas is a thin leaf-like shaped gland with two major tissues (islet of Langerhans - secrete insulin/ glucagon for regulation of body glucose and acini secrete the digestive enzymes, bicarbonate ions and water) located behind the stomach. The destruction of the pancreatic cells caused the liberation of its constituents such as amylase and lipase into the internal environment with the relative activity in serum dependent on the amount/ time frame of tissue loss. The three basic tests for pancreatic function are:

a. Glucose test
b. Amylase test
c. Lipase test

1. **Blood Glucose:** Glucose is the end-product of carbohydrate metabolism in the body and its utilization/storage depends on the secretion of the hormone insulin by the islets of Langerhans in the pancreas. The absence of which results in various degrees of hyperglycaemia (increased blood glucose level). Primary hyperglycaemia resulting from absolute or relative deficiency of insulin is called diabetes. There are two types of diabetes Type-1 diabetes is caused by the destruction of pancreatic beta cells by auto-immune disease leading to absolute insulin deficiency, less common in adult but more in children accounts for 5-10% of all diagnosed cases. Type-2 diabetes is caused by insufficient insulin production or tissue resistance to insulin produced leading to a relative insulin insufficiency, often associated with obesity, accounts for 20% of children diabetes. The prevalence of diabetes varies among groups by race, gender and age. Severe hyperglycaemia causes polyuria, polyphagia and weight loss with increased ketone bodies in serum resulting in ketosis/ ketonuria. Metabolic acidosis may occur with death from multiple complications. Increased blood glucose level above 500mg/100ml should be a concern in human. The determination of the blood CO_2 level would help to distinguish potentially fatal uncontrolled diabetes with ketoacidosis (indicated by a low blood CO_2 level) from hyperglycaemia with normal CO_2 level (though rare) found in the aged (associated with dehydration/ hypernatraemia) which is non-ketotic/ non-acidotic and not necessarily associated with diabetes (though both are dangerous). Apart from diabetes, other conditions that could be responsible for hyperglycaemia include steroid therapy, hyperthyroidism- in which case the mild hyperglycaemia is associated with hypocholesterol levels in serum, and in adenoma of the pancreas (resulting from the production of glucagon only), use of diuretics which induce hypokalaemia (hypokalaemia is known to suppress insulin secretion), certain conditions causing raised levels of blood catecholamines/ steroids, etc. Hypoglycaemia, though less common in adults may be caused by severe liver disease, pituitary hypofunction, adrenocortical hypofunction, hypothyroidism, substantial ingestion of alcohol after a period of fasting (starving), weight loss diets or oral hypoglycaemic

agents, etc. False hypoglycaemia may result from delay in specimen analysis (when preservative techniques are ignored), a markedly jaundiced specimen, reactive (insulin induced) hypoglycaemia excessive secretion of insulin following a rising blood glucose level which cause most of the glucose to be stored or metabolized, the insulin continues to act and hypoglycaemic state results. The different blood tests which can be carried out to confirm a diagnosis of diabetes include:

(i) **Random blood sugar test (RBS)** - a blood sample is taken at a random time. Blood sugar values are expressed in milligrams per deciliter (mg/dL) or millimoles per liter (mmol/L). Regardless of when you last ate, a RBS level of 200mg/dL(11.1 mmol/L) or higher suggests diabetes, especially when coupled with any of the signs/symptoms of diabetes, such as polyuria (frequent urination or excessive volume of urine), polydipsia (excessive thirst) and unexplained weight loss.

(ii) **Fasting blood sugar test (FBS)** - a blood sample will be taken after an overnight fast (8 12hours). A FBS level less than 100mg/dL(5.6 mmol/L) is normal. A FBS level from 100 to 125mg/dL(5.6 to 6.9 mmol/L) is considered prediabetes. If it's 126mg/dL (7 mmol/L) or higher on two separate tests, then diabetes is confirmed. Fasting plasma (or fasting serum) glucose is recommended rather than the previously used oral glucose tolerance test.

(iii) **Oral glucose tolerance test (GTT)** - after fasting overnight, the fasting blood sugar level is measured. Then you drink a sugary liquid (75g glucose load), and blood sugar levels are tested periodically for the next two hours. A blood sugar level less than 140mg/dL(7.8 mmol/L) is normal. A reading of more than 200mg/dL(11.1 mmol/L) after two hours indicates diabetes. A reading between 140 199mg/dL (7.8 mmol/L and 11.0 mmol/L) indicates prediabetes.

(iv) **Glycatedhaemoglobin (A1C)- test** this blood test indicates the average blood sugar level for the past two to three months. It measures the percentage of blood sugar attached to haemoglobin, the oxygen-carrying protein in red blood cells. The higher the blood sugar levels, the more haemoglobin that will have sugar attached. An A1C level of 6.5% or higher on two separate tests indicates diabetes. A result between 5.7 and 6.4% is considered prediabetes, which indicates a high risk of developing diabetes. Normal levels are below 5.7%. For those who are already diabetic A1C blood test is done periodically and estimates glucose levels in the blood over the previous two to three months. It's used to help identify overall glucose level control and the risk of complications from diabetes, including organ damage.

There is no cure for diabetes. The condition, however, can be managed so that patients can live a relatively normal life. Treatment of diabetes focuses on two goals: keeping blood glucose within normal range and preventing the development of long-term complications. Type 2 diabetes can be controlled with weight management, good nutrition, and exercise. Unfortunately, type 2 diabetes tends to progress, and diabetes medications and insulin are often needed. For individuals with diabetes mellitus who engage in self-monitoring, blood glucose measurements are to be preferred to urine sugar testing because it is not affected by variations in renal threshold for glucose, and the blood glucose analysis can quickly warn patients of hypoglycaemia and most target levels are set based on blood glucose level. If a whole blood meter such as glucometer is used in specimen analysis, the value obtained must be multiplied by 1.12 to obtain plasma or serum equivalent. Conversely, the plasma glucose value is multiplied by 0.89 to obtain equivalent of whole blood meter value. This conversion is only valid if haematocrit values of the samples fall between 35% and 55%.

2. **Amylase:** Out of the number of digestive enzymes secreted by the pancreas only amylase and lipase are commonly employed in evaluation of pancreatic function. Amylase is use for carbohydrate digestion and it's the only plasma enzyme normally found in the urine. Its assay in serum and urine are used in the diagnosis/ treatment of acute pancreatitis which cause a transient rise in serum amylase activity within 24 hours of the onset. A four-fold rise in serum amylase level above the upper normal reference limit may be observed in this condition with maximum level being attained within 72 hours. Although serum amylase levels are also elevated in mumps/ renal insufficiency, abnormally high levels of amylase (and lipase) would indicate that a significant number of pancreatic cells have been lysed in a short period. However, serum levels of amylase do return to normal by the 5th day, as against lipase which persists longer. In acute pancreatitis, the white cell count is raised. A decrease in level of albumin, calcium and haematocrit may indicate a critical phase of the disease especially if accompanied by increased urea levels. Serum bilirubin may increase if there is an obstruction to the common bile duct by an oedematous pancreas. Acute pancreatitis is usually caused by gallstones, alcoholism and certain medications. In chronic pancreatitis, since only a small number of pancreatic cells die over a long period of time, serum amylase is not significantly increased at any point in time so amylase activity measurement is not very effective in its diagnosis. There is transient increase in serum amylase in traumatic lesions of pancreas, temporary irradiation, pancreatic abscess, surgical trauma, etc. Urinary amylase (diastase) assay can be of additional help in the diagnosis of acute pancreatitis since it is elevated longer in urine than serum by a couple of days. In addition to aiding food digestion, amylase also digest white blood cells, so amylase-deficient individuals suffer majorly from abscesses/ inflammatory conditions such as dermatitis, eczema, asthma, hives

(nettlerash), etc. Although there are several assay methods for amylase, the use of a dye-labelled substrate which directly measures the amount of starch hydrolysed is preferred.

3. **Lipase:**-Lipase is an enzyme responsible for breaking down lipids into fatty acids and glycerol. It acts on emulsified form of lipid which is made possible by bile salts from the gall-bladder. Lipase is essentially produced in the pancreas, but small quantities may also be secreted by the gastric, pulmonary and intestinal mucosa, as well as the lingual salivary gland. Lipase activity has been found in leucocytes, adipose tissue cells and milk. Serum lipase activity increases within 48hours in acute pancreatitis. Serum lipase parallels the amylase with the lipase rising later and persisting in the serum longer than amylase making its assay more useful in late diagnosis of acute pancreatitis. Lipase remains elevated for about 2 weeks after onset of attack. Abnormal lipase levels are seen in advanced renal disease (though not in mumps), obstruction of pancreatic duct caused by carcinoma or calculus. Lipase deficiency may result in high blood cholesterol, hypertriglyceridaemia, muscle spasms, vertigo, diabetes and coronary heart disease, etc as a result of impaired cell permeability, inability of nutrients to get into cells and waste product taken out of cells. In chronic pancreatitis where the pancreas is progressively damaged there is a reduction in the amount of enzyme secreted but lipase levels in serum is found to be normal, making enzyme assay of this kind of limited use in investigating this condition. Imaging techniques are usually employed in the resolution of suspected cases. Investigation of biliary tract with the use of endoscopic retrograde pancreatography, or treatment with opiates which induce contraction of the sphincter of oddi may cause atrauma-induced increase in serum lipase level.

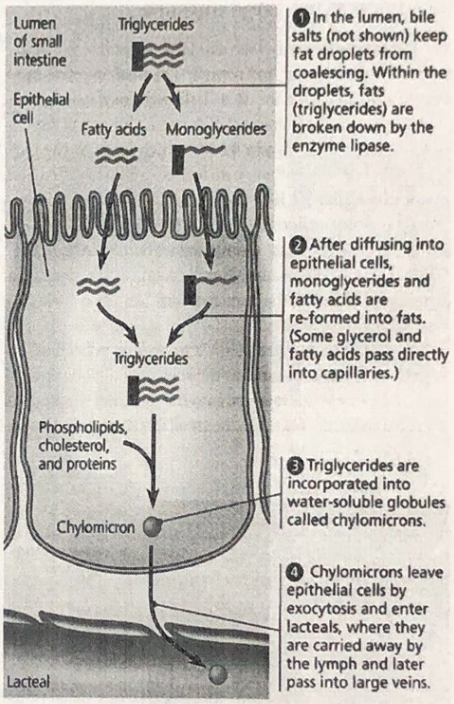


Figure 13.0: Fatty acid metabolism

CHAPTER FOUR

ANATOMY AND PHYSIOLOGY OF THE DIGESTIVE SYSTEM

The digestive system (digestive tract) consists of a musculomembranous tube extending from the mouth to the anus. The functions of the gastrointestinal tract (GIT) include the following:

- Ingestion of solid, liquid or semi-solid food substances
- Grinding of coarse food particle to fine texture
- digestion with the aid of enzymes ptyalin, rennin and digestive juices such as proteinase, lipase, maltase, sucrose)
- absorption of food in the small intestine, and
- elimination of solid wastes (faeces).

The GIT reduces the nutrients in food to compounds that are simple enough to be absorbed and used for energy/ building other compounds for metabolic use. It consists of a tube lined with mucous membrane which is continuous with the external skin at the mouth and at the anus. It is made up of four layers from within (inside) the digestive tract to the outward (outside) of the wall. They are -

1. Epithelial layer (stratified squamous to the glandular part of the stomach and simple columnar from there on)
2. Lamina propria (including the muscularis mucosae and submucosa)
3. Muscles (striated into or through the oesophagus, smooth the rest of the way usually with the inner part circular and the outer part longitudinal)
4. Serous covering (an outer serous covering the visceral peritoneum from the caudal to the diaphragm, covering most of the digestive tract)

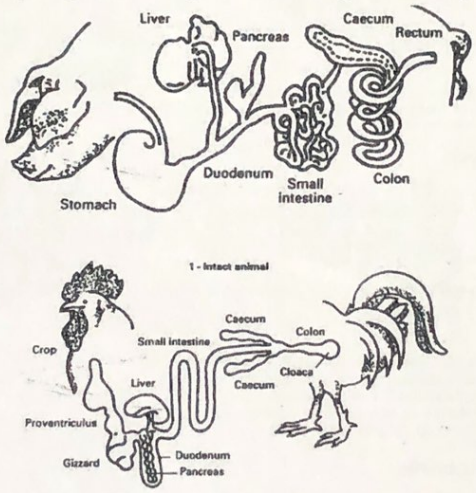
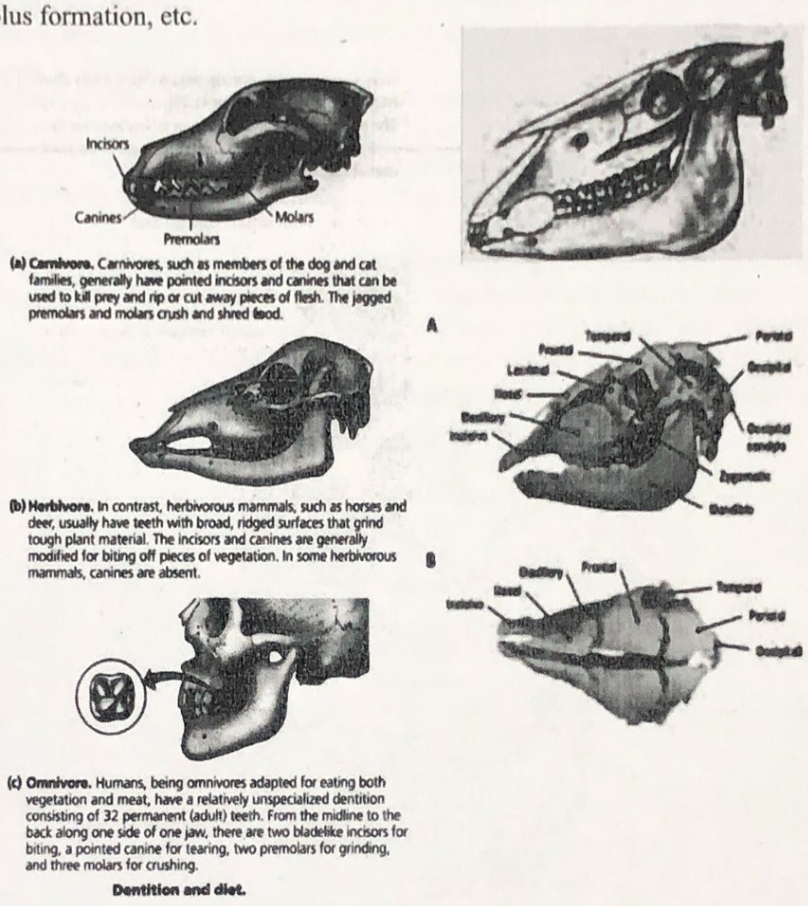


Figure 14.0: Structure of non ruminant digestive system

PARTS OF THE DIGESTIVE SYSTEM

1. Mouth
2. Pharynx
3. Oesophagus (and crop in poultry)
4. Stomach (Fore- stomach in ruminants and Glandular stomach for monogastric)
5. Instestine (Small intestine and Large intestine)
6. Accessory glands (salivary glands, liver and pancreas)

MOUTH (Oral cavity): Primarily for storage, grinding food, mixing with saliva, sometimes use for grasping (prehensile), defensive and offensive weapon. The teeth/ tongue are surrounded by lips, cheeks, and muscles to operate the jaws. The mouth functions in: Prehension, Mastication, Salivation, Bolus formation, etc.



(a) **Carnivore.** Carnivores, such as members of the dog and cat families, generally have pointed incisors and canines that can be used to kill prey and rip or cut away pieces of flesh. The jagged premolars and molars crush and shred food.

(b) **Herbivore.** In contrast, herbivorous mammals, such as horses and deer, usually have teeth with broad, ridged surfaces that grind tough plant material. The incisors and canines are generally modified for biting off pieces of vegetation. In some herbivorous mammals, canines are absent.

(c) **Omnivore.** Humans, being omnivores adapted for eating both vegetation and meat, have a relatively unspecialized dentition consisting of 32 permanent (adult) teeth. From the midline to the back along one side of one jaw, there are two bladelike incisors for biting, a pointed canine for tearing, two premolars for grinding, and three molars for crushing.

Dentition and diet.

Figure 15.0: Dentition in relation to type of food and skull bone

Teeth: It develops from an invagination of the epithelium known as the **dental lamina**, which produces the enamel organ (a cap-like covering of a connective tissue elevation called **dental papilla**). The connective tissue around the beginning tooth forms the **dental sac**. Both deciduous (temporary) teeth and permanent teeth develop in the same manner from similar embryonic structures. Teeth can be classified according to: permanency (milk or temporary and permanent teeth); function (cutting incisors, tearing canine, grinding molar/premolar), manner of growth (true teeth as in carnivore, constantly growing teeth as in swine, rodent, rabbit and constantly erupting teeth as in horses). In ruminants, the upper part lacks incisors and canine, rather they have a hard pad or dental pad that is used against the lower incisors for tearing grasses. Birds have no teeth but beak and claws which serve to practically reduce feed to sizes that could easily be swallowed. Teeth help to increase the surface area of food. The gap between the teeth of rabbit is called diastema.

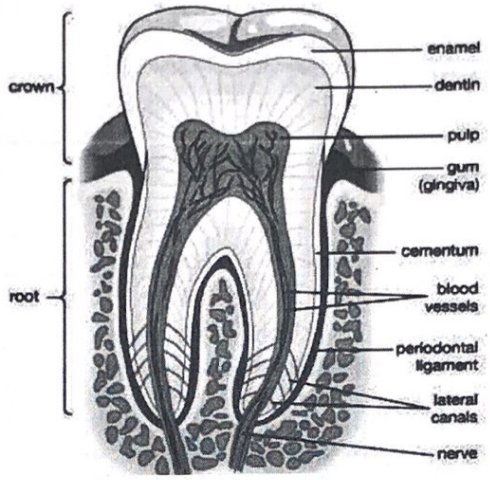


Figure 16.0: Structure of a tooth

The outer cells of the dental papilla produce the **dentine** while the inner cells form the **pulp** which is innervated by vessels/ nerves. The enamel is formed by the ameloblast layer of the enamel organ. It is the only part of the tooth derived from epithelium and is the hardest substance in the body. The inner layer of the dental sac forms the cementum, and the outer layer of the dental sac forms the periodontal membrane that connects the tooth to the bone of the socket (alveolus).

Teeth are of cutting or shearing type (**incisors**), tearing (**canine**) and of grinding type (**premolars/ molars**). Teeth that have a short crown are called **brachydont teeth**, while those with a long crown are known as **hypsodont teeth**.

The major portion of the tooth is made up by **Dentine**. It is produced by odontoblasts cells that line the pulp cavity and send processes out through the dentine. It also contains collagenous fibrils and fine canaliculi.

Enamel covers the crown in brachydont (short crown) teeth while the enamel not only covers the crown but is invaginated into the longitudinal grooves and infundibula (cups) in hypsodont (long crown) teeth of herbivores.

Cementum is modified bone that covers the roots of brachydont teeth, while in hypsodont teeth the cementum covers the root/ crown and fills in the grooves and infundibula where the enamel is not in apposition with anything else.

Deciduous (milk) teeth erupt first and are later replaced by permanent teeth. The time of eruption is the most accurate aid in determining the age of animals where no accurate records are available. Any animal that has molar is relatively old because molar is not present in milk teeth.

The front teeth are called incisors and denoted by letter I. It is numbered from the center of the mouth or symphysis, laterally. The first pair of incisors is called I_1 (or central), the next pair I_2 (first intermediates), next I_3 (second intermediates) and the last/ most lateral pairs I_4 (corners). In non-ruminants, only one pair of intermediate incisors is found.

Canine (C) teeth are also called eye teeth, bridle teeth, tusks and tushes. Normally not more than one pair of canine teeth occurs in each jaw at any given time, and canines may be completely absent in the ruminant and Mare. Cheek teeth are called premolars, P and molars, M. Deciduous cheek teeth are premolars and are numbered from front to back P_1, P_2, P_3 and P_4. Molars appear caudal to the premolars and are numbered M_1, M_2 and M_3. The dental formula for deciduous teeth is indicated by a D preceding the key letter as DI, DC and DP. There are no deciduous molars. The dental formulas for:

(a) Cattle, sheep and Goat 2(DI0/4 DC0/0 DP3/3), 2(I0/4 C0/0 P3/3 M3/3) = 32
(b) Pig 2(I3/3 C1/1 P4/4 M3/3) = 44
(c) Rodent 2(I2/2 C0/0 PM4/4) = 20
(d) Horse 2(I3/3 C1/1 P3-4/3 M3/3) = 40 or 42
(e) Dog 2(I3/3 C1/1 P4/4 M2/3) = 42
(f) Cat 2(I3/3 C1/1 P3/2 M1/1) = 30

These formulas indicate the teeth found on one side of the mouth. The numerator of the fraction represents the teeth in the upper jaw, while the denominator indicates the teeth in the lower jaw. This fraction must be doubled to include all the teeth in the mouth.

Table 6: Typeof teeth and the dental formula in livestock

Type of teeth	Horse	Cow	Sheep	Pig
Deciduous formula	2(DI3/3 DC0/0 DP3/3)	2(DI0/4 DC0/0 DP3/3)	2(DI0/4 DC0/0 DP3/3)	2(DI3/3 DC1/1 DP4/4)

Incisor	DI^1 birth to 1week	DI^1 birth-2wks	DI^1 birth- 1wk	DI^1 2-4wks
	DI^2 4- 6weeks	DI^2 birth-2wks	DI^2 1-2wks	DI^2 1.5- 3mths
	DI^3 6- 9weeks	DI^3 birth-2wks	DI^3 2-3wks	DI^3 at /or before birth
	DI^4 -	DI^4 birth-2wks	DI^4 3-4wks	DI^4 -
Canine	DC -	DC -	DC -	DC before birth
Premolar	DP^1 -	DP^1 birth-few days	DP^1 2-6wks	DP^1 5months
	DP^2 birth-2wks	DP^2 birth-few days	DP^2 2-6wks	DP^2 5-7weeks
	DP^3 birth-2wks	DP^3 birth-few days	DP^3 2-6wks	DP^3 4-8days(upper part)
	DP^4 birth-2wks	DP^4 birth-few days	DP^4 2-6wks	DP^4 upper 4-8days lower 2-4days
Permanent Teeth	**2(PI3/3 PC1/1 PP3-4/4 PM3/3)**	**2(PI0/4 PC0/0 PP3/3 PM3/3)**	**2(PI0/4 PC0/0 PP3/3 PM 3/3)**	**2(PI3/3 PC1/1 PP4/4 PM3/3)**
Incisors	PI^1 2.5years	PI^1 1.5-2years	PI^1 1-1.5years	PI^1 1year
	PI^2 3.5years	PI^2 2-2.5years	PI^2 1.5-2years	PI^2 16-20months
	PI^3 4.5years	PI^3 3years	PI^3 2.5-3years	PI^3 1year(8-10months)
	PI^4 -	PI^4 3.5-4years	PI^4 3.5-4years	PI^4 -
Canine	PC^1 4-5years	PC -	PC -	PC about 1year
Premolar	PP^1 5-6months	PP^1 2-2.5years	PP^1 1.5-2years	PP^1 1-1.5years
	PP^2 2.5years	PP^2 1.5-2.5years	PP^2 1.5-2years	PP^2 1-1.5years
	PP^3 3years	PP^3 2.5-3years	PP^3 1.5-2years	PP^3 1-1.5years
	PP^4 4years	PP^4 -	PP^4 -	PP^4 1-1.5years
Molar	PM^1 9-12mths	PM^1 about 6months	PM^1 about3-5mths	PM^1 4-6months
	PM^2 2years	PM^2 1-1.5years	PM^2 about 1year	PM^2 about 1year
	PM^3 3.5-4years	PM^3 2-2.5years	PM^3 1.5-2years	PM^3 1.5-2years

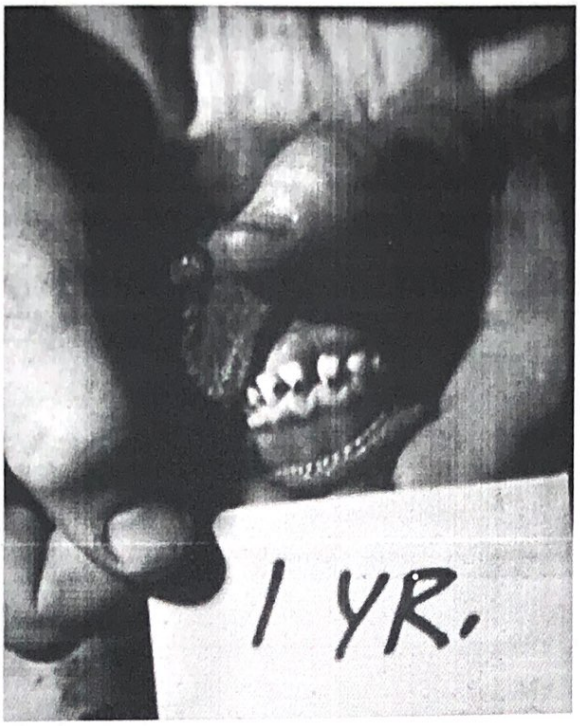


Figure 17.0: A one year old ruminant dentition

Tongue: The tongue consists of a mass of muscle covered with stratified squamous epithelium that presents a large number of papillae dorsally covered by mucous membrane. The tongue serves as a prehensile organ as well as an aid to chewing/ forming bolus in cattle. The tongue is attached to the lower jaw by three muscles: the **hyoglossus muscle** attaches to the basihyoid bone, the **genioglossus muscle** attaches to the symphysis of the mandible (chin), and the **styloglossus muscle** attaches along the inside of the stylohyoid bone.

The tongue is demarcated into four sections: **Filiform**, **Fungiform**, **Foliate** and **Vallate** or **Circumvallate**. Filiform, fungiform and circumvallate papillae are found in all domestic animals, and foliate papillae are present in the horse, pig and dog, but not in Sheep/ Cow.

1. Filiform papillae do not bear taste buds, but all other types of papillae do have taste buds. Filiform papillae are somewhat hair-like in appearance. In Ox they consist of a connective tissue core covered by a highly cornified epithelial layer. These papillae are shorter/ softer in the horse than other domestic animals, giving the tongue of the horse a velvety feel.

2. Fungiform papillae are assumed to resemble a fungus, or low toadstool. They contain taste buds in all animals.
3. Foliate papillae resemble the foliage or leaves of plants. They are found in horse, pig and dog where they contain taste buds and serous glands. The foliate papillae of the horse and dog also contain mucous glands.
4. Vallate (Circumvallate) papillae are large circular projections surrounded by a deep groove. They contain taste buds and serous glands in all domestic animals. It also contain mucous glands in the horse.

NOTE: Vallate and Fungiform papillae have taste buds while filiform, conical and lenticular papillae do not. Taste buds may also be found on the epiglottis, larynx, pharynx and soft palate. The tongue of the cow has a transverse groove in front of a dorsal prominence, which consists largely of a thickened mucosa.

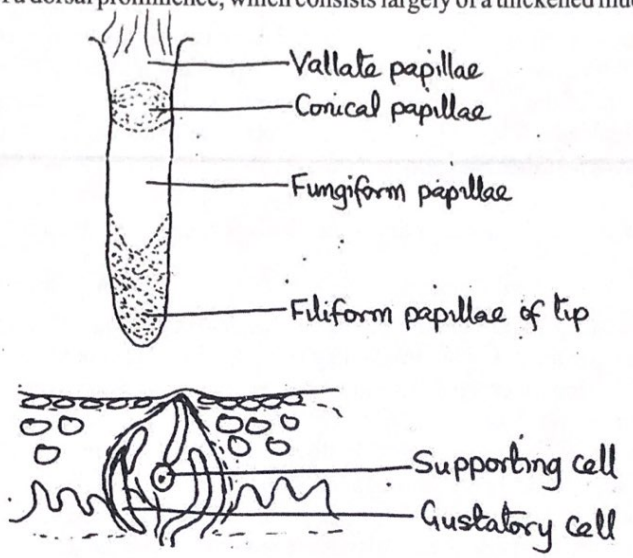


Figure 18.0: Dorsal view of a tongue (above) and the structure of the taste bud (below)

Taste buds: The end organs for the sense of taste consist of fusiform gustatory cells intermingled with sustentacular (supporting) cells arranged in somewhere barrel-shaped groups. Hair-like processes of the gustatory cells project through a pore at the superficial portion of the taste bud. Nerve fibers terminate around the gustatory cells.

Vallate and fungiform papillae of the tongue contain the most taste buds although some are also found on the palate, pharynx, and larynx. The sensation of taste is supplied to the anterior (roastral) two-thirds of the tongue by the chorda tympani branch of the facial nerve, which accompanies the lingual branch of the trigeminal nerve.

The posterior (caudal) one-third of the tongue receives taste by way of the lingual branch of the glossopharygeal nerve. Other sensations (heat, cold, touch, pain) to the tongue are supplied by the lingual branch of the trigeminal nerves. Probably the taste buds located on areas other than the tongue are supplied by the vagus nerve. In man, the four specific taste modalities include sweet, salt, bitter and sour (acid). Other tastes sensations involve mixture of these basic tastes or combinations of tastes/ smell.

The base of the tongue is sensitive to bitter taste, the lateral sides of the tongue respond primarily to sour stimuli and salt, while the lip of the tongue is sensitive to all four modalities but more sensitive to sweet/salt.
Taste appear to be an important factor in the ability of an animal to select food containing elements or factors in which the animal is deficient, e.g. Vitamin deficient rat will select food high in the particular vitamin they need. Rat with the adrenal glands removed prefer saline solutions to pure water to replace the salt that has been lost. If the parathyroid glands are removed, the rats prefer solutions containing calcium. The ability to correct dietary deficiencies is lost when the sensory nerves for tastes are cut.

The taste buds are chemoreceptor for the obvious reason they are stimulated by chemicals.

Lip: Lips of sheep, rat horses are soft and flexible, and in picking up food while the lips of cattle/pig are stiff / immobile only serve to close the mouth. The lips are covered with skin externally and mucous membrane internally over a layer of muscle, connective tissue, and labial glands. As the skin joins the mucous membrane, the hair and skin glands disappear, and the labial glands begin. Relatively large conical papillae are found projecting inward on the mucosa of the lips of ruminants. The upper lip forms part of the planumnasolabiale of cattle and the planumrostrale of pigs. Serous glands are present on these areas as well on the planumnasale of sheep, but not on the dog. (The moisture on a dog's nose comes from exhaled moisture Saliva from the tongue). Tactile hans, used as sensing probes are present in the lips of some animals including dogs / cats.

Cheeks are muscular structures covered with skin and lined with mucous membrane. They aid the tongue in positioning food between the teeth for chewing. The cow has numerous conical papillae lining the cheeks. The cheeks have the same layers as the lips, with the skin externally, the mucous membrane internally, and the muscle, the connective tissue, and the buccal (salivary) glands between the mucous of the ruminant cheek bears rather large cornfield papillae that may aid in mastication

Hard palate forms the roof of the mouth and is continued caudally by the soft palate which separates the mouth from the nasopharynx. The hard palate contains transverse ridges formed by thickenings of the mucous membrane. A network of veins forming cavernous tissue underlies the mucosa.

The lining of the oral cavity of the soft palate is covered by stratified squamous epithelium overlying mucous glands in the submucosa. The pharyngeal side of the soft palate is covered with pseudostratified columnar ciliated epithelium. The submucosa and lamina propria of the mucosa both contain glands.

Tonsils are more or less circumscribed masses of lymphoid tissue, named according to their location. Palatine tonsils in man and the dog are paired oval bodies located in pickets on the lateral wall of the pharynx ventral to the soft palate and lateral to the base of the tongue. These are the structures commonly referred to as tonsil. In the horse, cow and sheep these palatine tonsil are located in about the same relative position, but they are in the submucosa and are completely covered by mucous membrane except for the crypts or fissures over the tonsils. In other words, the tonsils do not project into the pharynx at all in these animals. In the pig the palatine tonsil are located in the substance of the soft palate.

The lingual tonsils consist of accumulations of lymph follicles on the base of the tongue. These tonsils are most prominent in the horse, cow and pig.

PHARYNX is a common passage for food and air, lined by mucous membrane and surrounded by muscles. Openings into the pharynx are the mouth, two caudal nares, two eustachian (auditory) tubes, the oesophagus, and the larynx. Inspired air passing through the nasal cavity enters the caudal nares and later crosses the pharynx to enter the larynx. The pharynx can be arbitrarily divided into nasal, oral and laryngeal portions. Foods, of course, enter the pharynx from the mouth and are forced into the esophagus by contraction of the pharyngeal muscles. During this period, the larynx is reflexly closed. Thus the paths of food and air must cross in the pharynx.

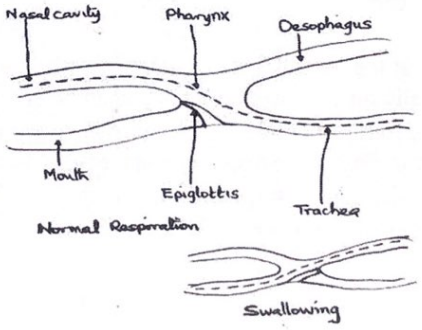


Figure 19.0: Structure of the pharynx

The eustachian tubes provide free exchange of air from the pharynx to the middle ear, so that pressure is equalized on both sides of the tympanic membrane (ear drums).

The nasal portion of the pharynx is lined with pseudo-stratified columnar ciliated epithelium, and the oral portion is lined with stratified squamous epithelium. Mucous glands predominate in the oral portion, and mixed glands are found in the nasal part, with the serous type predominating.

The muscular wall of the pharynx is continuous with the muscle of the oesophagus pharyngeal muscles are named according to the structures where each originates. These include the pterygopharyngeus (from the pterygoid bones), the hyopharyngeus (from the body of the hyoid bone), the thyropharyngeus (from the thyroid cartilage of the larynx), the stylopharyngeus (from the styloid process of the hyoid bone), and the cricopharyngeus (from the cricoids cartilage of the larynx). Most of these muscles insert on a fibrous raphe (band) on the midline at the dorsum of the pharynx during swallowing, the stylopharyngeus muscles shorten the pharynx, and the rest of the pharyngeal muscles (because of their circular arrangement), by contracting in series from cranial to caudal, force the bolus of food from the pharynx into the esophagus. The pig has a pharyngeal diverticulum that opens into the dorsal wall of the pharynx. Care must be exercised not to enter this diverticulum in passing a stomach tube or giving pills.

OESOPHAGUS is a muscular tube extending from the pharynx to the cardia of the stomach just caudal to the diaphragm from the pharynx. It passes dorsal to the trachea and usually inclines somewhat to the left in the neck. It again passes dorsal to the trachea, where it enters the thorax and continues caudal between the trachea and the aorta at the diaphragm. The oesophagus then passes through the hiatus esophagus of the diaphragm and joins the stomach within the abdominal cavity at the cardia. The cardia, or cardiac sphincter of the stomach is so named because of its proximity to the heart.

The muscular wall of the oesophagus consists of two layers that cross obliquely, then spiral, and finally form an inner circular and an outer longitudinal layer. The muscle changes from striated to smooth in the caudal one-third of the oesophagus in the horse and just in front of the diaphragm in the pig; it is striated throughout its entire length in the dog and ruminants. In avian species, the oesophagus has a diverticulum referred to as **crop** (see fig. 25.0).

STOMACH

(MONOGASTRIC) STOMACH: It is located just behind the left side of the diaphragm (a dome-shaped muscular sheet that separates the thoracic and abdominal cavities). Externally, the stomach is subdivided into: cardia (entrance), fundus (body) and pylorus (termination). The cardia and pylorus have sphincters (cardiac sphincter and pyloric sphincter) which control the passage of food through the stomach. The cardia and pylorus are quite close together, giving the stomach a shape somewhat like a best pear. This arrangement produces a very short concave side between the cardia and pylorus, which is known as the lesser curvature, and a much longer convex side, known as the greater curvature. The large bulge near the cardia is called the fundus but should not be confused with the fundic gland region of the interior of the stomach. Internally, the stomach presents a cavity that is lined with mucus and differentiated into four regions: **oesophageal region**, **cardiac-gland region**, **fundic-gland region** and **pyloric-gland region** with the last three being the glandular region. The glandular surface area of the stomach is increased many times by in-folding of the epithelium into depressions called **gastric pits** (foveolae) with a thick lamina propria outside the epithelium, which is almost filled with glands that empty into the depths of the gastric pits. Three types of glands found in the stomach are: Cardiac glands (located closest to the cardia), Pyloric glands (located in the region of the pylorus) and Fundic glands (gastric glands) (found throughout the remainder of the stomach).

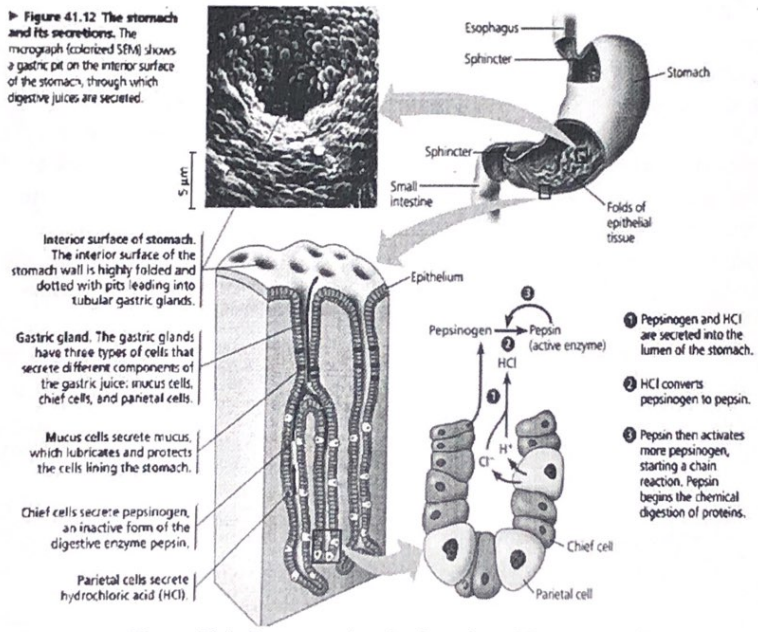


Figure 20.0: Structure showing location of the gastric pit

1. Oesphageal region Is an area of stratified squamous epithelium immediately surrounding the cardia. Its size varies with the species and is non-glandular. In the non-ruminant stomach, the oesophageal region compares to the fore stomach of the ruminant (rumen, reticulum and omasum) in that it is lined with non-glandular stratified squamous epithelium. The rest of the stomach can be more or less accurately divided into a cardiac gland region, a fundic-gland region and a pyloric gland region. The oesophagus region is large in the horse, small in the pig and absent in the dog.
2. Cardiac-glands region - It contain cardiac gland which are are simple or compound tubular glands that produce mainly mucus. Cardiac glands are found in the cardiac-gland region of the mucous membrane lining the stomach, which should not be confused with the cardiac of the stomach. The cardiac is the location of a sphincter at the junction of the oesphagus and stomach and in the ruminant is a great distance from the cardiac glands region. The cardiac glands region of the ruminant is poorly defined and contains cardiac, pyloric and fundic glands. The cardiac gland region is large in the pig but smaller in the horse, and the remainder of the non-ruminant stomach is divided between fundic and pyloric- gland regions.
3. Fundic-glands region - It occupies much more area than just the fundus of the stomach. It includes the entire area between the cardiac-glands region and the pyloric-glands region. A fundic glands is a simple tubular glands that consists of a mouth that opens into a gastric pit, a neck that is constricted, the main portion (body of the glands), and the blind extremity (fundus). Several fundic glands may open into the same gastric pit. The neck of the gland contains **mucous neck cells** that produce mucous, and some **parietal cells** that are involved in the production of hydrochloric acid. Parietal cells are also found in the body, where they are most numerous and to a lesser extent in the fundus of the gland. The **chief** or **zymogenscells** contain an intracellular network of canaliculi which connect directly or indirectly with the lumen of the gland and produce enzymes or precursors of enzymes. They are found mainly in the body and fundus of the fundic glands. The chief cells are located close to the lumen of the glands, where they appear to crowd the parietal cells out toward the periphery of the gland. Pepsinogen is produced by the chief cells and is broken-down by HCL to form pepsin. This region compares to the proventriculus of chicken.

   RUMINANT STOMACH: the ruminant stomach is composed of four compartments called:

   (a) Rumen
   (b) Reticulum
   (c) Omasum
   (d) Abomasum (true stomach)

The true (glandular) stomach in the ruminant is preceded by three divisions, or

diverticula (lined with stratified squamous epithelium), where food is soaked and subjected to digestion by microorganisms before passing through the digestive tract. The rumen, reticulum and omasum are collectively known as the fore-stomach. The cardia is located craniodorsally in the dome-shaped atrum ventricle, which is common to both the rumen and reticulum. The sulcus ruminoreticularis (oesophageal or reticular groove), which extends from the cardia of the stomach to the omasum, is formed by two heavy muscular folds or lips, which can close to direct material from the oesophagus into the omasum directly, or open and permit the material to enter the rumen and reticulum.

a. Rumen is a large muscular sac that extends from the diaphragm to the pelvis and almost entirely fills the left side of the abdominal cavity. The rumen is subdivided into sacs by muscular pillars, which appear from the exterior of the rumen as grooves. The dorsal and ventral sacs are separated by a nearly complete circle, which is formed on the frontal plane by the right and left longitudinal pillars, connected by the cranial and caudal pillars. The dorsal sac is the largest compartment. The dorsal sac overlaps the ventral sac and is continuous cranially with the reticulum over the rumino-reticular fold, which separates the floor of the rumen from the floor of the reticulum. Caudally the dorsal sac is further subdivided by the dorsal coronary pillars, which form an incomplete circle bounding the dorsal blind sac. The caudal part of the ventral sac is a diverticulum (ventral blind sac) separate from the rest of the ventral sac by the ventral coronary pillars. The mucous membrane lining the rumen is glandless stratified squamous epithelium. The most ventral parts of both sacs of the rumen contain numerous papillae up to 1cm in length, but papillae are almost entirely absent on the dorsal part of the rumen. The smooth muscle of the wall of the rumen consists essentially of two layers. The superficial layer runs largely craniocaudal in direction and bridges most of the ruminal grooves. The fibers of the inner layer of muscle run more transversely and also make up most of the substance of the ruminal pillars. The two muscle layers are continues with the muscle of the oesophagus. They run obliquely and cross at right angles. The wall of the sulcus ruminoreticularis consists largely of smooth muscle. Striated muscle from the oesophagus predominates at the cardia but fades out rapidly in the groove. Both transverse and longitudinal smooth muscle fibers are found in the floor of the groove. The lips contain mainly longitudinal fibers which form a loop around the cardia at the dorsal end of the groove and enter the sphincter of the reticuloomasal orifice at the ventral end of the groove. Most of the transverse fibers enter the wall of the reticulum. The mucous membrane of the reticular groove resembles that of the reticulum in the ventral part and that of the rumen in the dorsal part. The lower part of the oesophageal groove connects the atrium ventricle with the omasum. Rumen becomes functional in about 6-8weeks in calf.

b. Reticulum or honeycomb is lined with mucous membrane containing many intersecting ridges which subdivide the surface into honeycomblike compartments that appear in hexagonal shape. The surface is stratified squamous epithelium. It is the most anterior/ smallest of the fore-stomach and is directly behind the diaphragm. This location immediately behind the diaphragm, places it almost in apposition to the heart, so any foreign object such as wire or nail that may be swallowed tend to lodge in the reticulum, and are in a good position to penetrate into the heart (hardware disease). The rumen and reticulum communicate at the junction (rumino-reticular orifice) where the oesophagus enters the stomach. The oesophagus has a groove (oesophageal-reticular groove) which prolongs the oesophagus at the rumino-reticular orifice into the omasum. The purpose of which is to cause milk to by-pass the rumen and move into the omasum/ abomasum in young animals.

c. Omasum is a spherical organ filled with muscular laminae that descend from the dorsum or root, located to the right of the rumen and reticulum just caudal to the diver with the floors covered with stratified squamous epithelium.. The mucous membrane covering the laminae is studded with short, blunt papillae that grind roughage before it enters the abomasum. Omasum of the sheep/ goat is much smaller than that of the cow and not in contact with the abdominal wall in normal condition in these small ruminants. Omasum is nearly filled with laminae, bearing pointed papillae arranged in such a manner that food is moved from the **reticulo-omasalorifice**, between the laminae, and on to the **omaso-abomasalorifice**. Each lamina contains three layers of muscle including a central layer continuous with the muscle wall of the omasum, and a layer of muscularis mucosa on each side of the central muscle. The fibers of the muscularis mucosa run at right angles to the fibers of the central muscle. At the junction of the omasum and abomasum is an arrangement of folds of the mucous membrane (**vela terminalia)** which may act as a valve that prevents return of material from the abomasum to the omasum. It is derived from the omasum in the cow, but from the abomasum in the sheep.

d. Abomasum is the first glandular portion of the ruminant digestive system. It is located ventral to the omasum and extends caudad on the right side of the rumen. The pylorus (terminal part of the abomasum) is a sphincter (thickening of circular smooth muscle fibers) at the junction of the stomach and small intestine. The epithelium of the abomasum changes abruptly from the stratified squamous epithelium of the omasum to a tall simple columnar epithelium capable of producing mucus. Presumably the mucus covering the stomach epithelium helps prevent the digestive juices from digesting the stomach cells. In general the gland regions of the abomasum correspond to the gland regions in the simple stomach of the non-ruminant and the fore-stomach of the ruminant correspond to the oesophageal region of the simple stomach.

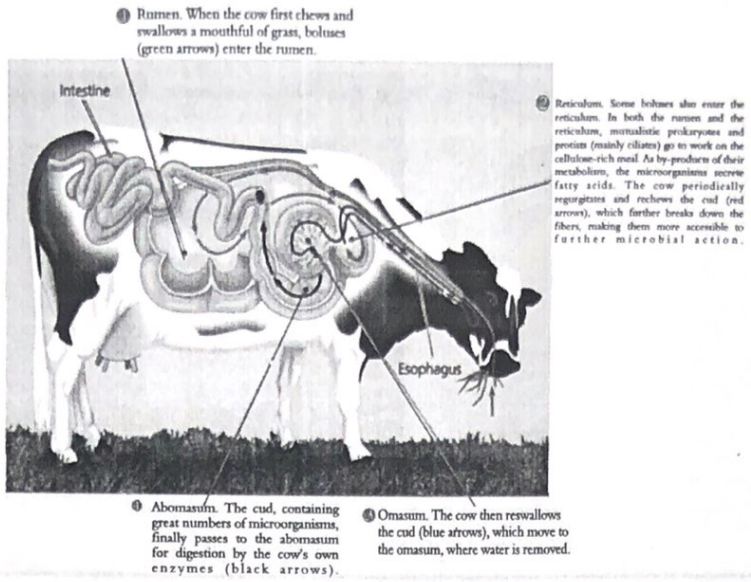


Figure 21.0: Ruminant digestive system

SMALL INTESTINE is divided on the basis of histologic or macroscopic structural differences into three parts Duodenum, Jejunum and Ileum.

Duodenum is the first part of the small intestine closely attached to the body wall by a short mesentery (**mesoduodenum**). The common bile duct (from the liver) and the pancreatic duct (from the pancreas) enter the first part of the duodenum a short distance behind the pylorus. The duodenum leaves the pylorus of the stomach and passes caudal on the right side toward the pelvic inlet. The duodenum then crosses to the left side behind the roof of the great mesentery and turns forward to join the jejunum.

Jejunum is indistinctly separated from the duodenum. It begins approximately where the mesentery starts to become rather long. The jejunum and ileum are continuous, and there is no gross demarcation between them. The ileum is the last part of the small intestine. It enter the large intestine at the osteumileale (ileal opening).

It is impossible to give a definite location for the jejunum and ileum, but they tend to be located toward the left ventral portion of the abdominal cavity in non-ruminants. The rumen displaces most other viscera to the right side in ruminant. The terminal part of the **ileum**, however, joins the caecum (horse), colon (dog), or caecum and colon (ruminant and pig) in the right caudal part of the abdominal cavity.

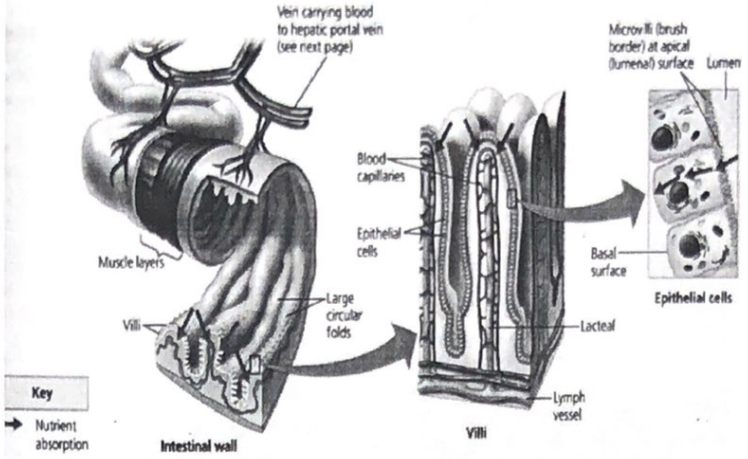


Figure 22.0: Structure of the intestinal wall

LARGE INTESTINE consists of the **caecum** (a blind sac) and the **colon** (consists of ascending, transverse, and descending parts). The descending colon terminates as the **rectum** and **anus.**

In ruminants, the blind end of the caecum projects caudad. Cranially, the caecum is continuous with the colon. This junction is marked by the entrance of the ileum at the ileal orifice.

The large intestine of the pig begins with the caecum with the blind end projecting forward and ventrally near the midline. The dorsal end of the caecum in pig is continuous with the colon at the ilio-caecocolic junction, where the entrance of the ileum marks the division between the caecum and colon. The ascending colon of the pig presents a spiral arrangement of coils giving somewhat cone-shaped appearance once. When the colon leaves this spiral, it passes forward and crosses to the left as transverse colon and then continues caudad as descending colon to the rectum. As in other animals, the rectum terminates at the anus.

In ruminant, the ascending colon passes forward apparently between the two layers of mesentery that support the small intestine. The proximal loop (ansaproximalis) is located between the caecum and the coiled colon (ansa spiralis). The ansa spiralis is arranged in coils. The first portion spiralis toward the center of the coils (centripetally) and the next part spirals away from the center (centrifugally). The last parts of the ascending colon, the ansadistalis, connect the ansa spirals with the transverse colon. The transverse colon crosses from right to left and continues caudal to the rectum and anus, the terminal part of the digestive tract.

.The arrangement of the intestinal tract of the sheep and goat is smaller to that of the cow. However, the last centrifugal gyrus (loop) of the ansa spiralis is outside the cranial mesenteric artery and mesenteric lymph nodes, and is much closer to the jejunum than in the cow. In the rabbit, the large intestine and caecum are separated. The ileum enters the caecum and the colon at the joint referred to as sacculusrotundus. The caecum which is about 20inches in length and 1inch in diameter has at its end a small closed sac called appendix.

ACCESSORY DIGESTIVE ORGANS

1. Salivary glands

	Major ones	**Minor ones**
Salivary glands	Parotid (close to the ear)	Labial
	Mandibular	Buccal
	Sublingual	Lingual
		Palatine glands

2. Liver: Kupfers cells help in phagocytosis. All domestic animals except the horse have a gall bladder for storage of bile.
3. Pancreas has both endocrine and exocrine portions. The exocrine portion produces $NaHCO_3$ and digestive enzymes which empty into the duodenum through the pancreatic duct close to the opening of the bile duct.
   The endocrine portion consists of pale staining cells (islets of Langerhans) scattered throughout the connective tissue stroma of the gland. They produce the hormones **insulin** (beta cells) and **glucagon** (alpha cells), which pass directly into the blood stream.
   The alveolus is the secretory unit of the pancreas with acidophilic zymogen granules in the cytoplasm.

CHAPTER FIVE

ANATOMY AND PHYSIOLOGY OF TELEOSTS

1. **Skin (integumentary system):** As in other animals the skin is the primary barrier against the environment. It allows normal internal physiological functions i.e. it contributes to the homeostasis mechanism. In bony fish the layer of the skin consist of the following from outside inward
   - **Cuticle (outermost)**
   - **Epidermis**
   - **Basement membrane**
   - **Dermis**
   - **Hypodermis (innermost)**

i. Cuticle: This is made up of slippery material, mucopolysaccharide which is secreted by the epithelia cells. It also contains epithelia cells which have sloughed (peeled) off from the epidermis. It also contains mucus which has been produced by the goblet cells in the dermis. The cuticle contains lysozymes and immunoglobulins and also free fatty acids which have antipathogenic activity. The thickness of the cuticle varies from fish to fish.

ii. Epidermis: This is immediately below the cuticle and is made up of layers of cells which are all living and are nucleated unlike the mammalian epidermis. Fish which do not have scales have a thicker epidermis. Epidermal cells and goblet cells produce mucus. The epidermis also contains lymphocytes and macrophages.

iii. Basement membranes: The foundation on which the above is laid.

iv. Dermis: Comprises an upper loose network of collagen fibres which is continuous with the basement membrane of the epidermis. The lower part of the dermis is thicker and gives strength to the skin. The dermis apart from connective tissue contains various types of cells and blood vessels. The cells include **chromatophores** which are involved in absorption and reflection of light to cause colour changes to match the environment or colour changes due to sexual activity. There is another type of cell called **melanophores** containing the dark pigment called **melanin**.

v. Hypodermis: The hypodermis is loose and contains mainly adipose tissue. It contains more blood vessels than the dermis.

THE MUSCULOSKELETAL SYSTEM OF FISH

The typical streamlined shape of fish is a requirement for swimming. The slippery streamlined body reduces friction. Swimming by fish is the most energy saving means of transportation.

Axial skeleton: It includes a rigid cranium joining with other bone of the skull which is mainly the jaws i.e. lower /upper jaws (mandible/maxillae), the branchial and opercular apparatus. The arrangement in fish is more complex than in mammals and most of the bones of the skull move during feeding and breeding movements because all the component of the skull is interdependent. The axial skeletons also include the vertebra. The number of vertebrae varies even in the same species of fish and it is affected by environment conditions during lava development. The vertebra column is held together by longitudinal ligaments above and below the spine. In many species intermuscular bones of various arrangements also come out from the vertebra column through the divisions of the muscle. **Myotomes**- division or group of muscles. Some of the fins are attached to the pectoral or pelvic girdles and others to the vertebra column.

MUSCLES: The muscular system of fish is composed of zig-zag muscle bands called **myotomes** or **myomeres**. They appear like the letter W lying on its side. The muscles are attached to broad sheet of tough connective tissues which are connected to the vertebra column.

THE RESPIRATORY SYSTEM

Teleosts (bony fish) breathe by means of gills. The gills comprise 2 sets of four **holobranchs**. Two on each side of the head. This (holobranch) form the side of the pharynx. Each holobranch consists of two **hemibranchs** projecting from the posterior edge of the branchial arch. The **branchial arch** is also called the gill arch. They project in such a way that the free edges diverge and touch the hemibranchs of the nearest (adjacent) holobranch. A hemibranch consist of long thin filaments called the **primary lamellae** which project from the gill arch like the teeth of a comb. The surface area of the primary lamellae is increased by the presence of folds which are shaped like half-moon across the dorsal and ventral rows. These folds are known as the **secondary lamellae**. They greatly enlarge the epithelium of the gills. The area of the gill is comparable to the total surface area of the skin or more, to increase the efficiency of their function. Each gill filament contains blood capillaries within the lamellae. The epithelium of the filament is thin to allow gas exchange but this also makes the epithelium liable to invasion by pathogen. The gill arch is bony and curved and from the anterior edge project bony rays which gives the arch support. Running through the arches are branches of the aorta and this branches are in turn divided into fine branches which supply the primary lamellae and the capillaries which supply the secondary lamellae.

Physiology of Respiration

The gills are covered by a movable operculum on each side. This provides protection to the delicate gills but they also make it possible for water to be pumped through the mouth across the gills.

The flow of water across the gill is opposite to the direction of blood flow and this arrangement allows for the maximum possible extraction of oxygen from the water. This arrangement is also known as counter-current flow → ←. Very active fish such as Herring and Mackerel can get enough oxygen only by continuous swimming forward to force water into its open mouth. If such fish is placed in an aquarium with limited space for swimming it will die for lack of oxygen. Energy required for extraction of oxygen from water by fish is high compared with air breathing. Some fish can exist outside water by breathing air e.g. Claria. Such fish use their primitive lung or their skin as a major respiratory surface or their swim bladder or a combination of their gills and the oral cavity which is richly supplied with blood capillaries.

OSMOTIC REGULATION

The salt concentration in fresh water is much lower than that of the blood of fresh water fish. Although the scales and the mucus covering of this type of fish reduce the amount of salt lost to the environment, water tend to enter their body by osmosis, and salt will be lost through diffusion into the water body particularly across the thin walls of the gills. To overcome this problem, freshwater fish

1. Pump out excess water through the kidneys by forming very dilute urine.
2. They actively absorb sodium and chloride ions from the water using special salt absorbing cells located in the gill epithelium. Fresh water fish are **hyper osmotic** regulators. Marine fishes on the other hand are **hypo - osmotic** regulators, they tend to loss water and gain salt. They actually drink sea water to be able to retain their water content. Therefore to get rid of the excess salt they do the following:

i. They actively secrete salt through salt secreting cells located on the gill epithelium.
ii. The remaining ions which are usually divalent ions e.g. Mg^{2+}, SO_4^{2-} are left in the intestine and passed out with faeces.
iii. Some divalent ions are also excreted by the kidneys.

THE CIRCULATORY SYSTEM

The heart is covered by a pericardium and it lies ventral to the pharynx. It has four chambers- the sinus venosus, the atrium, the ventricle, the bulbus arteriosus. Deoxygenated blood enters the sinus venosus which is very small and may not be seen as a chamber of the heart. It has a thin wall. The blood now enters the atrium through valves. The atrium is dorsal (on top of) to the ventricle. Its wall is also thin but there is a muscular network within it which contains phagocytes and other white blood cells. So it performs an important body defence function. Blood from the atrium enters the ventricle which has a thick wall. The ventricle pumps blood into the bulbus arteriosus (beginning of the aorta). The bulbus arteriosus acts as a passive elastic reservoir which smoothens the blood pressure pulse from the ventricle and maintains blood flow even when the ventricle relaxes.

The ventral aorta runs forward from the heart distributing blood to the gills via the afferent brachial arteries. The efferent brachial arteries on the other hand receives blood (oxygenated) from the gills joined together to form the dorsal aorta. Blood is distributed from the dorsal aorta to the organs of the head, and branches from it supply other parts of the body including the muscles and the viscera (abdominal & thoracic). However, the muscles are mainly supplied by lymph (blood without RBC) instead of blood. There is high capillary permeability in fish producing much lymph which is rich in protein. Oxygen is carried in blood bound to haemoglobin like in mammals but because the blood temperature is usually low much of the oxygen carried in the blood of fish is dissolved in the water of the blood. Certain fishes in the Polar Regions don't have haemoglobins because they don't need it. The red blood cells of fish (like poultry which is oval in shape) are nucleated. Blood cells are formed in the spleen and the kidneys. Phagocytic cells are well distributed in fish in the skin, atrium and in the blood, however the liver has little phagocytic activity unlike in the mammal.

DIGESTIVE SYSTEM

Since the various fishes feed on a variety of diets there is a wide variation in the adaptation of the alimentary tract to suit these diets. Herbivorous fishes have longer alimentary tract than omnivorous and carnivorous ones. The mouth plays an active part in both digestion and respiration. The digestive function is limited to selection, seizure and transfer of food into the stomach. Except in a few herbivorous species chewing is not a function of the teeth. The oesophagus is short and muscular; it produces mucus to help the food down into the stomach. The stomach is highly distensible (expensible) and sigmoid in shape. The stomach has many fold and many layers of muscles. Some species have no stomach. The functions of the stomach are similar to the intestine producing many digestive enzymes. The intestine is a simple tube whose diameter does not increase to form a large intestine. It is straight, sigmoid or coiled in the different species. The rectum has a thicker wall down than the intestine. Its linning produces a lot of mucus. The liver is large and reddish brown in carnivores but lighter brown in herbivores. When the feed is less suitable for any particular fish then its liver becomes less brown in colour (lighter in colour). In certain species, the liver is combined with the pancreas and it is called **hepatopancreas** (as in snails), however in others the two organs are separate. The pancreas is located in different spaces in different species; it contains both the exocrine and endocrine.

REPRODUCTIVE SYSTEM

There is a wide variation in the reproductive pattern in teleost fish. The vast majority however are dioecious that is fertilization and development of the eggs and embryo takes place outside the body. Most species are either males or female but hermaphroditism occurs in some fish species. **Parthogenesis** i.e. development from an unfertilized ovum in some fish and **gynogenesis** which is the development

of an ovum which is stimulated by sperm penetration; however the sperm does not contribute any genes.
Copulation takes place in some fish resulting in either the discharge of fertilized eggs into the water or resulting in young live fish being released.

The testes are elongated, paired and whitish. They are suspended from the dorsal abdominal wall alongside or below the swim-bladder. They are small in young fish but larger in adults. A testis is made up of lobules or blind sacs called the seminiferous tubules, the tubules are lined with seminiferous cells. The development of spermatocytes is similar to that in mammals. The testes also produce the hormone testosterone.

The Ovary: The female genital tract in fish is highly variable depending upon the method of reproduction. It is simple in the lower teleost but complex in viviparous species (fertilized eggs retained and hatched). In viviparous species the ovary acts as spermatozoan store, the vagina and also the uterus where the young embryos are nourished. In immature female fish, the ovaries appears as a cluster of minute orange white spheres but in adults they may form as much as about 70% of the total body weight.

The Swim bladder
This is gas filled and may form about 7% of the body volume. It is a characteristic of teleost fish but it is absent in many bottom living fishes. It develops (in the lava fish) as part of the alimentary tract and it is a diverticulum. In many primitive fishes this connection is retained and it is referred to as the pneumatic ducts. The function of the swim bladder is to maintain buoyancy since the teleost fish is heavier than both fresh water and sea water fish. It is also used for sound and pressure reception. The neutral buoyancy mechanism involves maintaining a constant volume of air in the swim bladder. In those fish (physostomes) which comes to the surface of water occasionally, the swim bladder is inflated by swallowing air which is then forced through the pneumatic duct into the swim bladder. In fish that do not have access to the water-air interface (physoclists) the swim bladder is inflated by the release of gas from the arterial blood which finds its way into the swim bladder. The gas present in the swim bladder is a mixture of oxygen, nitrogen and carbon dioxide but the proportion is usually different from that of air.

CHAPTER SIX

COMPARATIVE STUDY OF THE AVIAN, SWINE, EQUINE AND RABBIT GASTRO - INTESTINAL TRACT

The digestive system of any organism generally connects the outside environment with the metabolic environment. Usually the type of food that is nutritionally useful to any species of animal is determined largely by the development and anatomy of the alimentary canal. The carnivorous species with relatively short alimentary canal are different from the herbivores which have long digestive system. The relationship of the length of the body to the length of the GIT (Gastrointestinal tract) is about ratio 1 - 4 for Cats/ Chicken, 1 - 6 for dogs, 1 - 16 for Swines, and 1-27 for Cattle, Sheep/ Goats. Ruminants needs very long GIT because of the fibrous nature of the feed which needs to be dealt with to expose them for utilization. Consequently, the food given to chicken resembles that for cats/ dogs rather than cattle and sheep.

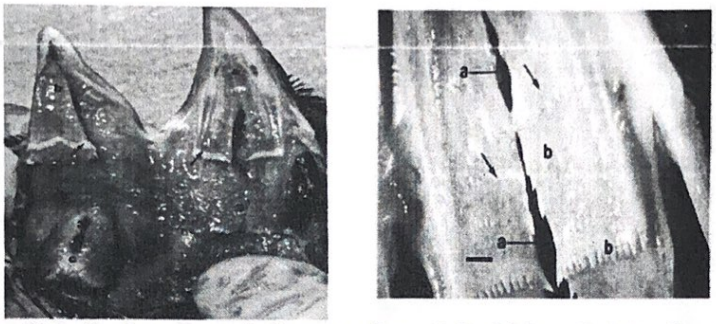


Figure 23.0: Oral cavity and pharynx of an adult chicken; Palate of an adult chicken (ventral view) shown on the palate (a) is the choana (1), the median fissure opening into the nasal cavities. The infundibular cleft (2) is the common opening of the auditory tubes. The tongue (b) in the chicken, turkey and pigeon cannot be protruded and is mainly an organ of swallowing. The laryngeal mound (c) has on its rostral surface the glottis (3) which is the entrance to the larynx. Many backward-pointing, thickly keratinized papillae (arrows) are distributed on the roof of the oropharynx, on the tongue and on the laryngeal mound. Due to the absence of a soft palate/ oropharyngeal isthmus, the oral and pharyngeal cavities together form a common cavity which is often referred to as the "oropharynx". The opening of the mouth is generally called gape. Swallowing is achieved by rapid rostrocaudal movements of the tongue and the laryngeal mound assisted by much sticky saliva and the caudally-directed papillae which propel the food backwards and prevent its regurgitation. During this process the choana, infundibular cleft/ glottis are reflexly closed. The method of drinking varies with the species. In the chicken, water is propelled into the mouth by rostro-caudal movements of the tongue. The head is then raised and the water passes into the oesophagus mainly by gravity. In pigeon, in contrast, the beak is immersed in water throughout and the head is not raised. The choana (a) and papillae (b) are shown in the second photograph. The numerous openings of the mucus-secreting salivary glands which form an almost continuous layer in the walls of the oropharynx are indicated by arrows.

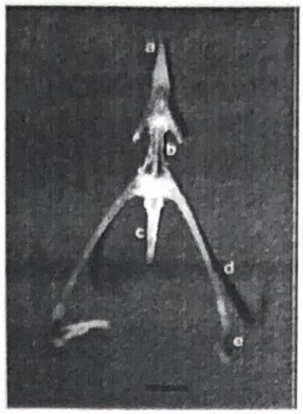
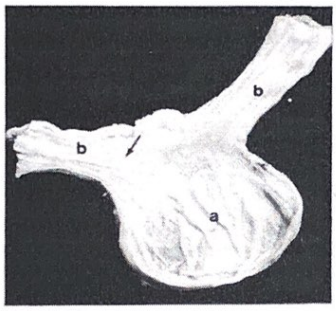


Figure 24.0: Hyoid apparatus of an adult chicken (Dorsal view, scale 5mm) entoglossal bone (a), rostral basibranchial bone (b), caudal basibranchial bone (c), ceratobranchial bone (d), epibranchial bone (e). The entoglossal bone lies in the body of the tongue and the rostral basibranchial bone in the base of the tongue. The hyoid apparatus and its associated musculature are responsible for tongue movements. Except in parrots, there are no true intrinsic muscles in the tongue.

The obvious characteristic of the mouth of chicken is the absence of lips/ teeth, both of which are replaced by horny mandibles which is referred to as the **beak**. The tongue of a chicken is shaped like the head of an arrow. The projections at the back of the tongue (Fig. 23.0) serve to force the food down the oesophaguswhen the tongue is moved to and fro. The salivary glands secrete mucoid saliva which lubricates the feed as it passes through the oesophagus. After the oesophagus is a pouch called **crop** (Fig.25.0) which forms an enlarged specialized area of the oesophagus which serves many storage functions.



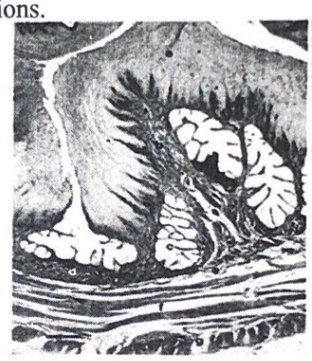


Figure 25.0: Interior of oesophagus and crop of chicken - The crop (a) is a ventral diverticulum of the oesophagus (b) which it divides into cervical and thoracic parts. The oesophagus is relatively distensible because the internal surface is longitudinally folded**; Light micrograph of a section through the entire wall of the oesophagus of achicken (Masson's trichrome, scale 250µm)** - (a) partly keratinized stratified squamous epithelium, (b) lamina propria, (c) mucus glands, (d) muscularis mucosae, (e) submucosa, (f) inner circular muscle layer, (g) outer longitudinal muscle layer. The structure of the crop resembles that of the oesophagus, except that the mucous glands are restricted to an area close to the oesophagus.

After the crop is the **proventriculus** or true stomach (Fig. 26.0) which is the acid secreting section of the digestive tract of the avian specie similar in function to the stomach of other animals such as pig, dogs/ cats. In other species like man, pig and equine the stomach usually opens into the small intestine (SI) but in birds' proventriculus open into the gizzard (Fig. 27.0) which inturn opens into the duodenum. Hcl/ pepsin which aid protein digestion are secreted by the walls of the proventriculus. There is no production of rennin in the avian stomach since they do not consume milk unlike mammals. Food usually stays for a short period in the proventriculus; consequently digestion here is of less importance.

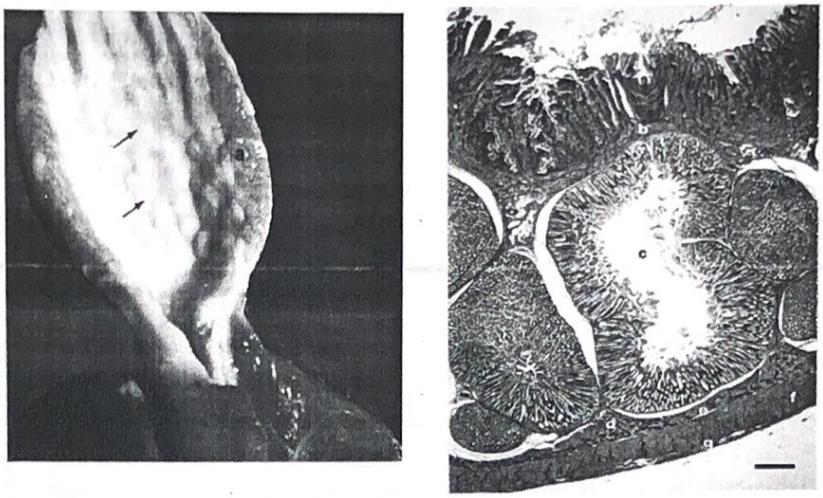


Figure 26.0: Interior view of the proventriculus of an adult chicken projecting into the lumen are well developed papillae (arrows) at the tops of which open the compound gastric glands. The glands form a thick layer (a) in the wall of the proventriculus. Gastric proteolysis occurs principally in the gizzard where the pH is low (1.5 2.5)**; Light micrograph of a section through the entire wall of the proventriculus of a chicken (Masson's trichrome, scale 500µm)** Columnar mucus-secreting epithelium (a), lamina propria (b), multilobular glands of the lamina propria (c), muscularis mucosae (d), submucosa (e), inner circular muscle layer (f), outer longitudinal muscle layer (g). The muscularis mucosae surround the glands of the lamina propria. The gland consists mainly of closely-packed alveoli radiating out from a central cavity. The central cavity is drained by secondary ducts, all the secondary ducts of a gland uniting to form a primary duct which opens into the lumen of the organ. The glandular epithelium of the alveoli is lined mainly by one type of cell, the oxynticopeptic cell, which secretes both pepsin and hydrochloric acid.

Posterior to the proventriculus is the **gizzard** (Fig. 27.0) which is an oval shaped organ lying between the proventriculus and duodenum. It is characterized by two pairs of red powerful muscles covered by a thick corny epithelium (Fig. 28.0). The chief function of the organ is to grind coarse particles aided by ingesting grits or pebbles. With properly ground ration, the role of the gizzard diminishes for proper digestion (ground feed stays for a short period than the coarse feed).

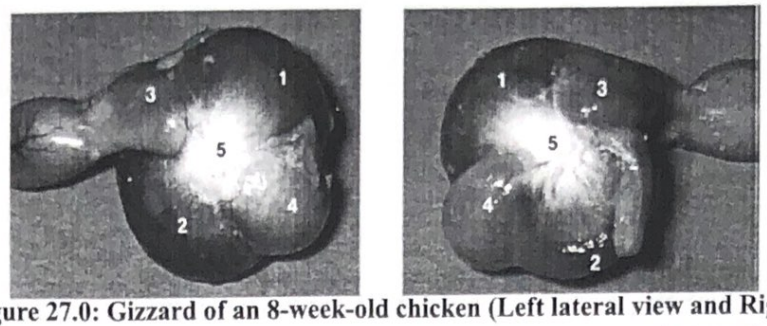


Figure 27.0: Gizzard of an 8-week-old chicken (Left lateral view and Right lateral view) most of the wall of the gizzard is made up of smooth muscle that is rich in myoglobin and arranged into four semi-autonomous masses. These are the dark-colouredcaudodorsal (1) and cranioventral (2) thick muscles, and the lighter-coloured craniodorsal (3) and caudoventral (4) thin muscles. The muscles attach to the right and left tendinous centres (5) in the lateral walls of the gizzard. Because the muscles are asymmetrically arranged, rotator as well as crushing movements are set up when the gizzard contracts.

Immediately after its attachment to the gizzard, the SI is folded into a loop called the **duodenum** (Fig. 32.0). In between the loop of the duodenum is the pancreas which is supplied with a number of ducts called pancreatic ducts (Fig. 32.0) which leads into the duodenum. The pancreatic juice is slightly alkaline and neutralizes the acid secreted by the proventriculus. Pancreatic secretions contain a series of enzymes which hydrolyze protein, carbohydrate and fats.

Trypsin →	Protein →	polypeptides
Lipase →	fat →	Glyceride/ fatty acids
Amylase →	starch →	disaccharides

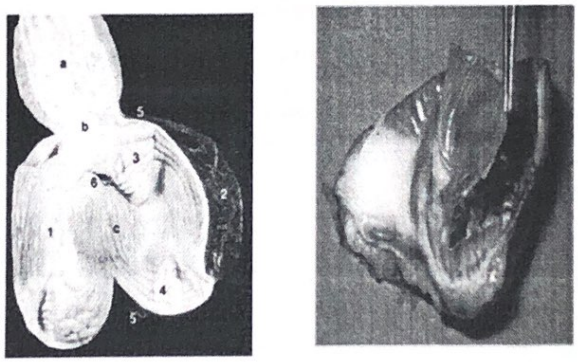
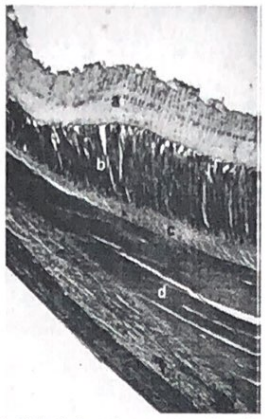


Figure 28.0: Interior of the stomach of an adult chicken separating the proventriculus (a) and gizzard (c) is the intermediate zone (b) which is characterized by a histological structure intermediate to that of the proventriculus and gizzard. Compound glands are absent here and the internal surface is relatively smooth. The bulk of the gizzard is formed by the body .

(1), in the walls of which are the thick muscles (2). At either end of the gizzard are the cranial (3) and caudal (4) blind sacs, in the walls of which are the craniodorsal and caudoventral thin muscles (5). The entrance (6) to the pyloric part of the stomach and the duodenum lies in the cranial part of the gizzard. Lining the inner surface of the gizzard is hardened membrane, the cuticle (sometimes called the koilin layer), which is a carbohydrate-protein complex secreted by the gizzard glands. The membrane is especially well-developed in birds, such as the domestic species, which feed on relatively hard items of food. In these birds it is asymmetrically developed, being thickest opposite the thck muscles of the body of the gizzard and thinnest opposite the thin muscles of the cranial and caudal blind sacs. Its green or yellow colour is due to bile regurgitated from the duodenum via the pylorus. The cuticle is a secretion of the mucosa and can therefore be easily stripped away from the organ. In poultry slaughter plant the cuticle has to be removed by machine before the gizzard is added to the giblets.



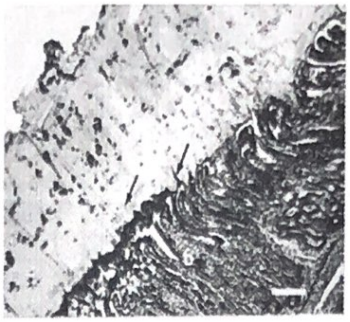


Figure 29.0: Light micrograph of a section through the entire wall of the gizzard of a chicken (Masson's trichrome, scale 250µm); Light micrograph of the inner layers of the gizzard of a chicken (Masson's trichrome, scale 100µm) cuticle (a), glands in the mucous membrane (b), submucosa (c), muscle (d). The cuticle is a carbohydrate-protein complex secreted by the mucosal glands and the surface epithelium. It is composed of vertical rods (arrows) and horizontal matrix. The vertical rods are secreted by the mucosal glands, the secretion hardening within the lumen as a thickening perpendicular to the surface. The horizontal matrix between the rods is a secretion of the surface epithelium which hardens after spreading around the rods. Within the horizontal matrix are trapped desquamated cells of the surface epithelium.



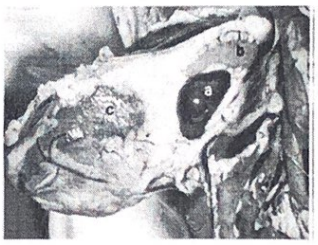


Figure 30.0: Spleen of a 6-week-old chicken; Spleen of an adult duck In all birds the dark-red spleen (a) lies on the right side of the junction between the proventriculus.

(b) and the gizzard (c). The shape varies from spherical in the chicken, to triangular in the duck, to elongated in the pigeon and canary. While having a similar function to that of mammals, the spleen does not appear to be important in storing blood.

Liver (Fig. 31.0) secretes the pigments called bile which is important for the proper absorption of fats in the S.I. Bile is conveyed to the lower end of the duodenum through the bile duct (Fig. 32.0). The duct on the right lobe of the liver is enlarged to form the gall bladder in which bile is consequently concentrated. Elaboration of bile is triggered off by the presence of food in the duodenum.

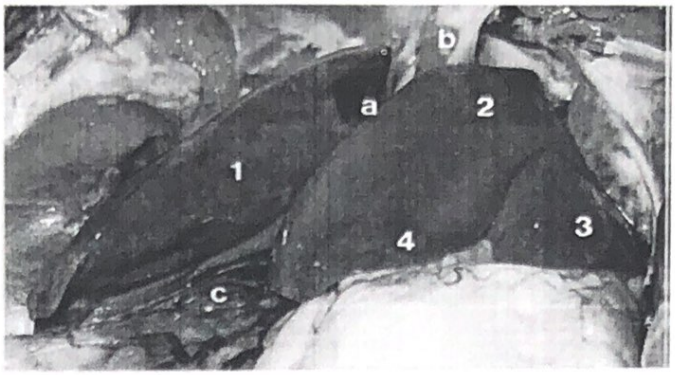


Figure 31.0: Liver of a 5-week-old chicken (ventral view) in birds the liver (a) lies in the cranial part of the thoracoabdominal cavity, its cranioventral portions surrounding the apex of the heart. It consists of right (1) and left (2) lobes, the two lobes in the chicken being almost equal in size. The left lobe in the chicken and turkey is subdivided into lateral (3) and medial (4) parts. In large birds it may be possible to palpate the liver beyond the edge of the sternum. The edges of the liver are normally thin and sharp but if the liver is enlarged they may become rounded. The colour of the liver depends on the nutritional state of the bird and while it is frequently red-brown, it may be light-brown or, if the bird is on a high-fat diet, yellow. The caudal vena cava (b) passes through the cranial part of the right lobe and receives the hepatic veins. The gall-bladder (c) lies on the visceral surface of the right lobe. In the poultry slaughter plant the gall-bladder is frequently cut by accident during post-mortem processing (a common cause of carcass contamination). The liver, but not the gall-bladder, forms part of the giblets.

The small intestine comprises two distinct parts i.e. the duodenum and the lower small intestine (Fig. 33.0). The various enzymes elaborated in the lower S.I. complete the digestive processes commenced by pancreatic enzymes. Thus the peptides are broken down to amino acids; fats to glycerides/ fatty acids and disaccharides to simple sugar for absorption.

Since there is no specialized area in the GIT of the avian species for extensive bacteria action it is important that any food fed to chicken must be readily digestible by the enzymes secreted in the GIT. The epithelia linning of the S.I. has tremendous surface area which makes rapid absorption of nutrients possible e.g. the chicken can digest/ absorb full meal in 3 hours.

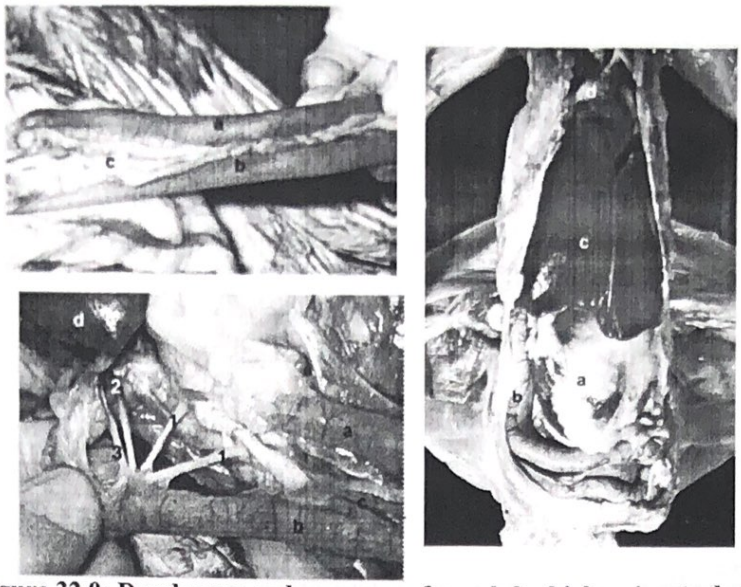
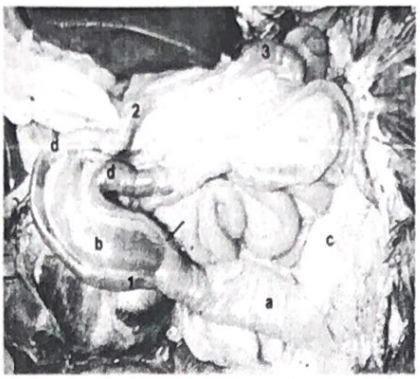


Figure 32.0: Duodenum and pancreas of an adult chicken (ventral view) the duodenum is arranged as a narrow U-shaped loop with descending (a) and ascending (b) parts, the two parts being held closely together by mesentery. The duodenal loop encloses the pancreas (c). It is always the most ventral part of the intestinal tract. Within the pale red or yellow pancreas of the chicken dorsal, ventral, third and splenic lobes have been identified; **Pancreatic and bile ducts of an adult chicken** the pancreatic (1) and bile (2, 3) ducts open into the distal part of the ascending limb of the duodenum (b). In the chicken the common hepatoenteric duct (2) drains the right and left lobes of the liver and is formed by the union of the right and left hepatic ducts on the visceral surface of the right lobe. The right hepatic duct gives off a branch which enters the gall bladder. The gall bladder is drained by the cysticoenteric duct (3). In the pigeon, which has no gall bladder there are two hepatoenteric ducts, the right duct opening into the middle of the ascending limb of the duodenum and the better developed left duct opening into the proximal part of the descending limb. The descending limb of the duodenal loop (a), pancreas (c) and liver (d) could be observed; **Thoracoabdominal cavity of adult chicken** (ventral view) gizzard (a), narrow loop of duodenum (b), liver (c) and heart .



Figure 33.0: Jejunum and ileum of a 6-week-old chicken the jejunum and ileum (a) in the chicken and turkey are relatively uncomplicated, unlike in most species of birds, and form short, garland-like coils at the edge of the long dorsal mesentery. Within the mesentery are the cranial mesenteric artery/ vein and their branches (b); the proximal loop of the small intestine is the duodenal loopn (a) which encloses the pancreas (b) succeeded by a number of jejunal loops (c).

The **caecum** (Fig. 34.0) is represented by two blind pouches found on both sides of the junction between the small intestine (S.I.) and large intestine (L.I.). It is about 10 15cm in length and it's frequently filled with faecal matters. With highly digestible rations fed to poultry the caecum has very little function in digestion. However in adult bird fed highly fibrous ration some level of digestion within the caecum is expected by the presence of numerous micro-organisms. However the usefulness of such digestive products is questionable since it is posterior to the active absorptive region of the GIT..



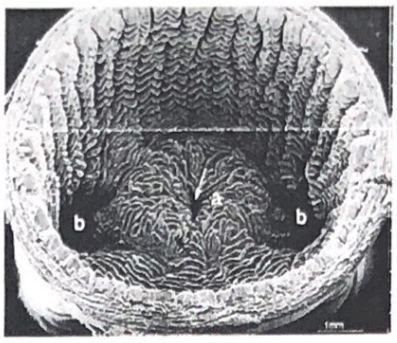


Figure 34.0: Large intestine of adult duck (ventral view) the large intestine consists of the rectum and right and left caeca. The rectum (a) extends from the ileum (b) cranially to the cloaca (c) caudally. The right and left caeca (d) arise from the lateral walls of the rectum (arrow) close to the junction with the ileum. The caeca in the duck, goose, turkey and chicken are extremely well-developed and are mostly dark-green in colour. They extend at first cranially before turning caudally to end close to the cloaca. Each caecum can be divided externally into a short proximal neck part or base (1), a long thin-walled middle part or body (2), and a short distal part or apex (3). Enlarged forms of caeca like these play a role in the bacterial fermentation of cellulose and along with the rectum are also important in water reabsorption from ureteral urine; **Scanning electron micrograph of the ileocaecal junction of adult duck (viewed from the rectum, scale 1mm)** the terminal part of the ileum, the lumen of which is indicated by a white arrow, protrudes into the rectal lumen as the ileal papilla (a). The right/ left caeca (b) open into the rectum lateral to the ileal papilla. Covering the entire intestinal tract are mucosal villi (black arrow) which act to increase the surface area of the gut available for the absorption of nutrients.

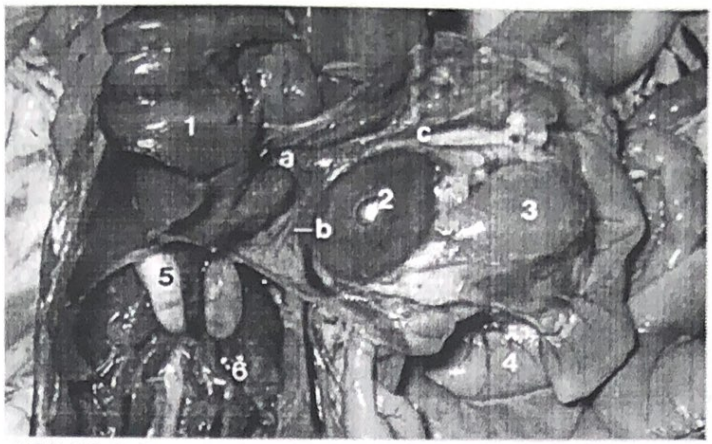


Figure 35.0: Right hepatic portal vein of a 10-week-old chicken (ventral view) the right hepatic portal vein is the largest of the two hepatic portal veins and drains the stomach, spleen and small/ large intestines. The right hepatic portal vein (a) is formed by the anastomosis of the common mesenteric vein (b) and the gastropancreaticoduodenal vein (c). The smaller left hepatic portal vein drains the region of the stomach. Liver (1), spleen (2), stomach (3), intestines (4), testes (5) and kidney (6) could be seen.

The **large intestine** in the avian species is usually short and consists of a short rectum usually about 3 4cm long which leads to the cloaca (Fig. 36.0). The cloaca is a chamber common to the digestive, urinary and reproductive passages which opens externally through a vent. The urine is discharged into this chamber and excreted with the faeces.

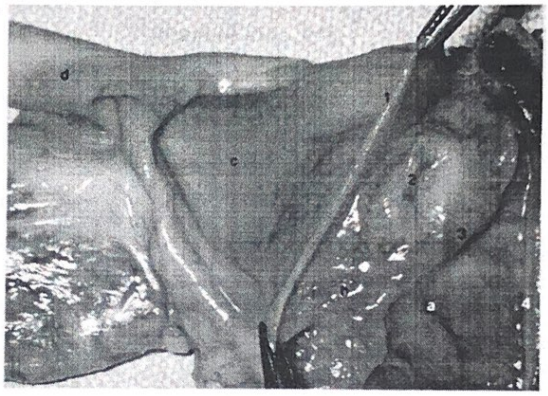


Figure 36.0: Cloaca of adult female chicken opened along the ventral midline and laid flat the cloaca consists of three compartments proctodeum (a), urodeum (b), and coprodeum (c). The coprodeum is directly continuous with the rectum (d). It is separated from the urodeum by the coprourodeal fold (1). The urodeum contains the openings of the ureters/genital ducts and in this photograph the opening of the left oviduct (2) on a slight mound can be seen. In young chickens, ducks and geese the opening is closed by a membrane.

This membrane is lost in chickens at the beginning of the first breeding season and in ducks at sexual maturity (1 11/2 years). A vestigial right oviduct is frequently seen in the chicken attached to the urodeum. The urodeum is separated from the proctodeum by the uroproctodeal fold (3). The proctodeum opens to the outside through the lips of the vent (4). If the coprodeum is full of faeces, the coprourodeal fold protrudes through the vent so that the faeces do not come into contact with the urodeum and proctodeum during defaecation.

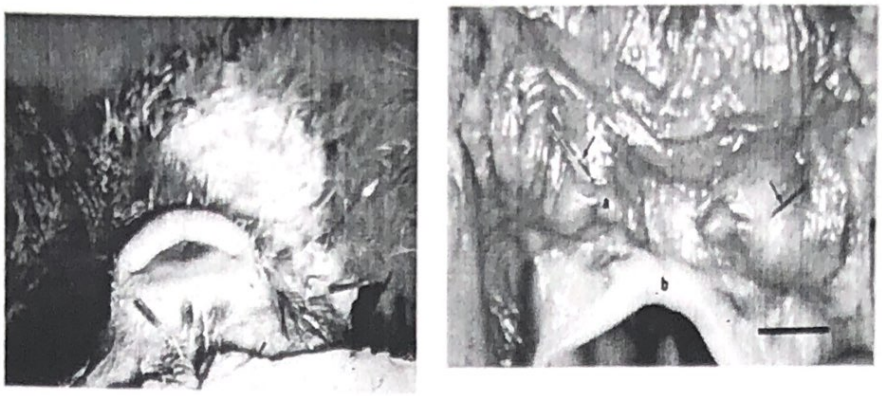


Figure 37.0: Vent of a chicken(bordered by dorsal and ventral lips which are inverted into the proctodeum, scale 5mm) - during the passage of faeces or egg the lips are everted; **Urogenital ducts and phallus (caudal view) can be seen in the second plate**. The urogenital ducts open into the urodeal compartment of the cloaca. The ureters are relatively dorsal, their position indicated by arrows. Urine deposited in the urodeum is moved by retroperistalsis into the rectum and cloaca where reabsorption/ conservation of water take place. Urine/ faeces are excreted from the cloaca together, the dark faeces being surrounded by a white ring of uric acid. The ductus deferens opens at the end of a small projection papilla (a) which is more ventrally situated. The phallus (b) lies on the crest of the ventral lip of the vent and consists of a median phallic body flanked on either side by a lateral phallic body/ lymphatic folds. The phallus is composed ofinterconnected lymphatic channels and in the detumescent state is directed towards the interior of the cloaca. To sex day-old chicks manually, the ventral lip of the vent has to be everted to expose the region of the phallus. Tumescence of the phallus is due to lymph which flows into the phallus from vascular structures in the ventrolateral walls of the urodeum called the paracloacal vascular bodies. This everts the ventral lip of the vent so that the phallus is extruded. This type of phallus is called non-intromittent because it does not enter the cloaca but is merely applied to the protruded oviduct of the female

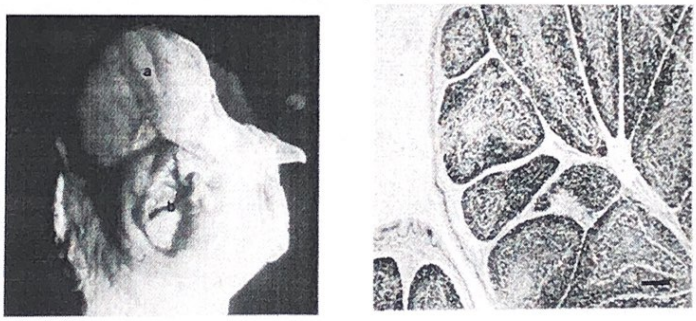


Figure 38.0: Cloacal bursa of a chicken (Cranial view) - The bursa (bursa of Fabricius) a dorsal median pear-shaped diverticulum in the wall of which is much lymphoid tissue (a) can be seen sitting on top of the rectum (b);**Light photomicrograph of the bursa**- shows the inner surface longitudinally folded with each fold containing follicles (consists of a dark outer cortex and a pale inner medulla) of lymphoid tissues separated by connective tissue. The lumen is lined by columnar epithelium. The bursa is the site of differentiation of B-lymphocytes (scale, 250μm).

Basically the degradation of carbohydrate, fats and protein are the same with chicken, swine and even horses. Swine can utilize fibre more than the chicken. Even in ruminants, apart from the complex stomach all other digestive processes are the same from the SI to LI in all the animals. In ruminants, when fibrous feeds are fed in the complex stomach, they are broken down into volatile fatty acids (VFA) which are abounded by the villi. There, odd numbers of VFA are broken down to propionic acids while the even numbered ones are broken down to ketone bodies. Swine has a larger caecum than poultry, a horse has a larger caecum than swine, and rabbit has a larger caecum than horses. The larger the caecum the more the microorganisms present and the more the animals are able to handle fibrous food.

The ruminant animal has complex stomach comprising of rumen, reticulum, omasum/ abomasums. Once the ruminant animal consume high level of fibrous substance, within the rumen/ reticulum we have high level of fibre breakdown. When protein and high carbohydrate are fed to ruminants, both the protein/ carbohydrate are converted by the microorganism to microbial protein and VFA and later degraded before they are absorbed in the GIT. High quality protein is transformed to microbial protein i.e. transformed to bacterial protein which is of low quality than the consumed protein.

RUMINANTS GASTRO-INTESTINAL TRACT AND PHYSIOLOGY

The ruminants can handle fibrous food because the GIT is adapted to handling such feedstuff. If high quality protein is fed to ruminants, it is degraded in the rumen & co by microbes into microbial protein (which is of low quality than the original protein), then converted to volatile fatty acids which will undergo the normal processes of degradation back to glucose. Therefore it is not economical to feed high quality protein to ruminants. The same thing goes for carbohydrate; it is degraded by the microbes and later to VFA's.

THE RUMINANT GIT AND FERMENTATION: The ruminant animals consume a great level of fibrous feed resources and therefore as a matter of necessity requires such feed resource to stay for a prolonged physiological processes before being broken down to utilizable forms.

The ruminant animal such as the sheep, goat and cattle are characterized mainly by the possession of complex digestive tracts which is an anatomical adaptation to their dietary habit.

The ruminant animal handles a considerable amount of fibre and therefore requires considerable and prolonged physiological processes before being broken down to utilizable forms. The various compartments of the ruminant stomach and their anatomical designation are: Rumen or the first stomach; Reticulum or the second stomach; Omasum or the third stomach; and Abomasum or the fourth stomach or true stomach or the secretory stomach.

The **rumen** and **reticulum** are contiguous (sharing a common boundary) and hence communicate freely with each other. The **reticulo rumen** i.e. the rumen and reticulum together, is by far the most extensive portion of the ruminal stomach. It is endowed with numerous muscular folds which help in mixing up of ingested food substances. It forms the region where food substance is further reduced in size by various digestive processes. The reticulo-rumen serves has an initial storage unit where food substances are initially reduced in size by various digestive processes.

The **reticulum** and **Omasum** communicate by means of the **reticulo omasal orifice** (passage). In young ruminant animal the development of an **oesophageal groove** or tube allows the liquid diet intake of the young to pass directly into the abomasums without passing through the reticulo - rumen. This is a major adaptation and as the young grows, it is withdrawn and food has to pass through the adult channels.

The **Omasum** is an oval organ which lies to the right of the reticulum. Its lumen is filled with a number of laminae. The omasum is linked with the abomasum by means of a groove called **sulcus omasi**.

The **abomasum** or true stomach is similar in several respects to the simple stomach of carnivores and it is linked to the duodenum by the **pylorus**. It must be emphasized that the matured rumen is richly supplied with a number of projections called **papillae** which gives the rumen a fur like appearance.

The **small intestine** of the ruminant animal is similar to that of the monogastric and terminates at the **ileocaecal** junction (ileum lower part of SI).

The **caecum** is a blind sac like outgrowth of the large intestine where-in some food may enter for some degree of digestion, although the function is still largely unclear.

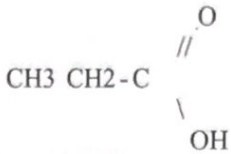
PHYSIOLOGICAL ASPECT OF RUMINATION (FERMENTATION IN THE RUMEN)

In the ruminant GIT, the rumen and reticulum mainly perform the function of storage and fermentation, while both the omasum and abomasums helps to prepare

food for gastric digestion by enhancing the removal of excess water prior to acid enzyme digestive phase in the glandular stomach.

Within the rumen, there are billions of micro-organisms whose function are not only to break-down cellulose into digestible polysaccharides but it also could synthesize a host of chemical substances which are utilizable as food by the ruminants. The main fermentation products are short chain fatty acids which include Acetic acid, C_2 (about 20% of the VFA produced), Propionic acid C_3 (about 20% of the VFA produced) and Butyric acid, C_4 (about 18%) and certain other alcohols.

The short chain VFAs are usually rapidly absorbed through the rumen wall. The VFA provide energy sources and may also be synthesized into fatty acid and ketone body. Propionates are of great importance in ruminant nutrition because the configuration



makes it highly glucogenic within the liver cells and consequently produces about 50% of glucose which enter the ruminant metabolic system. Butyrates are also absorbed but they are largely ketogenic and therefore less useful nutritionally as propionates. The formation of the butter fat of milk also takes this process. Cows and most ruminants are endowed with the ability to synthesize protein from fatty acids because of the symbiotic activities of the rumen microorganisms. This represents a marked contrast to the metabolism of non ruminant mammals that lacks such specialization. It must be emphasized that the ability to efficiently handle dietary fibres is not found in the young ruminant animal at birth, thus a young calf feeding on the mother's milk metabolizes food in much the same way as the monogastric animal until it is about 4months of age when the rumen would have developed sufficiently to assume its full nutritional responsibility.

While several advantages exist in the ruminant digestive system as far as food is concerned, the process of fermentation generates so much gas which must be eliminated through belching. Any interference with the mechanism of elimination of gases produce a syndrome in cattle called **bloat**. This is a disease condition which is usually associated with cattle grazing on lush legume pasture. Some of the method of reducing the incidence of bloat is the use of oil or the introduction of canula i.e. a tube introduced into the digestive tract. Another problem with ruminant metabolism is the formation of ketone bodies which is a specific disease of ruminants. The formation of ketone bodies brings about a disease condition known as **ketosis**. Ketosis is believed to be as a result of the inability of the liver to detoxify or metabolize short-chain fatty-acids in the blood-stream due to increased stress arising from lactation, pregnancy or other factors.

CHAPTER SEVEN

ENVIRONMENTAL PHYSIOLOGY

Environment is the physical, social and cultural factors and conditions influencing the existence or development of an organism or assemblage of organisms. The act of surrounding, or the state of being surrounded. It has also been defined as all of the surrounding things, conditions and influences affecting the growth or development of living things. These definitions refers to two characteristics

1. Surroundings the physical aspects of an animal's surroundings with such considerations as temperature, photoperiod, ventilation, floor and space allowance. Studies are done on their relationship to their effect on animal comforts and well-being rather than simply productivity.
2. Conditions of life or growth this include human factors which though not completely absent in the first characteristics (surroundings) achieves a particularly special status under the second definition in that the farmers actions, rather than mere presence, have a profound effect on the animal at all stages of life, influencing both its physical and mental well being. The human factor is increasingly seen as being of major significance in intensive husbandry systems, where physical aspects of the environment have been carefully considered and the optimum provided for the animals. Phenotypic variations in economic traits due to environment are also of great importance. Environment includes all such factors as disease, nutrient supply, temperature effects, accidents and others which the individual may encounter from the time of conception until its death.

Phenotypic variations due to environment are important because

a. They are not transmitted from parents to their offsprings
b. They overshadow variations due to heredity
c. The proper environment is necessary for an individual to reach its genetic potential
d. Rapid improvements can be made in the efficiency of livestock production by supplying uniform and superior environmental conditions to breeding animals and those used for commercial production.

STRESS

In psychology, a demand, threat, or other event that requires an individual to cope with a changed situation. Stresses are an unavoidable part of life. They range in severity from relatively mild forms, such as a traffic jam, trekking long distance, under-feeding, a disparaging remark from one's employer, or a family argument, to severe ones such as the death of a spouse or the fear of dying in battle, birth stress,

management stress, environmental draw-back, etc. People's responses to stress vary widely depending on their cultural and family background, their personal experiences, their mood at the time, and on other stresses present at the same time. Most people are able to cope satisfactorily with the everyday stresses of living. However, when problems escalate faster than they can be solved, a person's adaptive capacity may be overloaded and chronic illness, anxiety, or depression may result.

In 1926, Hans Selye, a Canadian scientist who pioneered in stress research, began investigating the effects of stress on the body. Selye had noticed that people showed characteristics of fatigue and general discomfort following either a physical or an emotional stress. He proposed that the response to stress involves three possible stages:

The first stage, called the **alarm reaction**, is associated with the secretion of epinephrine (adrenalin) by the adrenal glands. It involves complex physiological mechanisms that increase general alertness and arousal to prepare the organisms for "fight" or "flight". During the alarm reaction, pulse and respiratory rates speed up, blood pressure rises.

In the **resistancestage,** the pituitary gland (Fig. 39.0) produces hormonal and neural changes throughout the body until the stressful situation has passed. These changes include an increase in strength and energy and heightened body defenses. Long continued severe stress leads to the **exhaustionstage**, in which a person's physical condition may deteriorate to the point where death from shock, heart failure, or other disorders ensues.

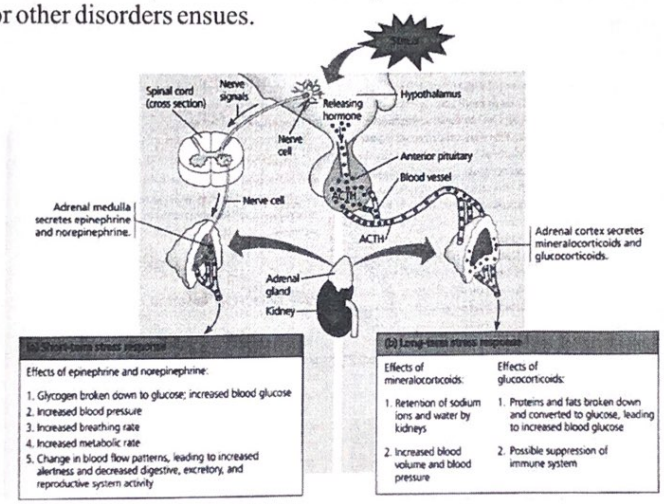


Figure 39.0: Stress and the adrenal gland

STRESS AND STRESSOR: Stress is theexperience of adversity or of events largely beyond ones control. Distress, the overpowering pressure of some adverse force or influence, chiefly stress of weather. A condition of things compelling or characterized by strained effort. It can cause depression or anxiety in vulnerable individual. It is also widely believed to cause physical illnesses like high blood pressure, heart attacks, and strokes (a sudden motor or sensory disorder, resulting from loss of blood flow to a region of the brain and usually occurring when a small clot blocks off a blood vessel or the vessel itself bursts. If this happens on the right side of the brain the left side of the body is affected. Speech is also affected if it occurs on the left side of the brain. The age of the patient and the severity of the stroke are among the factors determining the degree of recovery. The effect of climatic stress on the development and survival of an animal is influenced by the breed or species of animal.

CLIMATE: This is the average weather condition of an area for a long period of time. It is made up of some physiological factors such as temperature, humidity, light, wind- speed, etc. It is a composite phenomenon usually taken for granted but exerting vast influences on almost everything which occurs in nature. Climatic stresses include:

1. High environmental temperature
2. High humidity
3. Solar radiation
4. Short daylength, and
5. Draught.

Temperature: The mammal controls body temperature within a narrow limit **homeothermy.** A deviation in body temperature evokes behavioural adjustments to correct such deviations.

Degree of Stress	Hypothermia	Hyperthermia
Mild	Hyperphagia (increased feed intake), Shivering, Rate, Group huddling, Body flexure, Increased motor activities.	Anorexia, Increased water consumption, Increased dispersion, Body extension, Dispersion, motor activities. motor activities.
Moderate	Decreased general activity, Reduced mental alertness, Extreme body flexure	Lethargy and lassitude, Panting and gasping, Extreme body spreading.
Severe	Depressed respiration, Cessation of shivering, Depressed metabolism, Death.	Weakness and stupor, Posture imbalance "staggers", Convulsions,

Humidity: is the amount of water in the atmosphere. The response of an animal to high environmental temperature depends to a large extent on the moisture content of the surrounding air. Since a large part of the body heat is dissipated by evaporation of water from the surface of the skin and oral mucosa, the absolute water load already present in the air often determines the course of adjustments open to the animal.

Humidity augments the effects of increasing heat load. Sweating alone does not effect cooling to the animal but evaporation from the skin surface has additional cooling effect. Therefore, heat stress is a composite effect of temperature and humidity. However, it is known that the degree to which the temperature and relative humidity affect heat stress depends on the animal and this is apparently related to the animal's ability to sweat. Pigs have no sweat glands and thus can only expel sweat through the respiratory tract by panting with a loss of much water vapour. In buildings for pigs, ventilation is necessary and wallows provide means of effective artificial sweating through sticky and porous mud layer which acts like clay pots in admitting air to cool the animal's body. Thus in wallowing, the water that sticks to the pig's body evaporates and gives cooling effect to the animal.

In order to reduce the climatic variable affecting production and performance such as growth, egg production, reproduction, milk production, feed conversion, etc. It is important to know the exact climatic factors responsible for heat stress. The physiologically effective temperature is more critical to the comfort of the animals than the actual temperature. It depends on the sweating ability of the animal.
In man, the dry bulb thermometer contributes about 15% while the wet bulb contributes the rest of the relative humidity (RH) (Lind, 1969). With cattle 35% was found for dry bulb and 65% for wet bulb. In pigs 65% dry bulb and 35% wet bulb (Lingram, 1965). For poultry, dry bulb effect is 64% and wet bulb is 36% (Egbunike, 1978). Sweating dwindles the importance of dry bulb in determining heat stress, but animals with higher capacity to sweat are more influenced by wet bulb.
Different animals have different critical temperature e.g. pigs when young are very susceptible to cold, pneumonia, etc. Therefore external heat source may be provided. As the pig grows, it readjusts. It should be noted that young pigs are not thermogenic and have no layer of fats whereas adult pigs are highly susceptible to heat.

EFFECT OF HIGH TEMPERATURE ON ANIMALS

1. Feed consumption: Animals tends to consume less feed and consequently growth rate is reduced.
2. Male animals experience a decrease or depression in spermatogenesis and poor seminal quality. In extreme cases, there may be cessation of spermatogenesis depending on the degree of the high temperature.

There is a temperature gradient between the environment of the testis and the blood temperature by about 2 - 7°C, thus mammals have their genitals outside while non-mammals have theirs inside.

Puberty: Because of reduced feed intake and increase in hormonal dysfunction, the endocrine system does not function properly. There is a decrease in libido in males. In the female, there is also delayed puberty, reduced ovulation and on top of this, there is a high proportion of abnormal eggs with shrunken cytoplasm or ruptured membrane. The sum total of reduced spermatogenesis, poor semen quality, etc will result in reduced conception rate and fertility rate. Even when fertilization occurs, there is an increase in embryo mortality or abortion, foetal resorption, foetal malformation and reduced litter size. High temperature also results in incidence of silent heat and short oestrus.

Light: It has been shown that while the rate of the spermatogenic cycle is unaffected by photoperiod, the quantity that normally mature is affected e.g. in rams, a 12 hour daily illumination provides optimum stimulus of spermatogenesis and a constant photoperiod results in reduced spermatogenesis. In pigs, it was observed that the daily sperm production was highest in March and April; the photoperiod increases very fast, while between Octobers to January, it is decreasing. It is not actually the length of time that affects the above parameters but the "change" from a minimum level to a maximum level. We have minimal problem with photoperiod in the tropics with about 11 hours of darkness and 13 hours daylight. In temperate region such as Sweden which is dark for only 1hour photoperiod is of effect. It is not the length of light but its effects on the animal or the change in photoperiod i.e. the break in photoperiod is what triggers the animal back to physiological activities. Therefore, photoperiod manipulation can be used to increase egg and milk production.

ADAPTABILITY OF LIVESTOCK TO CLIMATIC STRESS

The ability of livestock to adjust and accommodate a stressful condition and thereby survive in it is termed **adaptability.** Adaptability varies between species and between individual stocks belonging to the same species. It varies also with age and health of stock, level of nutrition and other management conditions.

Acclamatization: When a stock adapts well to a stressful environment and remains there perpetually unharmed, the stock is said to have **acclimatized**. Acclamatization is therefore, according to Sastry and Thomas (1976), **is a long term adaptive physiological adjustment which results in an increased tolerance to continuous or repeated exposure to environmental stress**.

HOMEOSTASIS: A well adapted stock does not manifest significant loss of health or productivity, the condition whereby the animals' internal body processes are kept constant in spite of the stress is termed **Homeostasis**. The physiological process of maintaining this constancy is called **Homeostatic process**. Homeostasis involves constancy of the following body processes:

1. Body weight
2. Body temperature
3. Blood pressure/ chemical composition
4. Respiratory/ pulse rate
5. Water balance/ composition.

Homeostatic process involves

a. Sensing of stress by the bodies sensory receptors at the sense organs skin, nose, eye, ears and tongue.
b. Transmission of the perceived stress to the hypothalamus of the brain and to the central nervous system (CNS).
c. Immediate response takes place which are behavioural, respiratory and cardiovascular.
d. Delayed responses later follows, which are hormonal, enzymatic and metabolic i.e. essential hormones are produced to counteract the effect of the stress, enzymes can be secreted for digestive purposes especially under cold conditions. Metabolic activity may increase or reduce; causing panting for evaporative cooling, increased water intake, etc.
e. Homeostasis is eventually maintained in the body which includes energy balance, circulation balance and biochemical balance.

EFFECT OF CLIMATIC STRESS ON LIVESTOCK

Direct effect of climatic stress on livestock:

1. High ambient temperature
2. High humidity
3. Solar radiation
4. Short day length
5. Draught

1. High ambient temperature

i. Rise in rectal temperature and consequent discomfort, heat prostration and possibly heat stroke.
ii. Lack of appetite and consequent reduction in feed intake.
iii. Increase in water intake.
iv. Decline in productivity and quality of products like milk, eggs, meat, skin and wool.
v. Low spermatogenesis, fertility and libido, especially in bulls, boars, rams and bucks, etc.
vi. High mortality.

2. *High humidity:* This has adverse effect directly on livestock only when the ambient temperature is also high. Under such condition, evaporative heat loss is reduced because of the high environmental humidity or high relative humidity. Feed intake is consequently reduced and productivity falls.

3. *Solar radiation*: Direct effect of solar radiation on stock, heats them up, thereby producing problems similar to those under high ambient temperature (surrounding). In addition, solar radiation can irritate the skin, causing sur burns, skin cancer and other photo-sensitive disorders. Stocks with light skin or coat colour having glossy texture are less affected by solar radiation than those with dark/ coarse skin or dark coat colour or sparse coat covering. Standing animals absorb less solar radiation than those lying down (because of surface area exposed)
4. *Short daylength:* Photo-periodism controls initiation of breeding cycle in some livestock like sheep. It controls onset of oestrus. Short daylength can delay oestrus.
5. *Draught:* Fast moving air on exposed stock can cause cold and consequently increase in feed consumption to generate extra body heat in combating the cold Draught causes discomfort to stock and can cause mortality of young stock.

Indirect effect of climatic stress on livestock

1. Dry season causes low productivity of grasses for grazing animals, as well as insufficient grains for monogastrics.
2. The available herbage at this period is usually coarse, less nutritive and less palatable.
3. Bush- burning is rampant during the dry season, causing destruction of the sparse vegetation and sometimes livestock housing.
4. Drinking water is usually scarce during the dry season as many wells, streams are dry.
5. During the rains, hay- making is difficult; humidity is usually high, causing mould growth on feeds/ equipment.
6. Ecto and endoparasites are on the increase during rains as well as fungi, bacteria diseases.
7. Draught capable of causing mortality is common during the raining season Sometimes, flooding is experienced.

STRESS AND METABOLIC DISORDER: When an animal is stressed the carbohydrate contained in the body will be used in generating energy to combat the stress factor (stressor) giving rise to excess carbondioxide production which is toxic to the body. It also leads to conversion of protein to carbohydrate which results in the production of lactic acid which is also toxic to the body tissue. Since protein is an essential component of the immune system, the immune system is therefore reduced. Also since protein is necessary for tissue maintenance, the body tissue will be broken down.

WATER BALANCE: The animal body is made up of protein, fats, minerals, water and a little amount of carbohydrate. Water makes the major component of the body.

It is essential for the chemical processes of the body such as digestion and secretion of saliva. Water constitutes 70 -75% of the body mass in domestic animals, 30-80% in pigs. In cattle, water concentration decreases from 95% in early embryonic stage to between 40-60% at maturity. The total amount of water in a non-herbivorous animal of 70kg body mass is approximately 50litres. A fat animal has lower body water content than lean animals. The body fluid can be divided into three compartments **extracellular, transcellular** and **intracellular fluids**.
Extracellular fluid or intercellular fluid (fluids between cells). Extracellular fluids include all the fluid lying outside the cell of the body such as plasma, interstitial fluid, lymph, gastrointestinal fluid and cerebrospinal fluid (CSF). It forms about 26% of the body mass. It can be divided into two major categories Plasma and interstitial fluids.

a. Plasma: This forms about 5% of the body mass and it lies within the vascular system. When a sample of blood is treated to prevent clotting using an anticoagulant such as EDTA (Ethylene diaminetetraacetic acid) and permitted to stand undisturbed, the cells gradually settle to the bottom of the container leaving a straw coloured fluid above it. This fluid portion is called plasma. It is referred to as the internal environment, it directly or indirectly bathe all cells of the body and protects them from external injury. Plasma is made up of 92% water and 8% of other substances.
b. Interstitial fluid: This forms about 15% of the body mass and lies in the spaces within or between cells of the body mass. It is outside the vascular system. However, plasma is constantly mixing with the interstitial fluid through tiny pores or holes known as **tenestrae** (membrane window). In the capillaries of the blood vessels, the colloidal osmotic pressure exerted by plasma protein tends to retain water in the capillary.

Transcellular - These are smaller fluid compartment that are usually included as extracellular fluid although they are separated from the main body of the extracellular fluid. They are referred to as special fluids because they are found in special compartment e.g. cerebrospinal fluid (brain), aqueous humour fluid (eyes), synovial fluid (joints), urine/ water of the alimentary canal (gastrointestinal fluid) and serous fluid in the peritoneal (stomach), the pleural (lung) and pericardial (heart) cavities.

Intracellular It includes all the water within the cells of the body. It is considered as one large fluid compartment although it is an aggregation of billions of tiny compartment. The intracellular fluid compartment is about 50% of body mass.

Maintenance of intracellular fluid
Homeostasis is defined as the regulation or maintenance of a fairly constant internal environment in an organism.

All living organisms must be able to maintain a steady internal environment for healthy growth and efficient functioning of body cells. A living organism must be able to adjust to any change in the physical and chemical conditions of its body fluids. That is the tendency for internal variables such as body temperature, respiratory rate, pH (hydrogen ion concentration), water content, osmotic pressure, concentration of dissolved substances in body fluid (solute concentration) such as carbon dioxide, oxygen, urea, food substances like glucose, amino acids and mineral ion such as sodium, potassium, chloride etc. must all remain at a controlled level. The internal environment consists of the fluid that surrounds the cells. The composition and condition of which the animal can regulate within narrow and precise limit. The extracellular constituent requires maintenance at uniform and stable level since cells function at their optimum in an environment where homeostasis is maintained.

BODY PARTS INVOLVED IN HOMEOSTASIS

The parts of the body involved in homeostasis are:

a. Kidney
b. Liver
c. Skin
d. Ductless glands (hormones)
e. Brain (brain has the overall control of the homeostatic processes of the body).

a. THE KIDNEY They are a pair of reddish brown bean-shaped organs located at the posterior end of the abdomen, with the left kidney being nearer the diaphragm than the right kidney. It is made up of three parts the outer region (Cortex) containing pairs of the nephron's Bowman's Capsules; the inner region (Medulla) containing the U-tubes (loop of Henle)/ the connecting tubule; and the pelvis the point where urine leaves the kidney and the pyramids open into the pelvis.

The kidney performs a number of functions which include:

- Osmoregulation: The process whereby an animal regulates the balance between water and salts in its body fluids as the body does not have room for excess or shortage of water/ salt in body fluid. When the osmotic concentration of blood is higher than that of the cell content due to high sugar, mineral salts, amino acids/ other plasma solutes, the kidneys extract these substances from the blood or body cells and absorb more water. The presence of these substances even in small amount in the blood creates higher osmotic pressure in body cells thus decreasing the osmotic concentration of the blood and the kidneys extract more water than usual. By the action of Anti-Diuretic Hormone (ADH), more concentrated urine is produced thus keeping the osmotic concentration of blood and cells fairly constant.

Osmoregulation through the loss of water is also carried out by the skin though sweating and by the lung through expiration or exhalation of air/ water.

- Excretion: This is the removal of metabolic waste products from the body in form of urine. The process of urine formation occurs in three phases ultra-filtration, selective reabsorption and hormonal secretion.

  Ultrafiltration The blood brought to the kidney by the renal artery circulates through the glomerulus capillaries of each Bowman's capsule where water, urea, nitrogenous compounds, mineral salts, sugar, glucose and plasma solutes are filtered into the capsule. This process of filtering materials from the glomerulus into the Bowman's capsule is called **ultra-filtration**.

Selective reabsorption - As the fluid in the capsule or glomerular filtrate flows down the tubule, some water, sugar, amino acids and salts that are useful to the body are reabsorbed into the blood capillaries against concentration gradient by active transport at the proximal convoluted tubule and Henle's loop. A process known as selective reabsorption.

Hormonal secretion -The fluid in the tubule gradually becomes more concentrated as it flows through the distal tubule where more water is reabsorbed by the action of Anti-diuretic hormone (ADH) and urine is finally formed. The urine gradually trickles into the renal pelvis and by peristalsis through the ureter into the urinary bladder which later contract when full, discharging the urine out of the body through the urethra.

- Maintenance of acid-base balance: The kidney also performs the function of maintaining the acid-base balance in the body. When the concentration of acids becomes more than the concentration of bases, the kidney helps in excreting more acid with the urine while at the same time prevent the loss of bases from body fluid. If on the other hand, the concentration of a base becomes higher, more salts are excreted with the urine.

Kidney function can be affected by various conditions or factors such as environmental temperature, diseases, drug intake, habitats, etc.

Environmental temperature affects the normal functioning of the kidneys. There is tendency to urinate more/ sweat less on cold days while less urination/ more sweating is done on hot days, hence more of the excess water in the body is lost through the kidneys on cold days. This condition results in the kidneys being under stress since they have to remove excess water, salts and other waste products from the body.

Disease is a change or deviation from normality. Diseases such as nephritis, kidney stones, diuresis, dropsy, etc can affect normal functioning of the kidneys. Diuresis is a condition in which the cells of the kidney tubules are not reabsorbing water from the glomerular filtrate resulting in large amount of water being passed out in urine (watery urine).

It could also result from a patient suffering from diabetes insipidus (a condition due to reduced production of Anti-diuretic hormone, ADH) resulting in reabsorption of less from urine and the patient produces a lot of urine i.e. about 5-20 litres/day. It leads to loss of weight, emaciation and a high thirst for a lot of water. It could also occur as a result of shock or accident. It is corrected by surgery or administration of drugs such as diuretics. Nephritis (inflammation of the nephron) is a condition in which the blood vessels in the Bowman's capsule (glomerular) becomes inflamed and porous leading to its inability to carry out the function of ultra-filtration completely. It results in the passage of most of the useful materials in the blood fluid with urine. It could be caused by bacteria infection. It leads to inflammation of kidney, presence of amino acids in urine (proteinurea), weakness of body, pains and fever etc. It could be corrected by use of antibiotics, dialysis (use of artificial kidney) or kidney transplant. Kidney Stones is a condition caused by one abnormality or the other resulting in amino acids and calcium phosphate settling in the kidney to form kidney stone, which may block urine passage making the removal of urine extremely difficult, thus reducing the free flow of urine. It leads to severe abdominal pain, painful urination, high blood pressure, fever, chills and blood in urine. It could be corrected by surgery (nephrectomy) to open up the kidney to remove the stones and patient should take in more water/ avoid excessive intake of calcium/ protein. Oedema or Dropsy is a condition in which the cells of the Bowman's capsules are unable to absorb water from the blood in the tubules resulting in the retension of a lot of water in the blood or tissue. It leads to swelling of the face and ankles (joints), abdominal pains, constant weakness or fatigue and sluggishness. It could be corrected by reduced intake of water, kidney transplant if critical, etc.

Intake of drugs affects the normal functioning of the kidneys. Drugs such as diuretics which are taken to get rid of excess water out of the body are among the essential drugs that affects the kidneys.
of drugs affects the normal functioning of the kidneys. Drugs such as diuretics which are taken to get rid of excess water out of the body are among the essential drugs that affects the kidneys.

Habitat is any environment in which an organism lives naturally. Its an area where physical and chemical constituents required by a particular organism are met. There are two main types of habitat aquatic (marine, estuarine and fresh water) and terrestrial (marsh, rainforest, grassland and arid land) each with different characteristic features. Animals in the fresh water habitat lives in an environment that is **hypotonic** to their body fluid i.e. body fluid is more concentrated than its water environment, leading to a constant flow of water into their body. Fresh water animals are able to solve this problem of excessive intake of water by excreting salts/ other nitrogenous wastes into the urine resulting in a normal salt/ water balance in the body.

The marine animal lives in an environment that is **hypertonic** to their body fluid i.e. the body fluid is less concentrated than its water environment, resulting in a constant flow or loss of body water to the surrounding saline water. Marine animals solve this excessive loss of water by either the reabsorption of urea back into the blood resulting in an increased body salts and high concentration of body fluid or by drinking plenty of salt water to increase the concentration of blood fluid.

b. THE LIVER This is the largest organ in the body of a mammal with an average weight of about 1.25kg. It is dark-red coloured, comprises of two big lobes and three smaller ones, located on the right side of the abdomen below the diaphragm and partly covers the stomach. The liver secretes the bile which is stored in the gall bladder located on one of the lobe of the liver and connected to the duodenum by a short tube called bile duct. The function of the liver in the body include the following

- It regulates blood sugar by converting excess glucose into glycogen (animal starch) under the influence of the hormone **insulin**. It also stores excess glucose. Conversely, when the level of blood glucose is low, glycogen is converted back to glucose under the influence of another hormone called **glucagon. The pancreatic islets secrete at least 4 hormones insulin, glucagon, somatostatin, and pancreatic polypeptide.** Thehormones are released into the pancreatic vein, which empties into the portal vein a convenient arrangement, since the liver is the primary site of action of insulin and glucagon which are chiefly involved in regulating carbohydrate metabolism, though affect many other processes. Somatostatin, first identified in the hypothalamus as the hormone that inhibits growth hormone secretion, is present in higher concentration in the pancreatic islets than in the hypothalamus and is involved in the local regulation of insulin and glucagon secretion. Pancreatic polypeptide affects gastrointestinal secretion.
- It helps in the regulation of blood protein as the body cannot store excess amino acids in the body. The liver deaminates excess amino acids. Deamination is the process by which excess amino acids are splitted into its two components the amino group (RNH_2) and the carboxyl group (COOH).
- The toxic amino group is converted into an harmless urea and excreted while the carboxyl group is processed into carbohydrates and stored in the liver as glycogen.
- It helps in detoxication by converting poisonous/ toxic materials such as drugs, alcohol etc in the body into harmless substances that are later excreted.
- It helps in the synthesis of bile which aids the emulsification of fat to enhance its digestion/ absorption.
- It helps in the breakdown of worn-out red blood cells (erythrocytes) in adult.
- It stores iron obtained from the breakdown of red blood corpuscles.
- It helps in the formation of red blood cells in embryo, although RBC are later formed in the marrow of the long bones in adult.

- The liver manufactures most of the essential blood proteins such as fibrinogen, prothrombin, globulins, etc which play important role in blood clotting.
- It helps in the regulation of lipids. The liver removes lipids from the blood, converts them into glucose and oxidizes them to release energy during aerobic respiration.
- Helps in the storage of vitamins. The liver manufactures vitamin A from carotene and stores fat soluble vitamins A, B_{12} and D.
- It acts as the reservoir of blood as it contains many capillaries and blood spaces with a large volume of blood.
- It helps in the production of heat as the activities of the liver generate heat which Helps to maintain the body temperature.

Diseases that can impair the functioning of the liver include diabetes mellitus, liver cirrhosis, gall stones, infective hepatitis and cancer of the liver. Diabetes mellitus is a disease caused by the inability of the liver cells to convert excess glucose in the blood to glycogen due to insufficient action of insulin, owing either to its absence or to resistance to its action. Glucagon, acting unopposed, aggravates this condition. Consequently, excess sugar is left in the blood and lost through urine. Diabetes mellitus leads to continuous dehydration, exhaustion or muscular weakness, blur vision and loss of weight. It could be controlled by regular dose of intravenous insulin injection throughout life, use of drugs like Diabinese and avoidance of carbohydrate food. Liver cirrhosis is a condition in which damaged liver cells become replaced by dead/ useless fibrous tissue which makes the liver very firm/ irregular as a result of prolonged use of excess alcohol and hepatitis. It could lead to loss of appetite, general body weakness and death. It is controlled by avoidance of alcohol, surgery and liver transplant. Gall stones are hard objects formed in the bile duct as a result of the precipitation of cholesterol and block the passage of the bile. The blockage leads to obstructive jaundice with the skin acquiring a characteristically yellow appearance due to retention of bile pigment (bilirubin) in the blood. It leads to inability to digest fats/ oil properly, yellowing of skin/ eyes and general body weakness. It could be controlled by drug administration, dissolving of stones by oral intake of bile acid and by surgery to remove the stones. Hepatitis is an inflammation of the liver which may be due to viral infection or toxic substances (poison, drugs, etc) which makes the liver unable to store or reconvert already stored glycogen into glucose. It leads to loss of appetite (anorexia), severe headache/ nausea, yellow colouring of eyes/ urine and general body weakness. It could be controlled by the administration of drugs, taking of special diet and avoidance of alcoholic drinks. Cancer of the liver is an uncontrollable or abnormal swelling of liver cells (hepatocytes) which obstruct normal tissue function. It leads to exhaustion, general body weakness and death. It could be controlled by early surgery, chemotherapy or use of radiation and liver transplant.

c. THE SKIN Mammals skin is the largest organ of the body covering the entire body surface and it's the toughest body tissue apart from bones and cartilage. It is made up of two main layers the **epidermis** and the **dermis**, and in some divisions the **hypodermis**
(Fig. 40.0).

- The Epidermis is the outer layer of skin made up of three layers cornified (horny) layer, granular layer and malpighian layer. The cornified layer is the outermost layer of the epidermis made up of flat, hard, dead and scaly cells. It is impregnated with the protein keratin which makes it tough, flexible and waterproofed. Cornified layer cells vary in thickness from one part of the body to another and are constantly being worn-out and replaced by cells from the layers below. They are usually very thick on the soles of the feet and palms, and thin on the eyelids. The granular layer consists of living cells produced by the malpighian layer beneath which continue to move up towards the skin surface and in the process becomes greatly flattened, accumulate keratin and eventually dies. Malpighian (Germinative) layer is made up of actively dividing cells that gets its nutrients/ oxygen by diffusion from the dermis blood supply capillaries. It contains two pigments - **melanin** (gives the skin its colour) and **keratin** (gives the skin its toughness and flexibility). This layer has many infoldings which penetrate deep into the dermis to form hair follicles and sebaceous glands.
- Dermis is made up of connective tissues containing blood capillaries, hair follicles, sweat glands, sebaceous glands, sensory nerve endings and fat cells. The blood capillaries bring nutrients/ oxygen to the dermal and epidermal cells and remove waste as well as supply the hair follicles and sweat glands capillary networks. The hair follicle is a deep pit formed by the infolding of the malpighian layer which keeps dividing at the base of each pit to produce a long cylindrical hair. The nerve endings in the follicle respond when the hair is touched. A hair erector muscle is attached to each follicle whose contractionpulls the hair to an upright position and makes it to stand erect e.g. when cold, the hair erector muscles contract giving the skin 'goose pimples'. Sebaceous glands secrete an oily substance (sebum) into the hair follicles to keep the hair and epidermis waterproof/ supple and also keep out dust/ micro-organisms. Sweat glands are found in the dermis as coiled tubes which continue as sweat ducts and opens at the skin surface as sweat pores. Cells of the coiled tubes absorb water containing mineral salts and urea waste (sweat) from the capillaries surrounding them and secrete them into the tube. When sweat reaches the surface of the skin and evaporates, it cools the body. Below the dermis is a layer of connective tissue which contains variable number of fat cells (subcutaneous fatty or adipose tissue) which acts as a long term food store and an insulating layer to prevent loss of heat from the body.

The skin, being also a sense organ contains sensory cells which can receive stimuli from external surroundings and send them to the appropriate parts of the body for interpretation and action.

Types of sensory receptors: a sensory cell typically has a single type of receptor specific for a particular stimulus, such as light or cold. Distinct cells and receptors are responsible for particular qualities of a sensation, such as distinguishing red from blue. Sensory receptors can be classified into five categories based on the nature of the stimuli they transduce: mechanoreceptors, chemoreceptors, electromagnetic receptors, thermoreceptors and pain receptors. Mechanoreceptors sense physical deformation caused by forms of mechanical energy such as pressure, touch, stretch, motion, and sound. It consists of ion channels that are linked to structures (stretch receptors) that extend outside the cells such as hairs, cilia, tentacles, etc. as well as internal cell structures, such as the cytoskeleton. Bending or stretching of the external structure generates tension that alters the permeability of the ion channels which alters the membrane potential, resulting in a depolarization or hyperpolarization. When the muscle is stretched, the spindle fibres are stretched, depolarizing sensory neurons and triggering action potentials that are transmitted to the spinal cord e.g. knee-jerk reflex. Mammalian sense of touch also relies on mechanoreceptors that are the dendrites of sensory neurons. Touch receptors are often embedded in layers of connective tissue whose structure and location often affect the type of mechanical energy (light touch, vibration, or strong pressure) that best stimulates them. Receptors that detect a light touch or vibration are close to the surface of the skin; they transduce very slight inputs of mechanical energy into receptor potentials. Receptors that respond to stronger pressure and vibrations are in deep skin layers. Other receptors sense the movement of hairs e.g. cats and many rodents have extremely sensitive mechanoreceptors at the base of their whiskers. Because deflection of different whiskers triggers action potentials that reach different cells in the brain, an animal's whiskers provide detailed information about nearby objects.

Chemoreceptors include both generals (those that transmit information about total solute concentration) and specific receptors (those that respond to individual kinds of molecules). E.g. Osmoreceptors in the mammalian brain are general receptors that detect changes in the total solute concentration of the blood and stimulate thirst when osmolarity increases. Most mammals also have receptors for specific molecules, including glucose, oxygen, carbon dioxide, and amino acids. Two of the most sensitive/ specific chemoreceptors known are found in the antennae of the male silkworm moth; they detect the two chemical components of the female moth sex pheromone. The stimulus molecule binds to the specific chemoreceptor on the membrane of the sensory cell and initiates changes in ion permeability.

Electromagnetic receptors detect various forms of electromagnetic energy such as visible light, electricity, and magnetism. Photoreceptors, electromagnetic receptors that detect energy in the form of light, are often organized into eyes.

Some snakes have very sensitive infrared receptors that detect the body heat of prey. Some fishes generate electrical currents and use electroreceptors to locate objects, such as prey, that disturb those currents. The iron-containing mineral magnetite is found in the skulls of many vertebrates such as salmon, pigeons, sea turtles and humans, in the abdomen of bees, in the teeth of some mollusks etc that orient to Earth's magnetic field.

Thermoreceptors (detect heat and cold) is located in the skin and anterior hypothalamus cells where they send information to the body's thermostat located in the posterior hypothalamus. Mammals have different kind of thermoreceptors that are specific for a particular temperature range. Exposing sensory neurons to capsaicin (in cayenne pepper) triggers an influx of calcium ions whose receptor respond in similar way to high temperatures (42°C or higher) hence spicy foods are described as 'hot'. The capsaicin receptor and at least five other types of thermoreceptors belong to the transcient receptor potential (TRP) family of ion channel proteins. TRP type receptor specific for temperatures below 28°C can be activated by menthol, a plant product that is perceived to have a "cool" flavor.

Pain receptors extreme pressure or temperature, and certain chemicals can cause damage to animal tissues. Detection of stimuli that reflect such harmful conditions in animals is dependent on pain receptors (**nociceptors** Latin nocere means to hurt) which trigger defensive reactions such as withdrawal from the source of danger. Certain naked dendrites detect noxious thermal, mechanical, or chemical stimuli just like the capsaicin receptor which acts as a thermoreceptor, is also a nociceptor. Although, nociceptor density is highest in skin, some pain receptors are associated with other organs. Chemicals produced in an animal's body sometimes enhance the perception of pain e.g. damaged tissues produce Prostaglandins, which act as local regulators of inflammation. Prostaglandins worsen pain by increasing nociceptor sensitivity to noxious stimuli. Aspirin and ibuprofen reduce pain by inhibiting the synthesis of Prostaglandins.

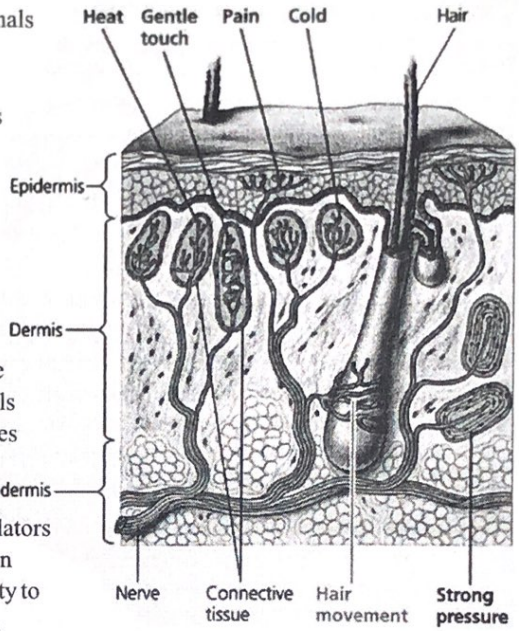


Figure 40.0: Structure of the mammalian skin

Function of the skin

a. Protection the skin protects the body against the entrance of microbes, mechanical injuries, ultra-violet rays of the sunlight and desiccation by acting as a shield. The pigment (melanin) absorbs u.v. rays from the sun which is harmful to the body.
b. Synthesis of vitamin D Liberal absorption of infra- red rays helps in the conversion of 7- dehydrocholesterol present in the skin to vitamin D.
c. Storage of food fats are stored under the dermis forming an insulating layer which is converted to energy when the need arise.
d. Assist in milk production the female mammary glands are modification of the skin to form teat which produce milk that are used to feed the young.
e. Provision of Sensory cells the skin is senstitive to its environment through the specialized sensory nerve endings scattered in the dermis which makes the skin to be sensitive to stimuli such as changes in temperature, pressure, pain and cold.
f. Regulation of body temperature animals are able to regulate their body temperature in their various habitat or environment leading to the classification of vertebrates into two categories (poikilothermic and homoeothermic animals). Poikilothermic animals are animals whose body temperature varies according to the temperature of the environment i.e. their body temperature is never constant e.g. fish, frogs, snakes, lizard etc. Homoiothermic animals are those animals whose body temperature remain constant regardless of the changes in the temperature of the external environment e.g. birds and mammals.

On a hot day, weather or environment, a rise in body temperature as a result of rise in environmental temperature stimulates the following processes to get rid of excess body heat:

i. Vasodilation: the capillaries near the skin surface dilate, while those in the deeper layers of the skin constrict, causing a large volume of blood to flow close to the surface of the skin so that heat is lost to the surroundings via conduction, convection and radiation.
ii. Sweating: the sweat glands become active and produce large amounts of sweat that flow out onto the surface of the skin. As this evaporates, heat from the body is used up, thus cooling the body.
iii. Decrease in metabolic rate: the body slows down its activities (hibernate) to reduce metabolic rate and heat generated by metabolic reactions, thereby decreasing the heat production within the body.
iv. Lowering of hair: in hairy mammal, the hair erector muscles relax, causing the hair to lie flat on the skin surface. This force out most of the air trapped among the hairs. Without this insulating layer, the animal loses more heat from its body.
v. Behavioural changes: many mammals keep cool by staying in the shade or

increase the intake of fluid, human wears light clothes with light coloured absorbent material.

On a cold day, weather or environment, a fall in body temperature stimulates the following processes to produce/ conserve heat:

i. Vasoconstriction: the capillaries near the skin surface constrict while those in the deeper layers dilate causing smaller volume of blood to flow near the surface of the skin so that heat loss is greatly reduced.
ii. Sweating: in human, sweat glands become inactive and produce very little sweat that flows out to the skin surface thereby reducing heat loss through evaporative cooling.
iii. Increased metabolic rate: the body's metabolic rate is increased, especially that of the liver in order to produce more heat. Shivering is also done to increase the body's metabolic rate along with other physical activities such as running, jumping, clapping, moving etc. to produce body heat during cold weather.
iv. Rising of hairs: in hairy mammals, the hair erector muscles contract to raise the hairs and trap more air. Since air is a bad conductor of heat, the layer of air next to the skin acts as an insulator and prevents heat loss from the body.
v. Behavioural changes: many mammals keep warm by staying in nests and huddling into a ball to reduce surface area or stay together with others when in group (huddling) to conserve heat.

Mechanism of homeostasis

Animals use various processes to maintain a normal internal environment such as feed consumption, thermoregulation, chemical balance, pH control, osmotic and electrolyte regulation and circulatory balance of all cardiovascular activities. The volume of extracellular fluid and circulating blood are adapted to specific needs by mechanism that affects the cardiovascular system and the transportation of salt and water. Homeostatic mechanismoperates in the animal through complex feedback control which brings about the coordination and integration of the various systems under different conditions. They are maintained in turn by physiological processes such as the use of diffusion pressure, gradient concentration, active transport and various regulatory mechanism controlled by the nervous and endocrine system.

Nervous coordination: The process whereby different parts of an organism are harmonized to work together to achieve a definite objective for proper effectiveness is called coordination. All metabolic processes in the body do not function in isolation but are closely linked and dependent on each other for effective functioning and are coordinated by two communication systems (nervous and endocrine system). Both the nervous and endocrine systems work together to bring about coordination of body activities (Table 7). The central nervous system (CNS) and endocrine secretions are mostly responsible for all the maintenance of homeostasis (internal environment). Within the CNS, the hypothalamus is the most important organ as it is the link between the nervous and endocrine systems as well as the control centre for thermoregulation, feed/ water intake, osmoregulation and all metabolic activities.

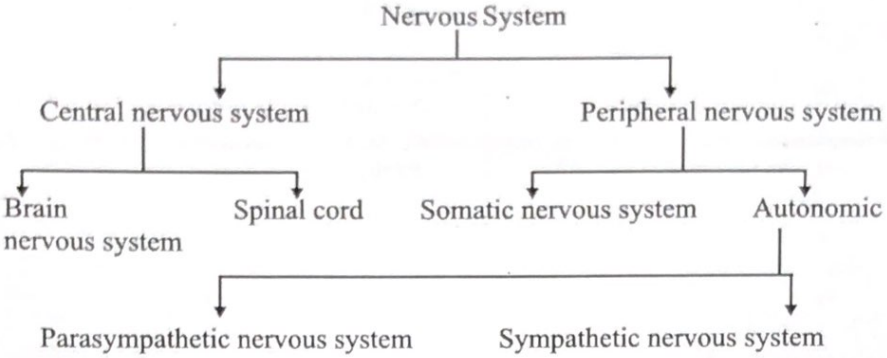
The hypothalamus also serves as an important link between the nervous and endocrine system through the neurosecretory tract which lead to the pituitary gland. In homeostasis, the pituitary gland is directly activated while the thyroid and adrenal gland is indirectly activated.

Table 7: Differences between the nervous and endocrine system

Nervous System	Endocrine System
1. Messages are mainly electrical impulses passed along the nerves	Messages are chemical substances (hormones) passed through the blood stream.
2.The response is fast.	The response is slow.
3.Response does not depend on the quantity of stimuli.	Response depends on the quantity of stimuli as too much or too little can cause metabolic disorder.
4.The effect is for a very short time.	The effect is for a long time.
5. Effectors (muscles and glands) receive the message.	Target organs receive the message.
6. It is mainly controlled by brain spinal cord.	It is controlled by hypothalamus or Pituitary glands.

The nervous system is made up of two parts Central Nervous System, CNS (consists of the brain and spinal cord) and the Peripheral Nervous System, PNS (consists of all other nerves - spinal and cranial, outside the brain and spinal cord (Fig.41.0). It is divided into two - the somatic and autonomic nervous system) (Table 7a). The **somatic nervous system** (SNS) controls activities which are mainly voluntary. It deals with external stimuli and their responses i.e. the animal's awareness of all external stimuli and the corresponding nervous responses. It is made up of 12 pairs of cranial nerves in the head and 31pairs of spinal nerves in the body of man. Among the cranial nerves, the optic and olfactory nerves consists only of sensory fibres while the remaining 10pairs of cranial nerves and 31pairs of spinal nerves contain both sensory and motor nerve fibres which conduct impulses from sense receptors to the spinal cord or brain and from there to effectors. The **autonomic nervous system** (ANS) is concerned with the body's involuntary activities such as heartbeat, breathing, digestion and sweat secretion. It consists of sensory and motor nerves which connect the brain or spinal cord to internal organs such as heart, liver and gastrointestinal tract. It is defined as the total visceral motor pathway. It is divided into two parts the **sympathetic nervous system (SNS)** and the **parasympathetic nervous system (PNS)**. The sympathetic nervous system consists of nerves which connect internal organs to the thoracic and lumber areas of the spinal cord.

SNS arises from neuron in the thoracic and lumbar spinal cord while the PNS arises from the sacral spinal cord and the 3rd, 7th, 9th and 10th cranial nerves. The parasympathetic division functions in promoting the even regulation of everyday activity like digestion and even blood flow. PNS stimulates the same organs as the SNS but its action is opposite (antagonistic) to the SNS (Table 7b; Fig. 42.0). It often acts to return the body to a normal state after stimulation by the SNS. The SNS is known as the fright and flight system, which when activated slows down digestion and increases the blood to the limbs. It dilates the pupil of the eye and slows down blood flow to the abdominal visceral. During the process, a hormone adrenaline may be released. SNS responds to emergency situations in the body by stimulating an increase in certain body processes. It stimulates many parts of the body for necessary action in times of danger.



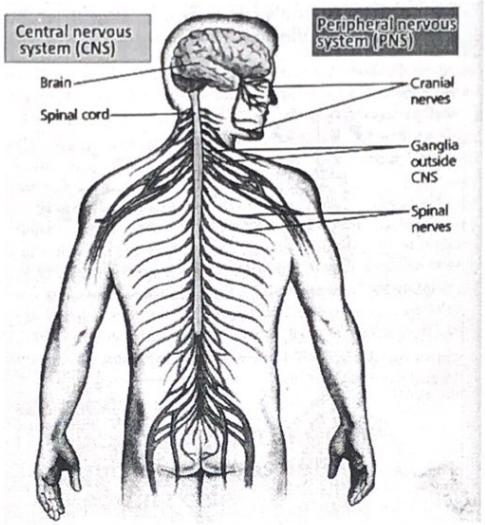


Figure 41.0: Major parts of the nervous system

Table 7a: Differences between somatic and autonomic nervous system

Somatic nervous system	Autonomic nervous system
1.Involved in voluntary body Activities.	Involved in involuntary body activities.
2.It always stimulates effectors.	It may stimulate or inhibit effectors. 3.It
affects skeletal muscles	It affects glands, cardiac muscles and smooth muscles.
4. Impulses speed along motor fibres that extend from CNS to effectors without synapses (no ganglia)	Impulses speed along motor fibres that extend from CNS to ganglia where they synapse and from ganglia to effectors.

Table 7b:Functions of the Sympathetic and Parasympathetic nervous system

Sympathetic Nervous System	Parasympathetic Nervous System
1. Accelerates heartbeat	Slows down heartbeat
2. Constricts arteries	Dilates arteries
3. Dilates the bronchioles	Constricts the bronchioles
4. Dilates the iris	Constricts the iris
5. Contracts the bladder and anal Sphincters	Relaxes the bladder and anal sphincters
6. Causes the relaxation of bladder Muscle	Causes the contraction of the bladder muscle
7. Slows gut movement	Speeds up gut movement
8.Raises blood pressure	Lowers blood pressure
9.Inhibits the secretion of salivary glands	Stimulates the secretion of salivary glands

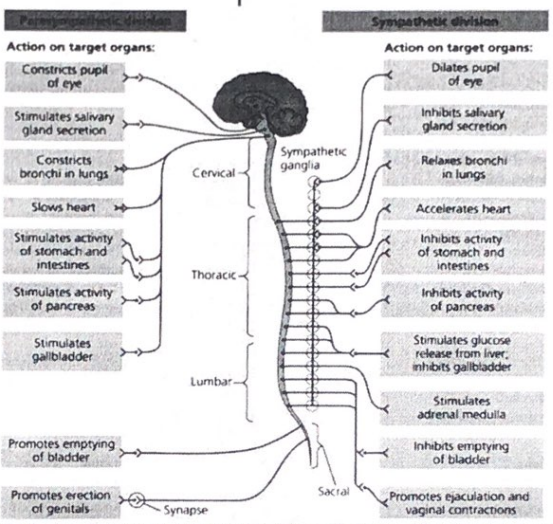


Figure 42.0: Points of the parasympathetic and sympathetic nervous system

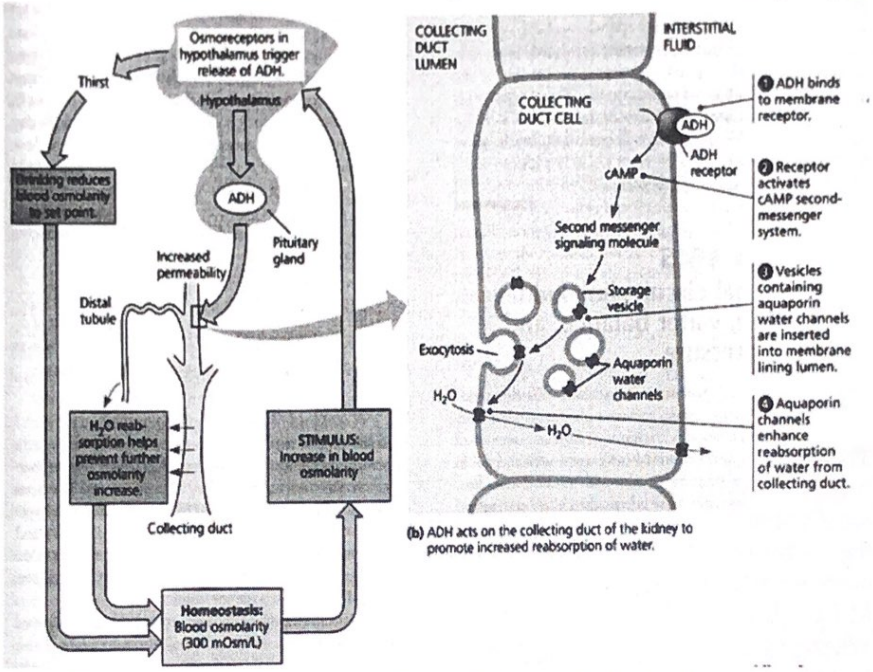
Maintenance of water balance

Animals maintain their water balance by reducing requirements and by offsetting all water loss through cutaneous and respiratory evaporation, through urination and defecation by an equal intake of water through food and water generated from oxidation of hydrogen fat. The regulation of body fluid volume by the kidney or urinary system is an important part in the maintenance of homeostasis. Although water is continuously lost from the body, total body water is kept at a relatively constant value. This means that the rate of water loss is approximately equal to the rate of water gained in the body. The routes by which water is lost from the body include:

- Urine
- Insensible water loss through skin/ respiratory tract
- Sweating/ panting in hot weather
- Faeces
- Normal/ abnormal spitting
- Sneezing/ coughing
- Parturition/ childbirth
- Milk in lactating animals

Sources of water gained in the body include:

- Ingested water through drinking and food
- Oxidative/ metabolic water resulting from cellular oxidation of hydrogen.



(b) ADH acts on the collecting duct of the kidney to promote increased reabsorption of water.

Figure 43.0: Maintenance of water balance in the body

Salt deprivation

Volume of extracellular fluid is indirectly influenced by the Sodium concentration in the body. This is due to the fact that an appreciable amount of water is passively absorbed along with Sodium ion during Sodium reabsorption in the renal tubules. Lack of Sodium (Na) in an animal's body for several days will lead to a small decrease in extracellular fluid because sodium ion concentration in the glomerular filtrate will be low. Conversely extracellular fluid will slightly increase in volume if large amount of salt is ingested for many days.

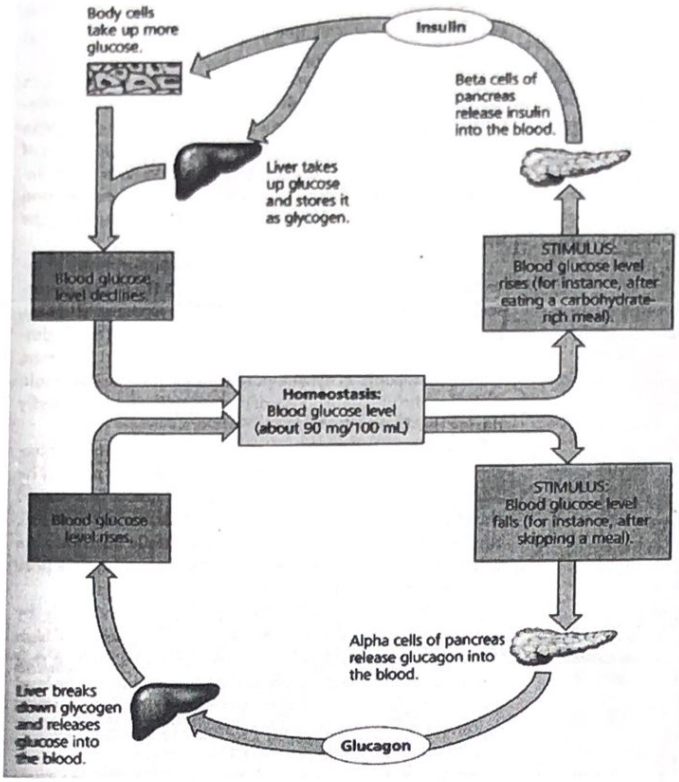


Figure 44.0: Maintenance of glucose homeostasis by insulin and glucagon

Temperature regulation in farm animals

Animal controls their physical and chemical thermoregulatory processes by means of temperature sensitive neurons (thermoreceptors) located in all parts of the body. These receptors are tonic in nature and are sensitive to increase or decrease in temperature. In order to maintain an internal environment

$M + G = L + DC$ or $M + G\ L = DC$

Where M is metabolic heat as a result of feed, G heat gained from the environment, L heat loss to the environment and DC temperature change.

A warm thermoreceptor will react to an abrupt temperature rise in the tissue by increasing the frequency by an amount corresponding to the rate of change in temperature. Likewise, a cold receptor will be activated by a drop in temperature. These thermoreceptors are classified according to their location (Fig. 45.0).

1. CNS thermoreceptor located in the hypothalamus
   CNS thermoreceptor located in the hypothalamus
2. Peripheral thermoreceptors located in the body e.g. skin
3. Extra hypothalamic deep body thermoreceptors - in the spinal cord, GIT and the walls of the major veins.

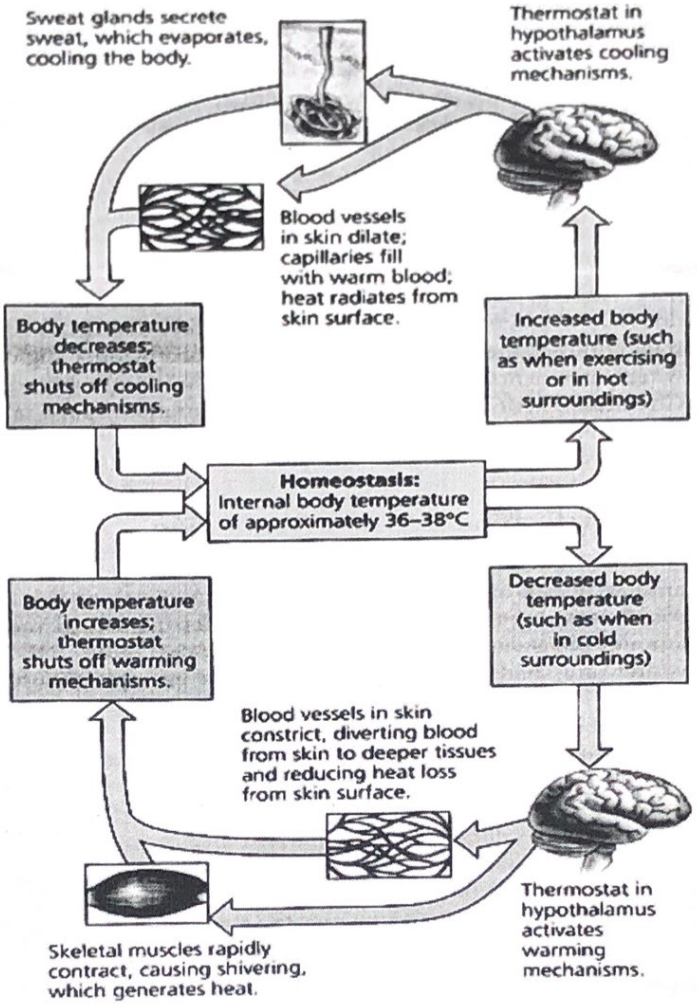


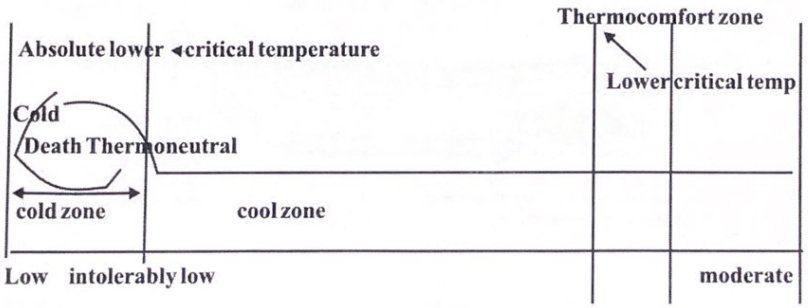
Figure 45.0: Thermostatic function of the hypothalamus

All informations from all these sites are channeled through the afferent nerves to the hypothalamus thermoregulatory centres (the decision making body) as to what line of actions to be taken.

There are two major stress that arises from temperature change namely **cold stress** and **heat stress.**Effective environmental temperature is an index of an animal's total thermal environment in terms of environmental heat demand. An animal is said to be undergoing heat stress when the effective environmental temperature is so high such that the animal must call on one or more thermoregulatory process in order to maintain homeothermy (balanced temperature).

COLD STRESS: The pattern of temperature regulation can be subdivided into the comfort or thermoneutral zone, cool zone, cold zone and intolerably cold zone.

a. Comfort or thermoneutral zone This is the zone whereby an animal is neither cold nor heat stressed. It is the zone where the heat produced by an animal is lost to the environment without the aid of any special heat conserving or dissipating mechanism.
b. Cool zone As effective environmental temperature falls below the lower limit of thermocomfort zone. The animal is then in a cool zone. The animal maintains its body temperature by invoking physical thermoregulatory processes.
c. Cold zone The drop in the effective environmental temperature below the lower critical temperature of the cool zone cause the animal to have an increase in the metabolic rate of heat production in order to maintain homeothermy. The following could influence the lower critical temperature (a) the level of feed intake; (b) the time of the day/ social activities thermoregulation and (c) thermal insulation.
d. Intolerably cold zone This zone lies below the cold zone. The demand for heat production will be at the maximum, and the maximum level can only be maintained for a short period before the body temperature begins to fall, sooner or later the animal dies.



Thermoregulatory mechanism to maintain cold stress can be sub-divided into three namely Physical mechanism, Chemical mechanism and Behavioural mechanism

An animal maintains cold stress through by physical mechanism through covered insulation, tissue insulation and by an increase in capillary blood flow.

Chemical mechanism - is done through secretion of certain chemicals known as catecholamine e.g. epinephrine and nor-epinephrine. This hormone (catecholamine) plays a sensitive role in the various thermogenic reactions to acute and chronic cold stress.

Behavoiural mechanism - An animal behaves apparently involuntarily in specific ways in order to alter the rate of heat exchange with its environment such as seeking for shade and by cuddling together.

CHAPTER EIGHT

REPRODUCTIVE PHYSIOLOGY

Reproduction is a complex science which involves the knowledge of anatomy, physiology, endocrinology, embryology, histology, cytology, biochemistry, microbiology, genetics, nutrition, etc. **It is the process through which offspring is produced from the fusion of male (spermatozoon) and female (ovum) gamete after mitotic and meiotic division.** Sexual reproduction is the formation of a new individual following the union of two gametes. In humans and majority of other eukaryotes plants as well as animals the two gametes differ in structure (anisogamy) and are contributed by different parents. Gametes need motility to be able to meet/unite and food to nourish the developing embryo. These two important needs are met by anisogametes i.e. the sperm are motile and small while eggs contain food.

Successful reproduction involves a series of physiological and psychology events which must be properly timed. The timing is achieved through the endocrine system by the production of several hormones. It involves heterosexual mating of pubertal animals, fertilization, gestation, parturition and lactation. Animal production revolves around reproduction since it must occur for replacement stock in meat production, egg production and milk production.

Evolution of Reproductive Science

Much advances in reproduction where made within the last 25yrs, however, in 384-322 BC Aristotle wrote the first scientific paper on embryology. His works serve to turn the human mind from superstition to observation due to the accuracy of his work. His work was so far ahead of his time to the extent that nothing was added until about 2000 years later. Although most of his work was based on speculation, he formulated two theories on embryonic development.

The first theory was that the embryo was pre-formed in man and during sexual intercourse deposited and developed in the female. Secondly, that the embryo was the result of a differentiation from a formless being. He decided on this theory because during that time slime and decaying matter where capable of producing living matter. He described the human embryo as been organized from the mother's menstrual blood. After is work further development followed the invention of the microscope. In 1668, Redi published papers based on observation which tends to disprove Aristotle's theory that human embryo develops from menstrual blood. In 1672 de Graaf described the ovarian follicle. In 1677 Hamin and Leuwenhoek, with the aid of the early microscope described the human spermatozoa. Between 1759 to1769 Wolf observes part of the chicken embryo take shape and his work disproved the pre-formation theory.

Wolf's work set forth the theory of epigenesis i.e. the development of the embryo by progressive growth and differentiation. So this led to some other scientist to think that embryo was pre-formed in the ovum and the development was stimulated by the male sperm while others thought that it was pre-formed in the sperm and development stimulated in the ovum.

After some time the work of Pasteur in 1864 disproved the theory of spontaneous generation by showing that bacteria cell reproduce by cell division. In 1900 Dreish provided the final proof that life came from single cells by separating daughter cells of a fertilized egg and showed that they could each develop into an embryo.

Purposes of Reproduction

1. Perpetuation of species: All species including man have very short desire or urge to maintain themselves e.g. the strongest urge in an individual is to preserve its life, no animal wants to die. Reproduction is nature's second strongest impulse to avoid death by producing its kind.
2. To produce food: Man uses animals through reproduction to produce food. Man has learned to manage or maintain animals and manipulate their reproductive function to produce food.
3. Genetic improvement: The management and manipulation of reproductive processes have been used as genetic tools e.g. Best animals have been maintained by mating the best to the best. Artificial insemination is a tool that has been developed to use the same of the best male animal to inseminate the best female animal resulting in the best offspring. Another technique is super ovulation which is to stimulate the female animal at an earlier age to produce more eggs/offspring; ovum transfer, storing fertilized ova, test tube fertilization, environmental manipulation to hasten pubertal development etc are some other improvement techniques.

   Existing technologies directed towards enhancing reproductive performance include the following processes:

   i. Frozen semen
   ii. Separation of male/female producing sperm i.e. sperm sexing
   iii. Oestrus synchronization
   iv. Superovulation
   v. Ovum transfer
   vi. Storing fertilized ova
   vii. Test-tube fertilization
   viii. Environmental influence on puberty

EMBRYONIC DEVELOPMENT OF THE REPRODUCTIVE ORGAN

The formation of the gonads depends on the movement of the primordial germ cells to the genital ridge. The primordial germ cells develop extra-gonadally in the yolk sac endoderm and migrate by amoeboid movement to the genital ridge.

For an individual to be a male the germ cells give rise to the seminiferous tubules while an additional migration and proliferation of epithelial/germ cells occur to develop into the ovarian cortex in females (Fig. 46.0). In another word, gonad forms from a group of granulated yolk cells which invade the germinal ridge and forms the ovary in females or testis in males. In female embryo, two invasions occur the first invasion is abortive, but the second invasion led to the formation of sex chords which later break off into primordial germ cells.

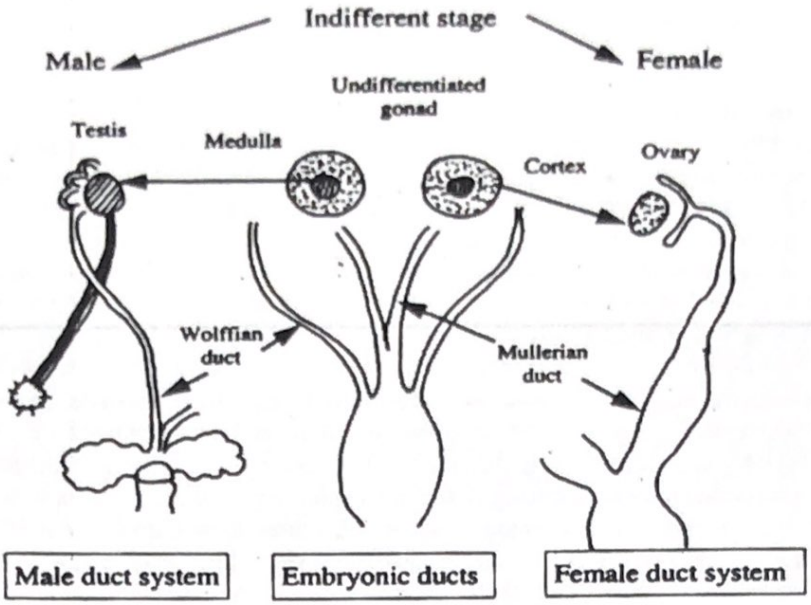


Figure 46.0: Embryonic development of the male and female reproductive system

The development of the **mesonephric (Wolffian)duct** and the **paramesonephric (Mullerian)duct** follow that of the gonad; with one duct developing while the other regresses.

Paramesonephric (or Mullerian) duct develops into functional oviduct, uterus and forms part of the vagina in the female.

The Mesonephric (or Wolffian) duct develops into ligaments on which the reproductive organs are attached. The mesonephrons is the initial excretory organ in the embryo but later degenerate and form ligaments on which the reproductive organs are attached. The Wolffian duct forms the epididymis and Vas deferens in male.

The Genital tubercle forms the penis in males and the clitoris in females. The vestibular fold forms the scrotum in males and the labia in females. The Urogenital sinus forms the prostate gland in male and part of the vagina in female, it also form parts of the cervix (Table 8).

Table 8:Development of the reproductive organ from the indifferent stage of the embryo

MALE	INDIFFERENT STAGE	FEMALE
Testis	**Gonad**	Ovary
Vas efferens	**Mesonephric tubules**	
Epididymis Vas deferens	**Wolffian duct**	
	Mullerian duct	Uterus Oviduct Trunks of the vagina
Urethra Prostate Bulbourethral Gland	**Urogenital sinus**	Urethra Part of vagina
Penis	**Genital tubercle**	Clitoris
Scrotum	**Vestibular fold**	Labia

THE MALE REPRODUCTIVE SYSTEM

The organs of the male reproductive system are specialized for the followings:

- To produce, maintain and transport sperm (male reproductive cells) and protective fluids (seminal plasma).
- To discharge sperm within the female reproductive tract.
- To produce and secrete male sex hormones.

The male reproductive system consist of external structures (penis, prepuce, scrotum and testis) and internal structures (vesicular glands, prostate glands, Bulbourethral or Cowper's glands, ampullae, the spermatic chord, and the male duct system consisting of vas efferens located within the testis with the epididymis, the vas deferens and the urethra, these are external to the testis). The testis which produces spermatozoa and the male hormone has its origin in the embryonic primary sex chord of the genital ridge, but the duct system originates from the Wolffian duct.

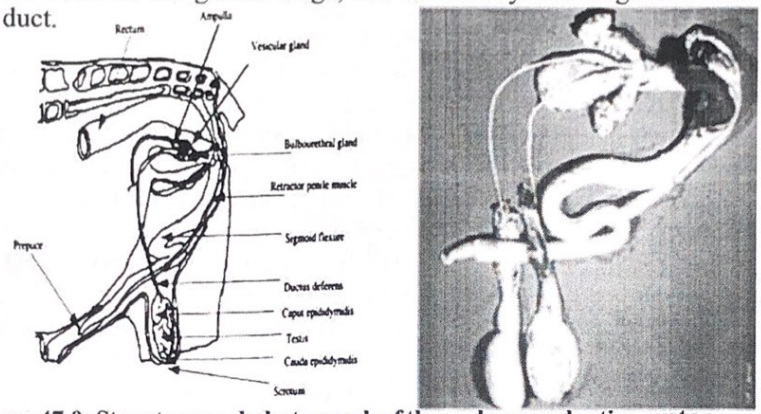


Figure: 47.0: Structure and photograph of the male reproductive system

Organ	Functions
1.Testis	(a) Produces spermatozoa (b) Produces Androgen
2. Scrotum	(a) supports the testis (b) Regulate testicular temperature (c) Protect the testis
3. Spermatic chord	(a) Supports the testis (b) Regulate testis temperature
4. Epididymis	(a) Stores spermatozoa (B) Concentrate spermatozoa (c) Maturation of spermatozoa occurs with it and it. (d) Transports spermatozoa.
5. Vas deferens	(a) For sperm transport
6. Urethra	(a) For sperm transport
7. Vesicular glands	Contribute the seminal fluids, energy substrates and buffers to the semen.
8. Prostrate glands	(a) Contribute fluids and inorganic ions to semen.
9. Bulbourethral glands	(a) Produces fluid which flushes the urine residues from the urethra.
10. Penis	(a) It's the main organ of copulation.
11. Prepuce	(a) It's a sheath that encloses the free end of the penis.

1. **Testis** is a Latin word for witness or "spectator" from which English words like "testify" and "testament" was derived. The use of "testes" for the male gonads reflects an ancient belief that these organs were witnesses to an act in which they took no part. However, the result of this act is always the product that brings joy to man as it is essential for the continuation of the species. Testis is the primary reproductive organ of the male because it produces the male gamete and regulatory hormones. It is an oval organ about the size of large olives that lie in the scrotum, secured at either end by a structure called the spermatic cord. The exact orientation of the testis in the scrotum relative to the body axis differs between species. It is firmly held by the scrotum with the long axis of the testis tangential to the body axis in boar while the scrotum is pendulous with the testis hanging loose and vertical to the body axis in bull, buck and ram. Although the testis varies with species as far as the shape, size and location are concerned, the essential structure is the same. Each testicle (testis) is made up of coiled mass of tubes (seminiferous tubules) which makes up about 90% of the testicular mass surrounded by a heavy fibrous capsule (tunica albuginea).

A number of septa or trabeculae pass inwards from the tunica albuginea to join almost in the centre of the testis to form a cord known as the mediastinum testis. The histology of the tubules changes with age. It is small, solid and widely separated in new-born while the tubule enlarges and the matrix dissolves to form the lumen at about 5 to 5.5months in bull. The seminiferous tubules increases about 5-fold with the testis weight increasing about 20-fold from birth to maturity in bull. Spermatogenesis occurs within the epithelium of the seminiferous tubules which consists of spermatogenic cells and supporting sertoli cells. Located in the connective tissue between seminiferous tubules can be found the intertubular or Leydig cells which secrete the male hormone, testosterone (an androgen).

Testicular descent

During embryonic stage or at birth the testis is situated in the abdomen, and as the embryo developed the gonad degenerate. Testicular descent involves abdominal migration to the internal inguinal ring, inguinal migration through the inguinal canal, and finally migration within the scrotum.

There are three (3) migration stages

1. Abdominal migration
2. Inguinal migration
3. Scrotal migration.

During the abdominal migration the peritoneal lining of the abdominal cavity invaginate in the area of the gubernaculum. The gubernaculum itself is a gelatinous mass that swells under the peritonium towards the scrotal swelling. This growth along with pressure gives rise to the formation of the vaginal process towards which the testis is drawn, carrying with it a peritoneal covering. The testis now moves into the inguinal region through the deep inguinal ring to the inguinal canal, and the superficial inguinal ring to the scrotum. After the passage of the testis through the inguinal canal into the scrotum, the canal constricts to propel the testis into the inguinal region. In some cases the inguinal ring fails to constrict, thus permitting the testis to return into the inguinal region or the testisfailing to enter the scrotum. This leading to a condition called cryptorchidism. Cryptorchid males are males whose testis fails to descend into the scrotum thereby impairing sperm producing function, such male shows normal sexual desire since hormone production is unaffected but they are sterile. Testis has a dual (exocrine and endocrine) function which are sperm production and secretion of male hormone (androgen). Occasionally some of the abdominal viscera pass through the orifice of the vaginal process and enter the scrotum resulting in a condition called scrotal hernia, which is common in pigs. Abnormalities involving differentiation of the gonads and duct system can result in varying degrees of intersexuality found in hermaphrodites.

Time of descent varies with species. In the bull it is half way in foetal life, same in the ram. In the boar it is last quarter of foetal life. In the stallion it is just before or a little bit after birth. In birds the testes remain within the body cavity.

Structure of the testis: In the bull it is ovoid and elongated organ and placed with their long axis vertical in the scrotum. Each testis weighs between 300 to 500g depending on age and usually between 12-16cm long and 6-8cm in diameter. The testis is surrounded with a serous coat called the tunica vaginalispropria and placed within a capsular tissue known as tunica albuginea. A section through the testis would reveal a cord of connective tissue called septula testis extends from the mediastinum between the seminiferous tubule giving the testicular tissue lobulated appearance.

In a cross section the mediastinum appears as a central striated figure enclosing the rete testis which serves as collecting tubes for the seminiferous tubules.

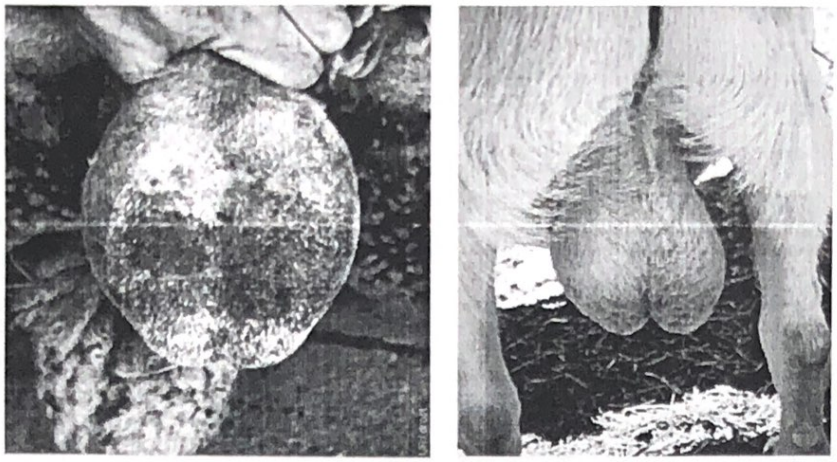


Figure 48.0: Scrotum of ram and buck

Functions of the Testis

Sperms are formed within the seminiferous tubules. About 6,000 sperms are formed in the testis per minutes with an average of 9 millions (9^{X} 10^{6}) per day. In most farm mammals sperm production is all the year round but in some like the sheep which produce sperm seasonally. Sperm production in bulls increases with age up to seven years. Production is usually greater than output as measured by the number of spermatozoa recovered at ejaculation. Newly formed sperms are non-motile; they move lumina of the seminiferous tubule through the rete testis by current of fluid originating in the tubules. The testicular fluids in which the sperm are suspended are secreted under considerable pressure and give the testicle their characteristic consistency.

A few drop of a cloudy fluid with a sperm concentration of 25,000-350,000mm^3 could be collected by puncturing the rete testis shortly post mortem (after death)

Thermoregulation of the Testis

Before the testis can perform its functions properly the temperature must be maintained below the body temperature. Some features enhanced the maintenance of testicular temperature; some of these are anatomical while others are physiological.

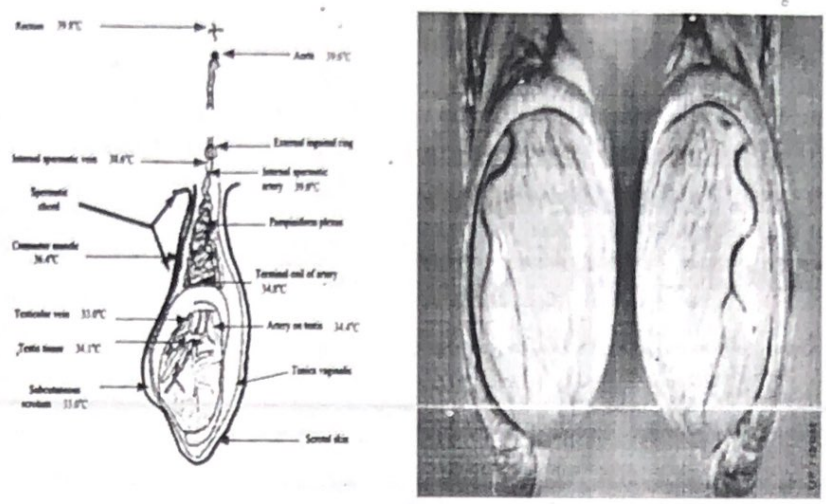


Figure 49.0:Structure of the testis and its thermoregulatory mechanisms; Normal ram testis

The anatomical features include:

a) The scrotal position: The scrotum is a cutaneous sac that conforms in shape, size and location to the testes it contains. The skin is thin, pliable and relatively hairless. The scrotum is exceptional in that it maintains the testes within it at a temperature between 2° and 7°C lower than the body or core temperature, in this connection in most mammals the testis are placed outside the body suspended between the hind legs.

b) Testicular descent: Testicular descent into the scrotum outside the body cavity enables the maintenance of lower temperature in the testis.

c) Scrotal skin: The scrotal skin lacks subcutaneous fats and is richly endowed with sweat glands.

d) Presence of tunica dartos: The scrotum skin has a muscular component known as the tunica dartos which enables the scrotum to alter its thickness and its surface area to vary the closeness of contact of the scrotum with the body wall depending on the weather. In hot weather it relaxes enabling the testis to move away from the body wall while in cold weather it contracts bringing the testis closer to the body wall to gain heat.

Presence of the cremaster muscle: In the horse the action of the tunica dartos is supported by the action of the cremaster muscle in the lowering and retracting of the scrotum. The cremaster muscle within the spermatic cord contract or relaxes to lower or raise the testis depending on the weather conditions.

f) Presence of temperature receptor: In ram, the presence of temperature receptors on the scrotal skin responds to help lower the whole body temperature when there is variation in weather.

g) Position of arteries/veins: The position of the arteries/veins close to the testis surface enables direct loss of heat from the testicular surface.

h) Position of the testis relative to the heart: The position of the testis relative to the heart in a region where little muscular activity occurs makes an unusual demand on return of blood. Venous return is promoted by the close contact between the arteries and veins. There is absence of the pulsatile flow of blood at the inguinal region (groin area) which might also be involved in lowering the testicular temperature.

i) Presence of the pampiniform plexus: In all mammals, the testicular arteries is a convoluted structure in the form of a cone the base of which rest on the dorsal plane of the testis to form what is called **pampiniform plexus**. This arrangement provides an effective countercurrent mechanism by which the arterial blood entering the testis is cooled by the venous blood leaving the testis. In the ram the temperature in the testicular arteries normaly falls by 4°C before it enters the testis and the venous blood rises by the same amount by the time it is leaving the testis.

2. **Epididymis**: Its an elongated fibrous body closely apposed to the testis and consists essentially of a single highly convoluted duct through which spermatozoa pass from the testis. It is made up of three (3) parts- the caput, corpus and cauda epididymis. In the bull the ratio of the three regions to each other are 1: 0.23: 0.45 for weight, 1: 0.95: 0.45 for length and 1: 0.33: 2.01 for sperm reserve respectively. Therefore, the epididymal head (caput) is the largest segment while the epididymal tail (cauda) contains the most spermatozoa and in fact acts as reservoir for sperm awaiting ejaculation. The caput contain 6-20 ductules joining the epididymal duct. It is a flattened structure applied to the upper pole of the testis and continued to the corpus and finally terminates at the opposite pole as the cauda epididymis. Histologically, the epididymal head has a high epithelium with narrow lumen that is lined with long straight stereocilia, it functions in the reabsorption of most fluid leaving the testis. The corpus epididymis on the other hand has lower epithelium with wider lumen, it functions in sperm maturation. While the epididymal tail has epithelium with very wide lumen lined with short stereocilia, it functions in sperm storage and maturation.

Functions of the Epididymis

The main function of the epididymis is sperm transport. Sperms are transported from rete testis to the efferent duct by fluid pressure in the testis. This is aided by cilia of the ciliated cells in the efferent duct. In the epididymal duct it is by peristaltic (contraction) movement of the musculature of the epididymal wall. The required time for transport of spermatozoa through the epididymis is constant for individual species. The duration of the epididymal journey for bull, ram and boar is 10days, 13-15days and 9-12days respectively depending on the frequency of ejaculation.

2. Concentration of sperm: The sperm leaves the testis in aqueous suspension, the water is reabsorbed into the epithelia cells during the passage through the epididymis especially at the caput and becomes highly concentrated as it get to the cauda epididymis.
3. Maturation of sperm: By the time the sperm leaves the testis they have cytoplasmic droplets but as it moves through the epididymis the droplet migrate from the head of the sperm to the end of the mid-piece and then becomes the distal droplet and by the time the sperm is fully matured it has no droplet. The maturation is caused by the secretion of the epithelia cell. In the bull the migration of the cytoplasmic droplet occurs only in the caput epididymis so that in the cauda region all sperm have their cytoplasmic droplet at the distal end.

4. Sperm storage: Sperm is stored in the tail (cauda) of the epididymis. The concentration is high in this section and the lumen of the duct is wide. Conditions in the tail are optimal for preserving the viability of the sperm. In this position the sperm are in a state of quiescent metabolism. Sperm can remain viable in the epididymis for about 60days but under long period of sexual rest the first few ejaculate may contain non-fertile sperms. Acrosome degeneration is one of the visible changes in sperm after a long sexual rest. A depot of the sperm in some cases may be discharged during masturbation or in sexually inexperience male during urination.

3. **Accessory sex glands:** These are lobular branched tubular glands whose secretions make up a large percentage of the fluid in which the spermatozoa is suspended. Accessory sex glands include the following -
   i. Ampullae
   ii. Vesicular glands
   iii. Prostate glands
   iv. Bulbourethral or Cowper's glands

The ampulla is a dilation of the urethral end of the ductus deferens involved in the ejaculatory process and secretes some fluid in which the sperm are carried. Though absent in the boar, it is well developed in the stallion, bull and ram.

Vesicular glands produce the seminal fluid which forms the bulk of the seminal plasma of the semen.

Prostrate glands are located caudally to the vesicular glands close to the point where the urinary bladder joins the urethra. It is made up of two parts an external body lying outside the thick urethral muscle surrounding the urethra and an internal body below the urethral muscle along the length of the pelvic urethra. It produces secretions containing citric acids in some farm animal species.

Bulbourethral or Cowper's glands are paired bodies lying dorsal to the urethra; secrete fluid which cleanses the urethra prior to ejaculation. Though small in other animals such as stallion, bull, ram and completely absent in dogs, it is large in boar and secretes the gel fraction of boar semen.

4. **Penis**: The penis is the male organ for sexual intercourse and it's a continuation of the urethra. The penis is a specialized tissue capable of being engulf with blood during erection and so is described as the erectile organ. It forms a Z-shaped structure known as the sigmoid flexure with retractor penile muscles that allows for extension of the penis when the retractor muscle relaxes in animals such as bull, ram and boar. The sigmoid flexure is completely or partially absent in other mammals. The body of the penis is cylindrical in shape and made up of three cavernous bodies that surround the pelvic urethra; these are ***corpus spongiosum penis***, ***corpus cavernosum penis*** and ***ischio-cavernous muscle***(Fig. 50.0).These chambers are made up of special, sponge-like erectile tissue. This tissue contains thousands of large spaces that fill with blood when sexually aroused. As the penis fills with blood, it becomes rigid and erect, which allows for penetration during sexual intercourse. The skin of the penis is loose and elastic to accommodate changes in penis size during an erection. The stiffening of the penis in ruminants is brought about mainly by the ischio-cavernous muscle which pumps blood from the cavernous spaces of the crura into the rest of the corpus cavernous penis. The sigmoid flexure, assisted by the relaxation of the retractor penile muscle is eliminated as a result of rising pressure in the cavernous spaces of the corpus cavernosum penis. Glans penis is the end of the penis and comes in different shapes among mammals. A bony structure called os penis is formed by ossification of a part of the corpus cavernosum in many species, especially the dog. Semen, which contains sperm, is expelled (ejaculated) through the end of the penis. When the penis is erect, the flow of urine is blocked from the urethra, allowing only semen to be ejaculated.

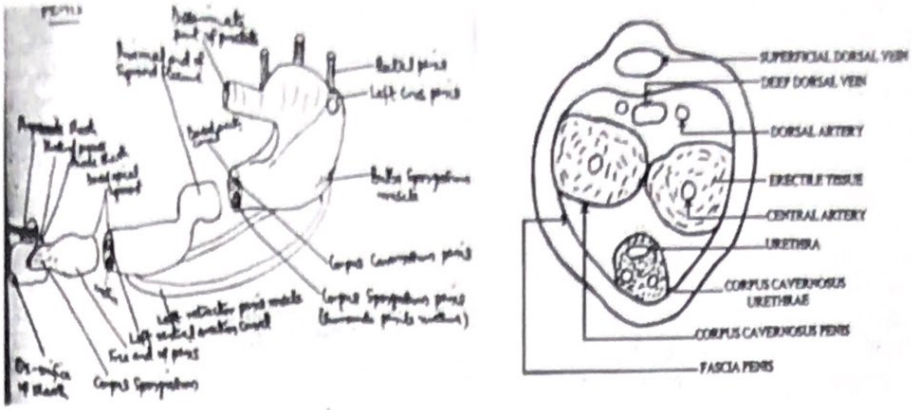


Figure 50.0: Structure of a bull penis and a transverse section of a penis

Functions of Penis

The major function is for copulation. Penis has to be erect before copulation. Erection is a process whereby a normally flaccid organ becomes turgid e.g. the clitoris, teat or nipple and the penis. Sexual excitation causes erection, causes decreased dilatation of the arteries supporting the cavernous bodies of the penis. In ruminants the penis stiffens due to the pumping of blood from the ischiocavernous penis muscle into the rest of the corpus cavernosum penis through the erection canal or the longitudinal cavernous spaces. Pressures of up to 7000mm Hg have been reported in the cavernous spaces during natural mating in the goat.

The Bulbocavernous muscle simultaneously pumps blood from the penile bulb and into the rest of the corpus spongiosum penis. Protrusion of the penis arises from the pressure in the cavernous space, the sigmoid flexure is eliminated, the retractor penis muscle then relaxes and the penis protrudes. In the bull, as the penis protrude the penile and the prepenile sheath is inverted over the protruded penis, the penis then spiraled due to the stretching of the penile integument. The urethral orifice turns counter clockwise through 300° as ejaculation occurs. Intromission in the bull lasts about 2seconds and the straightening of the penis occur abruptly as the dorsal epical ligament re-exact its action in keeping the penis straight as the bull ejaculate.

Withdrawer into the sheath follows as the pressure in the cavernous spaces subsides. Events are similar in the ram, but in boars spiral deviation of the penis is pronounced and copulation lasts about 7minutes during which time the boar ejaculates a large volume of semen. There is no spiral deviation in the stallion but intromission lasts for several minutes. In the dog, a bony structure called the ***os penis*** is formed by the ossification of part of the corpus cavernosum. The end of the penis (***glans penis***) has different shapes among mammals.

.Emission and Ejaculation

The physiology of ejaculation is not clearly known but it is known that emission of semen from ductus deferens is under sympathetic nervous control. The secretion is from the accessory gland and is through muscular action under the autonomic nerves. The secretion of the glandular epithelium is probably under similar control. Ejaculation involves contraction of the striated muscles which are innervated by the sacral nerves. The sequence of erection, emission and ejaculation is under complex nervous control.

Table 9: Regional variation of the bull epididymis

Trait	Ratio of bull epididymis Caput: Corpus: Cauda
Weight	1.0: 0.23: 0.45
Length	1.0: 0.95: 0.47
Sperm reserve	1.0: 0.33: 2.01

Source: Osinowo, 2006

Table 10: Dimensions and Weights of the Components of the male reproductive tract in farm animals.

Organ		Ram	Bull	Boar
Testis	Length (cm)	10	13	13
	Diameter (cm)	6	7	7
	Weight (g)	275	350	360
Epididymis	Length of duct (m)	50	40	18
	Weight (g)	38.6	36	85
Ductus deferens	Length (cm)	---	102	---
Ampulla	Length (cm)	7	15	---
	Diameter (cm)	0.6	1.2	---
Vesicular gland	Length (cm)	4	13	13
	Breadth (cm)	2	3	7
	Thickness (cm)	1.5	2	4
	Weight (g)	5	75	200
Prostate gland	Body (cm)	scattered lobes	3x1x1	3x3x1 (20g)
	Disseminate part (cm)	---	12x1.5x1	17x1x1
Bulbourethral gland	Length (cm)	1.5	3	16
	Breadth (cm)	1	2	4
	Thickness (cm)	1	1.5	4
	Weight (g)	3	6	85
Penis	Total length (cm)	40	102	55
	Length of free end (cm)	4	9.5	18
	Urethral process (cm)	4	0.2	absent
Sheath (preputial diverticulum volume about 100ml)	Length (cm)	11	30	23

Source: Adapted from Hafez and Hafez, 2000 and Osinowo, 2006.

Sperm maturation and storage capacity

It is well established that mammalian spermatozoa emerging from the testis cannot yet fertilize ova and only acquire this ability during their transit from the caput to the cauda epididymides, where they are stored and whence they can be expelled at ejaculation. Both maturation and storage of mammalian spermatozoa appear to depend on a special environment created by the androgen-dependent activities of the epithelium of the epididymis. These activities ensure the prolongation of sperm life to maximize the capacity for the storage of functional spermatozoa.

Thus sperm maturation is composed of an array of processes that occur during sperm transit along the epididymis and leads to the acquisition of fertilizing capacity by spermatozoa. It lasts from eight to fifteen days in the bull, ram, buck and boar and involves physiological, morphological, biochemical and biophysical changes, such as a loss of phospholipids, modification of accessible surface proteins and membrane-bound enzymes, an increase in motility and cyclic AMP levels and the appearance of carnitine. All factors responsible for the forward motility of sperm occur in the caudal region of the epididymis although forward motility has been observed in spermatozoa liberated from preceding regions of the epididymis in boar and rat. A specific protein called forward motility protein was identified in the caudal region of the epididymis to be responsible for this forward propulsion. Sperm motility depends on the coordinated propagated flagellar wave under acetylcholinestrase control, progressive sperm motility is gained with a concomitant loss of acetylcholinesterase activity in the caudal epididymis i.e. as spermatozoa gain ability for progressive motility there is a reduction in acetylcholinestrase (AChE) activity.

Moreover, since lactate dehydrogenase (LDH - lactate: NAD oxidoreductase, EC.1.1.1.27) consisting of five isoenzymes, LDH_{1-5}, catalyses the reversible reaction between lactate and pyruvate, and pyruvate is the preferred substrate that is essential for the activity and survival of spermatocytes and spermatids. It should be noted that LDH content of sperm reduced significantly as they move from testes to the caput epididymis after which only insignificant changes was observed. Testicular sperm which are composed more of the fastest anodically-migrating isoenzyme (LDH_1) while with sperm maturation, the least or slowest migrating isoenzymes (LDH_4 and $_5$) are progressively more dominant, i.e. from the distal corpus epididymis to the cauda epididymis LDH_5, followed by LDH_4 and $_3$ are more frequent than LDH_1 and $_2$. This preponderance of $LDH_{5,4}$ and $_3$ in this order in spermatozoa at the advanced stages of sperm maturation and during sperm storage in cauda epididymis as been suggested to indicate changes in the physiological state of sperm during epididymal transit from aerobic to anaerobic metabolism. This may be involved in the maintenance of quiescence in matured sperm stored in the cauda epididymis. LDH-C_4 is related to sperm count and motility by being involved in energy-supplying metabolic processes, constituting about 80%of the total LDH activity in mature spermatozoa.

The presence of sperm in the testes and epididymides of a pubertal or adult male animal is often referred to as sperm reserves or sperm storage. Testicular and epididymal sperm reserves are highly influenced by the age of the animals and the weight of the organs. There is a directly proportional relationship between organ weight and sperm reserves. The distribution of spermatozoa in the epididymis was more in the cauda followed by the caput and least in the corpus.

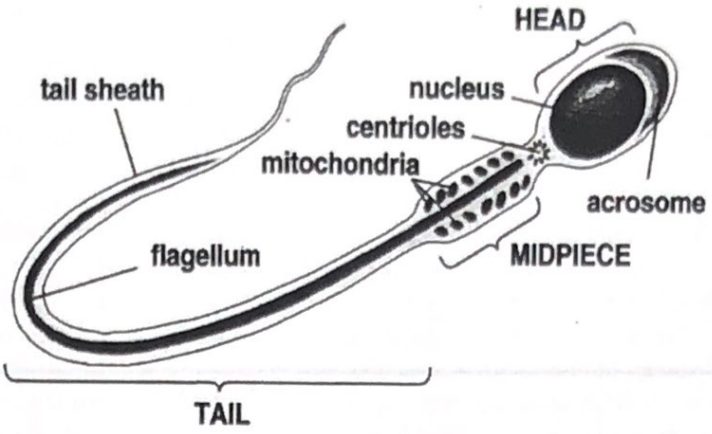


Figure 51.0: Structure of a spermatozoon

SPERM FORMATION PROCESS

Spermatogenesis is the sum of the transformation that results in the formation of spermatozoa from spermatogonia while maintaining spermatogonial numbers. It is the process by which spermatozoa are formed which commences at puberty. It occurs in the **seminiferous tubules** which contain two typesof cells **sertoli cells** and **spermatogonia.** The sertoli cells which are larger and less numerous, are glycogen rich cells that serves to provide mechanical support/ nutrition for the germ cells, control germ cell migration/ spermatid release, secrete androgen-binding protein and remove excess cytoplasm from the spermatid as it changes to sperm. The spermatogonia which are much smaller and numerous are the potential gametes. The total testicular population of stem A_1 spermatozoa is highly correlated with that of sertoli cell ($r > 0.65$). Therefore the factors controlling sertoli cell multiplications in early life could later indirectly influence the sperm production per testis (Fig. 53.0).

The total number of sertoli cells is established some time before puberty, between 1 and 2 months of age in all species. It varies from about 1×10^6 at 40 days of foetal life to 0.5×10^9 at birth and to 6×10^9 in adult rams. These multiplications are under gonadotropin control. An increase in the sertoli cell population correlates with that of plasma gonadotropin levels.

The total numbers of cyclic A_1 or active spermatogonia per testis increase during puberty and could be modified in the adult by endocrinological environment, season, hemi-castration or hypophysectomy.

A_1 spermatogonia renewal appears to be mainly under follicle stimulating hormone (FSH) control. FSH controls the last B-type spermatogonial divisions and spermatocyte formation, while luteinizing hormone (LH) or testosterone control those from A_1 to intermediate spermatozoa.

Spermatogenesis takes about 7 weeks (49 days) in rams, requires an additional 7 to 10 days in bulls (8 weeks). As spermatogenesis proceeds, the developing gametes migrate from the basement membrane of the seminiferous tubules toward the lumen. The output of spermatozoa per day is about eight billion for ram, four billion for beef bulls, seven billion for dairy bulls, fifteen to twenty billion for boars and ten billions for stallions. The actual production of spermatozoa may be fifty to one hundred percent higher because all that are produced cannot be collected.

Spermatogenesis can be divided into two distinct phases **spermatocytogenesis** and **spermiogenesis.**

Spermatocytogenesis the first step in spermatocytogenesis is a mitotic division of a spermatogonium forming a dormant and an active spermatogonium. The dormant spermatogonium remains in the germinal epithelium near the basement membrane to repeat the process later. The active spermatogonium undergoes four mitotic divisions leading to the eventual formation of sixteen primary spermatocytes. These mitotic divisions are completed in 15 to 17days in rams. Later each primary spermatocyte undergoes a meiotic division leading to the formation of two secondary spermatocytes. In this division the chromosome complement in the nucleus is reduced by half so that the nuclei in the secondary spermatocytes contain unpaired (n) chromosomes. This step requires approximately 15 days (Table 11). Within a few hours after their formation each secondary spermatocyte again divides into 2 spermatids. Thus, 4 spermatids are formed from each primary spermatocyte (64 from each active spermatogonium). All the stages of spermatocytogenesis require about thirty-one to thirty-two days to occur in rams.

Spermiogenesis - This is the phase when spermatids undergo metamorphosis (change in morphology) to form spermatozoa. During this phase spermatids are attached to sertoli cells. In metamorphosis, the nuclear material compacts in one part of the cell to form the spermatozoon head while the rest of the cell enlongates to form the tail. A cap around the head of the spermatozoon called **acrosome** which is rich in mucopolysaccharides and contains two enzymes **hyaluronidase** and **proteases** which digest the cuticle that surrounds the ovum is formed from the golgi body of the spermatid. Cytoplasmic droplet appears on the neck of the spermatozoon as the cytoplasm of the spermatid is cast off during the tail formation. The tail contains spermosin which is similar to myosin. The middle piece is rich in mitochondria.

The sperm synthesizes ATP by both aerobic and anaerobic routes. Newly formed spermatozoa are then released from the sertoli cell and forced out through the lumen of the seminiferous tubules into the rete testis from where it is passed into the epididymis via the vasa efferentia. Spermiogenesis is completed in 15 17days. Spermatozoa are unique cells that possess no cytoplasm and become progressively motile after maturation. Sperms in the seminiferous tubules are non-motile even when suspended in oxygen-rich fluid. They mature in the epididymis which is the major storage site. The sperm moves by lashing movements of the tail which are in amplitude until the final tail piece is reached. The fibrils are presumably responsible for the movements and the constancy of the amplitude of the movement suggests that energy must be imparted throughout the length of the tail. ATP and the enzyme responsible for breaking it down have been found throughout the tail. The process of spermatozoa maturation occurs in the epididymis where it gains the ability to be motile/ fertilize and lose its cytoplasmic droplet. Spermatozoa cannot penetrate the zona pellucida of the ovum and participate in fertilization except it undergoes a second maturation (capacitation) in the female tract which involves the removal of the lipoprotein layer from the spermatozoon surface, permitting the release of the enzymes hyaluronidase and corona penetrating enzyme necessary for penetration.

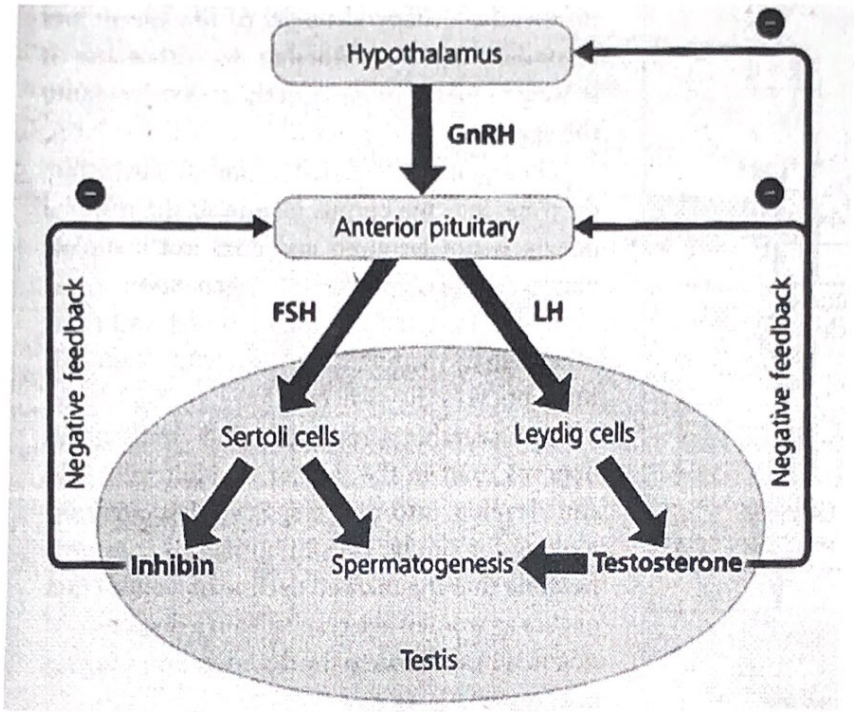


Figure 52.0: Hormones of the male reproductive system

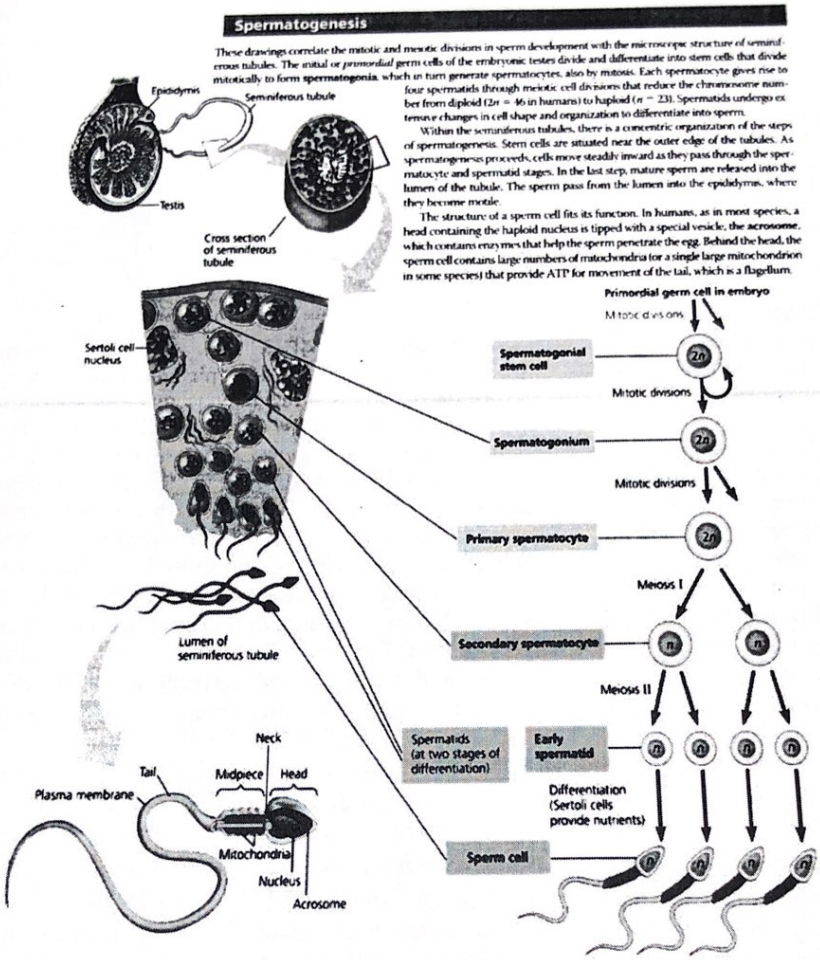


Figure 53.0: The Sperm production process

Table 11: Duration of seminiferous epithelial cycle and spermatogenesis in some species

Species	Seminiferous cycle duration (days)	Spermatogenesis duration (days)
Man	16.0	75.0
Bull	13.5	60.0
Stallion	12.2	-
Ram	10.3	42.0
Boar	8.6	-
Mouse	-	35.0
Rat	-	48.0

Source: Adapted from Cole and Cupps, Reproduction in domestic animals, p. 222

SEMEN

Semen is made up of spermatozoa and secretions from the accessory reproductive organs, which serve as a vehicle for spermatozoa as well as providing an environment with adequate pH for optimum metabolic activities. The secretions from the accessory glands provide substrates that are essential for metabolic activities and motility of the spermatozoa. The characteristics and composition of semen changes in disease conditions resulting in fertility and sterility problems. The seminal plasma is produced by the accessory glands with a small portion supplied by the spermatozoa concentrate. The seminal vesicle contributes 60% of the semen, the prostate gland 20%, the bulbourethral gland about 1% and the rest is made up of spermatozoa. Freshly emitted semen first clots then liquefies. The function of the clotting is unknown although in some mammals it forms a vaginal plug which prevents the leakage of sperm. The seminal plasma serves as a buffered nutrient medium which suspends and maintain the fertility potential of the spermatozoa. It is slightly acidic in rams and bulls with an osmotic pressure similar to that of blood (equivalent to physiological saline 0.9% NaCl).

Seminal plasma is composed of a number of organic and inorganic compounds. The major organic ion found in semen is bicarbonate, produced by the vesicular glands and it functions as a buffering agent, regulating pH changes of semen. Buffers are often not present in sufficient quantities to prevent a reduction in semen pH when in storage; therefore a good semen extender should have buffering capacity. Other organic compounds that serve primarily as energy substrate include fructose, sorbitol and glycerylphosphorylcholine (GPC). Fructose (a simple sugar) and sorbitol (a sugar alcohol) are produced by the vesicular glands while GPC is produced in the epididymis. Fructose is the major substrate utilized for energy production in bull and ram semen. It is utilized by spermatozoa as an energy substrate both under anaerobic (storage) conditions and aerobic (as in the female tract) conditions. Sorbitol and GPC can only be utilized under aerobic condition.

Lactic acid, a by-product of anaerobic metabolism of fructose builds up in stored semen and becomes available as energy substrate in aerobic condition. Inositol and citric acid are also found in large concentrations in seminal plasma but do not function as energy substrate. The buildup of lactic acid produced in anaerobic metabolism of fructose reduces the pH of semen, thus affecting sperm viability. Hence the need to slow down sperm metabolism during storage by reducing the temperature, regulation of pH, monitoring of osmotic pressure, regulating spermatozoa concentration, gases, light, hormones and microbial level.

The major inorganic ions found in seminal plasma are sodium and chlorine, though smaller quantities of calcium and magnesium have been reported. Potassium, though present in substantial amounts in whole semen was found mostly in spermatozoa than in the seminal plasma. These inorganic ions are necessary for the maintenance of osmotic pressure necessary for spermatozoa survival.

Table 12: Average chemical composition of semen from different species (mg/ 100ml).

Constituents	Bull	Ram	Boa	Stallion
Fructose	530	250	13	2
Sorbitol	75	72	12	40
Glycerylphosphorycholine	350	1650	175	70
Inositol	35	12	530	30
Citric acid	720	140	130	26
Ergothionine	0	0	15	75
Plasmalogen	60	380	-	-
Sodium	230	190	50	70
Potassium	140	90	240	60
Chlorine	180	86	330	270
Calcium	44	11	5	20
Magnesium	9	8	11	3

Source: Bearden and Fuquay, 1980.

Table 13: Ejaculate characteristics of semen from farm animals

	Bull	Cattle			
Trait	Dairy	Beef	Ram	Boar	Stallion
Volume, ml	6	4	0.8-1.2	225*	60*
Sperm concentration, $x10^9$/ml	1.2	0.6-2.0	2.0-3.0	0.2-0.3	0.15
Total sperm, $x10^9$ (billion)	7	4	3	45	9
Motile sperm, %	70	50-75	60-80	50-80	70
Mophologically normal sperm, %	80	65-95	80-95	70-90	70
pH	6.5-7.0	6.5-7.0	5.9-7.3	6.8-7.5	6.2-7.8
Protein, g/100ml	-	6.8	5.0	3.7	-

*Gel free portion in boar/stallion, the Cowper's or Bulbourethral glands produce the gelatinous mass that forms the post sperm fraction.

Source: Adapted from Setchell et al., 1977 and Osinowo, 2006

There is increased sperm producing capacity with age with weekly sperm output for bulls on 6x weekly collection increased 2.4 times from 14 billion to 33.6 billion at 1 and 2 years of age respectively. The volume and composition of semen vary from individual to individual and are related to the degree of abstinence and sexual excitement. After a few days of continence, 2-5ml of semen containing100million sperms per ml is ejaculated in man. Frequent ejaculation tends to reduce the volume and possibly the count. Prolonged continence has the same effect. A sperm count below 60million/ml seriously impairs fertility while fertilization is almost unknown with a count below 20million/ml in man. Sperm output depends on age, species, breed, season, scrotal size, frequency of ejaculation and degree of sexual preparation.

MATING BEHAVIOUR TEST

Effect of substances (extracts) on mating behaviour can be studied according to the methods described by Gopumadhavan*et al.*, (2003) and Tajuddin*et al.*, (2005). Healthy and sexually experienced male animal should be selected for the study. A male is considered sexually active if it attempted to mount any active female introduced into the pen. The male should be made familiar to the pen 14days before the test begins. Sexually active female is introduced to the male in the pen. The female should be induced into oestrus by a simple intramuscular injection of oestradiolvalerate at a dose of 1.5ml/doe intramuscularly 48h before the test. The receptivity of the female animal should be confirmed before the test by exposing it to male animal other than the test animal. The most receptive female should be selected for the test. The receptive female should be introduced into the male pen with one female to a male. The observation for mating behavior should be immediately commenced and continued for first 2 mating series. The test should be terminated if the male failed to show sexual interest. The occurrence of events and phases of mating should be called out and recorded on an electronic recorder as soon as it is observed. Their disappearance should also be called out and recorded. The frequencies and phases can be determined from recorder transcriptions.

a. Mounting frequency (Mf) Is the number of mounts in a given period of time (15min) i.e. number of mount before ejaculation.
b. Mounting latency (MI) Is the time taken for the first mount after the introduction of female into the male pen.
c. Intromission frequency (If) This is the number of intromission before ejaculation.
d. Intromission latency (IL) This is the time taken for the first intromission after the introduction of the female into the male pen.
e. Ejaculation frequency (Ef) This is the number of ejaculations in a given period of time (15min).
f. Ejaculation latency (EI) This is the interval between the first mount to the first ejaculation.

g. Total Sexual Behaviour (TSB) Male sexual behaviour such as genital grooming and sniffing at females should be visually monitored and recorded.

In the second mating series only the ejaculation latency should be recorded.

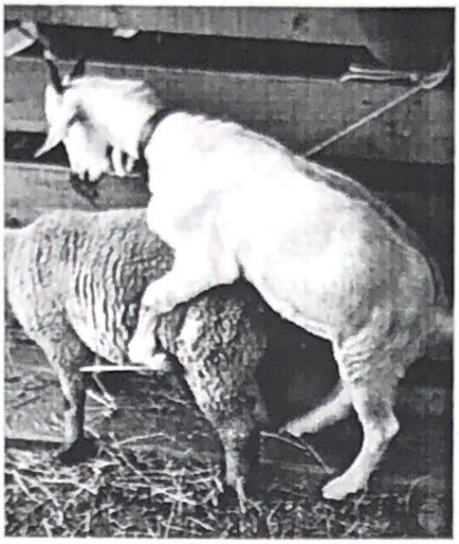


Figure 54.0: Mating of ewe by a buck

THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive system is made up of the ovary and the tubular genitalia (oviduct, uterus, cervix, vagina and external gernitalia). Structurally the tubular genitalia basically consist of serosa, longitudinal muscle layer, stratum vasculare, circular muscle layer and mucosa.

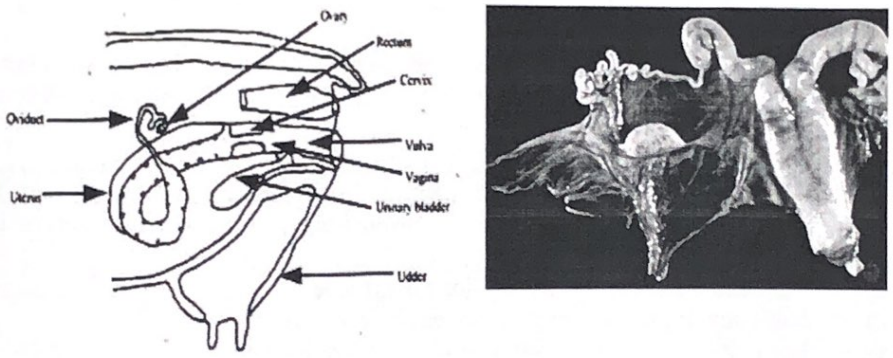


Figure 55.0: Structure and photograph of the female reproductive system

1. THE OVARY: is the primary sex organ of the female, it produces the ova and female sex hormones - estrogen and progesterone. Estrogens are produced by maturing follicle while progesterone is produced by the corpus luteum (CL) i.e. the remnant of the ovulated follicle. The ovary is made up of the cortex and medulla. The cortex and the medulla are surrounded by the germinal epithelium. The cortex is the site of both egg formation and hormone production.

The cortex contains follicles that are at various stages of development and with corpus luteum (CL) left after ovulation. The medulla contains many fibroelastic connective tissues, blood vessels and nerve supply which enter the ovary through the hilus. Also within the cortex are fibro-elastic connective tissues, collagen and reticular fibres, blood vessels, nerves and some smooth muscle fibers. At various stages there are ovarian follicles. Follicles are continuously developing in the ovary and the stages of the Graafian follicle lasts about 3days out of the 21days life cycle. When the Graafian follicle ruptures the ovum is released, leaving a shell called corpus haemorrhagicum.



2. THE OVIDUCT: This is the connecting tube between the ovary and uterus. It is divided into three major parts- infundibulum, ampulla and isthmus. It has various functions such as

a) Collection of eggs or ova from the ovary after ovulation.
b) Transportation of the ova to fertilization site (ampullary-isthmus junction)
c) Serves as a fertilization site i.e. provision of suitable environment for fertilization
d) Habouring of eggs till fertilization takes place
e) Early development of embryo takes place in the oviduct
f) The upper portion transport sperm to fertilization site
g) Sperm capacitation takes place at the lower portion.

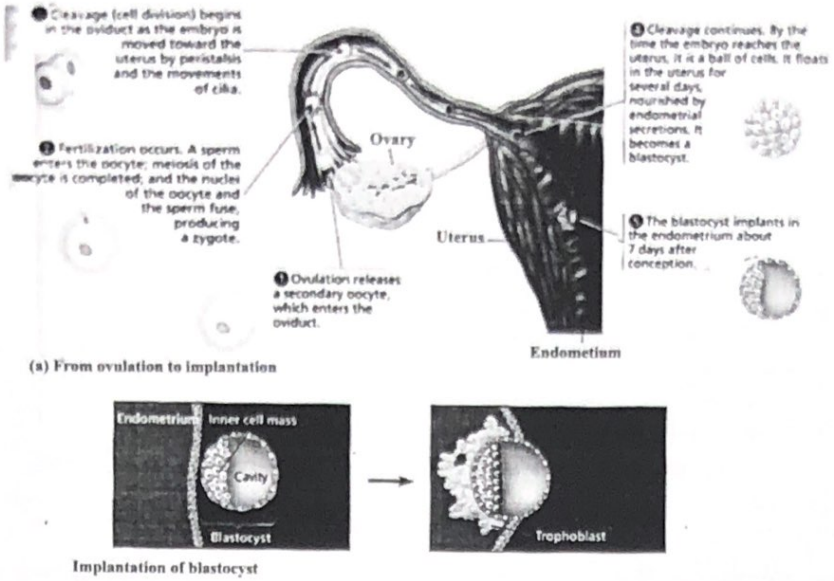


Figure 56.0: Egg formation and fertilization process

The oviduct has the following points:

1. Infundibulum: is the first region of the oviduct always in constant motion at the time of ovulation. It's the area that normally collects the egg after ovulation, it is funnel shaped. It has fimbrae at its end which either envelope the ovaries or are very close to the ovaries and helps in egg collection.
2. Ampulla: is the area below the infundibulum, it forms about half the length of the oviduct which merges with the isthmus at the ampullary-isthmic junction where fertilization/ early cleavage of zygote take place.
3. Isthmus: is the connection between the ampulla and the uterine horn or body. Secretory cells and ciliated cells are less in the isthmus. It is the area where fertilization takes place; the cells are for transport of ovule and sperm.
4. Utero-tubal junction: is the portion below the isthmus, connecting it to the uterine horn or uterine body. The utero - tubal junction controls the movement of sperm into the oviduct as well as the fertilized egg into the uterus. The transportation of the egg into the oviduct is enhanced by the contractile action of the unstriated muscle of the oviduct.

Physiology of the Oviduct

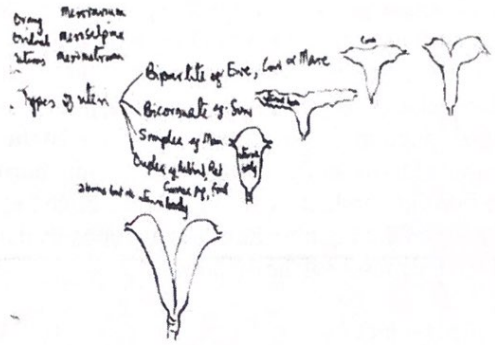
The fimbrae transport ovulated egg into the infundibulum. Eggs are then transported to the ampullary - isthmus junction where fertilization takes place. The oviduct fluid provides a suitable environment for fertilization, early embryonic development and capacitation of the sperm. Fertilization and cleavage of the fertilized egg takes place at the isthmus.

The lumen of the oviduct is lined by much folded mucous membrane with simple columnar/ ciliated epithelial lining. The embryo spends about three days in the oviduct before being transported to the uterus.

3. THE UTERUS: The uterine wall is made up of a serous membrane, myometrium (having internal circular muscle, external longitudinal muscle and vascular layer) and endometrium (having glandular layer, epithelial lining of lumen and connective tissue) It performs the following functions;
   a. It is the place where the embryos develop i.e. gestation.
   b. Functions for sperm transport i.e. contraction of the myometrium.
   c. For sperm capacitation, although this is not true in all species.
   d. Function by contracting for foetal expulsion i.e. Parturition.
   e. Its secretion of prostaglandin causes luteolysis.
   f. Uterine fluid provides nutrient for zygote before implantation.

In some species it is divided into the uterine horn and uterine body. Uterus generally consists of two uterine horns (cornua), a body and a cervix (cervical neck). Generally there are about 4 types of uterus

- **bipartite uterus**: has prominent uterine body and the horn are separated by a septum. Both sides are attached to the pelvic and the abdominal wall by broad ligament e.g. as found in the Ewe, Cow and Mare.
- **bicornuate uterus**: has a long folded or convoluted uterine-horn and a short uterine body. Attachment is similar to that of the Cow except that the uterine- horns are long and meander e.g. as found in Sow.
- **Simplex uterus**: has a prominent uterine-body and cervix with the absence of uterine-horns e.g. primates (man).
- **Duplex uterus**: Have no uterine-body but completely separated uterine-horns (two horns) with two cervices e.g. rabbit, fowl, rat, guinea pig.



Blood supply to the uterus

The uterus receives its blood and nerve supply through the broad ligament. The uterine artery comprise of the internal and external iliac arteries which supplies the uterus generally. The ovaries and oviduct are supplied by autonomic nerves. The pudic nerves supply sensory fibres to the vagina, vulva and clitoris.

Anatomy of the uterus: compose generally the same types of tissues. A section of the uterus shows that the cow and Mare have no caruncle.

Myometrium: Is the muscular portion of the uterine wall. Its contraction during mating greatly facilitates sperm transport from the site of ejaculation to the site of fertilization. Its vascular layer carries important blood vessels supplying the uterus. The degree and frequency of movement of the uterus depends on the stage of the oestrous cycle and the rhythmic movement of the ovary and oviduct. The contraction increases in amplitude and frequency at oestrus and at parturition but the uterine muscle is quiescent during pregnancy.

Endometrium: Is a highly glandular structure consisting of epithelia lining of the uterine lumen. It has glandular layers which are secretory connective tissue. The thickness of the endometrium varies in vascularity with the ovarian hormonal changes and the state of pregnancy in the animal. Caruncle is found in the inner surface of uterus serving as the areas for the attachment of the placental chorionic villi (cotyledon) i.e. the placentome is made up of cotyledon and caruncles. The uterine areas are highly enriched with blood vessels. Uterus of ruminant such as cow has between 70-100 caruncles arranged in 4 rows of non-glandular projections (caruncles) between the uterine-body to the uterine-horn. Each caruncle measures about 15mm in non-pregnant cows but may measure up to 10cm during pregnancy and at this time they appear spongy due to the numerous crypts that receives the placental chorionic villi. These villi develop in localized areas referred to as the cotyledons which invades the caruncle. The cotyledons and caruncles are together referred to as plancentum. It can now be seen that the caruncle is a maternal tissue whereas the cotyledon is the foetal tissue. The caruncle is the base of attachment between the mother and foetus. It is highly vascularised. The uteri of the mare and sow have no caruncles but the mucosae have longitudinal folds.

Uterine glands

These are scattered within the endometrium except in the caruncle. They are branched, tubular and considerably coiled (convoluted) especially toward the ends of the uterine glands. The density varies with species, parity (stage of birth or number of pregnancies) and stage of oestrous cycle. The variation during the oestrous cycle is due to the diameter changes and the amount of stroma changes. There are higher numbers of glands in the uterine-horns than in the mucosa adjacent to the cervix. In animals without caruncle which are said to have diffuse placentation during pregnancy the gland serves to nourish the foetuses. Endometrial gland secretions and uterine transudates form the uterine fluids which provide nutrition for the zygote till implantation is complete.

Cyclic changes in Endometrium

The endometrial changes are under the control of ovarian hormones. At proestrus, endomentrium is under oestrogen, vascularity increases, surface epithelial cells becomes columnar and the glands increases in size. In early dioestrus endometrium increases in thickness, epithelial cells becomes more columnar, gland increases in size, becomes highly branched, tubular and coiled, there is maximum secretory activity. At this stage it is under progesterone which is produced by the corpus luteum. In late dioestrus, endometrium and all the glandular epithelium cell shrinks; endometrium is shedded and development in the uterine wall dies off. In primates this is the time that there is a menstrual flow.

In summary, the endometrium undergoes cyclic changes under the control of ovarian hormones, increasing in thickness, vascularity and secretory activities towards oestrus and regresses in late dioestrus. There is cyclic shedding and regeneration of the surface epithelium of the endometrium. There is a tremendous change in uterine size, structure and position during pregnancy. After parturition, uterine involution occurs and uterus returns to its normal size.

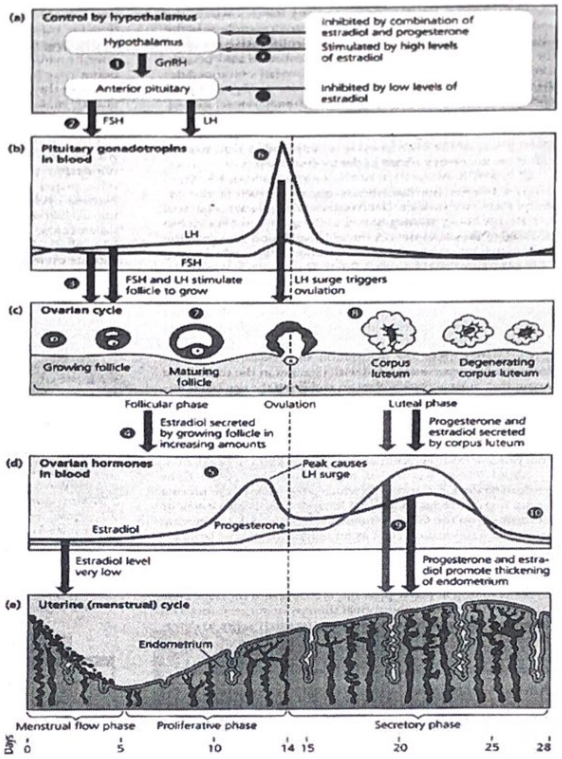


Figure 57.0: The reproductive cycle in primate

4. THE CERVIX: Is the portion between the uterus and the vagina. It is a cylindrical structure found within the pelvix of the female reproductive structure. The cervix is a sphincter-like structure projecting caudally into the vagina, characterized by a thick wall and constricted lumen. The structure differs between species. In ruminants cervical lumen is lined with transverse prominences or spirally interlocking ridges called annular ring. This rings which are prominent in the cow are usually four, which normally fit together to close the cervix securely. The four annular folds protect pregnancy in the cow (Fig. 58.0). Secretions and cilia are necessary for sperm transport. In the sow, the annular-rings are cork screw shaped, an arrangement adapted to the spiral twisting of the tip of the boars' penis. There is no secretory gland in the sow but there are secretions from the cervical mucosa. Usually the mucosa is made up of two types of columnar epithelia cells ciliated columnar epithelia cells and non-ciliated epithelia cells. The nonciliated cells are usually secretory, while the ciliated cells are not. The secretion is mucus which changes in amount and viscosity with the stages of the oestrous cycle. The cervical wall is primarily of fibral, elastic and collagenous tissue with small amount of muscle.

Functions

a. The primary function is to prevent intruders from entering into the uterine lumen due to its annular fold being tightly held together. However this tightly annular fold normally relaxes during oestrus to permit sperm entrance into the uterus.
b. Cervix also acts as sperm reservoir, so providing the upper tract with subsequent release of sperm.
c. It's also possible that the sperm trapped in the cervical crypts are never released, thus preventing excess sperm reaching the site of fertilization.
d. During pregnancy the cervix secretes mucus which hardens to form cervical-plug that normally seals off the cervical canal, thus preventing uterine infection.
e. During parturition the cervical-plug liquefies and the cervix dilates to allow for the expulsion of the foetus.
f. In sow, it is penile receptacle i.e. it receives the penis.

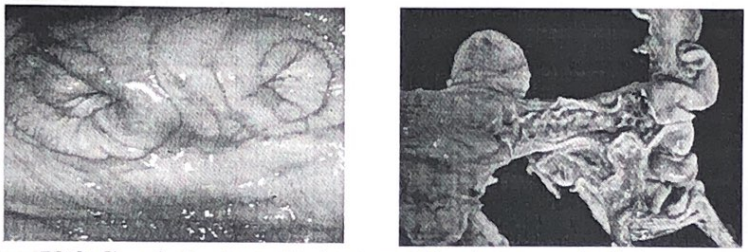


Figure 58.0: Cervix (external os); opened to show cervical ring (dark pebble-like structures)

5. THE VAGINA: The vagina is a well innervated tube that extends from the urethral opening to the cervix. It's the female organ of copulation that receives the penis and also serves as the passageway for foetus and placental expulsion (Fig. 59.0). Its walls are made of glandless, stratified, squamous epithelium consisting of mucosa, muscularis and serosa. In cow some mucous cells are present. In general, the mucosa responds readily to gonadal hormones (increase under the influence of oestrogen but reduce under the influence of progesterone). Cyclic changes occurring in the vaginal epithelium can be monitored by the **vaginal smear technique**. Ovulation can be induced by nervous stimulation of the vagina in rats and rabbit.

Function:

a. It is the organ of copulation for the female, it receives the penis.
b. Serves as passageway for foetus during parturition.
c. Serves as passageway for placental delivery.
d. In most species the vaginal environment is not conducive to sperm survival.

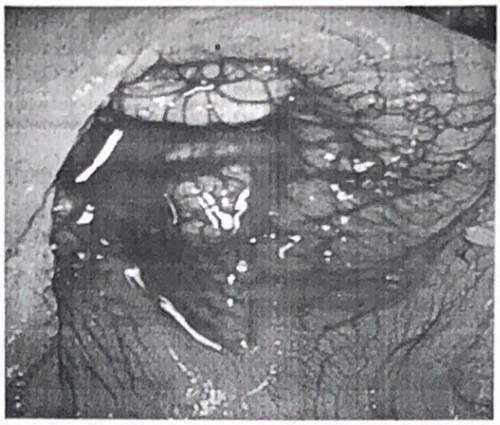


Figure 59.0: Vagina showing bleeding

6. THE EXTERNAL GENITALIA: The anatomical name for the area of the female external genitalia is the vulva (Fig. 60.0). It is made up of several structures which surround the entrance to the vagina, and each of which has its own separate function. It is the passage of urine, foetus, receives the penis and serves as sexual stimulation. Between the vulva and the vagina is the vaginal sphincter. The external genitalia are made up of **vestibule, labia majora, labia minora and clitoris.**

i. Vestibule: This is the area between the vagina and the outside wall of the vulva. The cleft below the clitoris and between the labia minora is called the vestibule (entrance). Just below the clitoris is the external opening of urinary tract (urethral orifice) which connects the bladder to the outside world. Below the urethral orifice is the hymen, which surrounds the vaginal orifice. The hymen is a thin incomplete fold of membrane, which has one or more apertures in it.

It varies in shape and elasticity but generally stretched or is torn, disappears or becomes vestigial when intromission occurs at first mating. Parturition causes a much greater tearing of the hymen and after delivery only a few tags remain. Just outside the hymen, still within the backward part of the vestibule are two pea-sized glands (vestibular or Bartholin's gland) which secrete fluid during sexual arousal and moisten the entrance to the vagina.

ii. Labia majora: or the large lips of the vagina. The labia majora are two large folds of skin which contain sweat glands and hair follicles embedded in fat. In young and old animals they are small and lack fat but well filled with fatty tissue in the reproductive years. The inner surfaces are free from hair and separated from the thin labia minora which guard the entrance to the vagina by a small groove.

iii. Labia minora: the small lips. They are delicate folds of skin which contain little fatty tissue. At one end it split into two folds with one passing over and the other under the clitoris and joins at the opposite end toward the anus to form the fourchette which could be torn during parturition. It is hidden by the enlarged labia majora in the reproductive years but in young and old animals the labia minora appear more prominent because the labia majora are relatively small. Its core is made of spongy connective tissue with large sebaceous glands on the surface. External female reproductive portions - the libiamajora and libiaminora have secretory function apart from serving as a passage for the penis.

Iv. Clitoris: This is the structure derived from the same embryonic structure as the penis. It is similar to the penis in both structure and anatomy. It is made up of tissue which fills with blood during sexual excitement. It is a sensitive organ that is well supplied with nerve and blood vessel and is involved in sexual stimulation. It becomes turgid when the animal is sexually

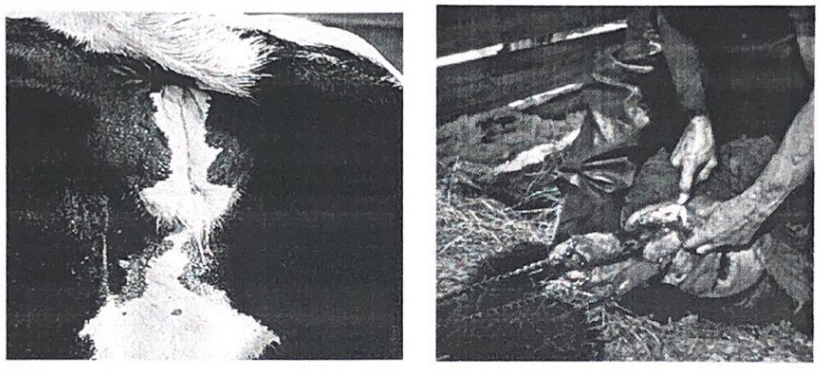
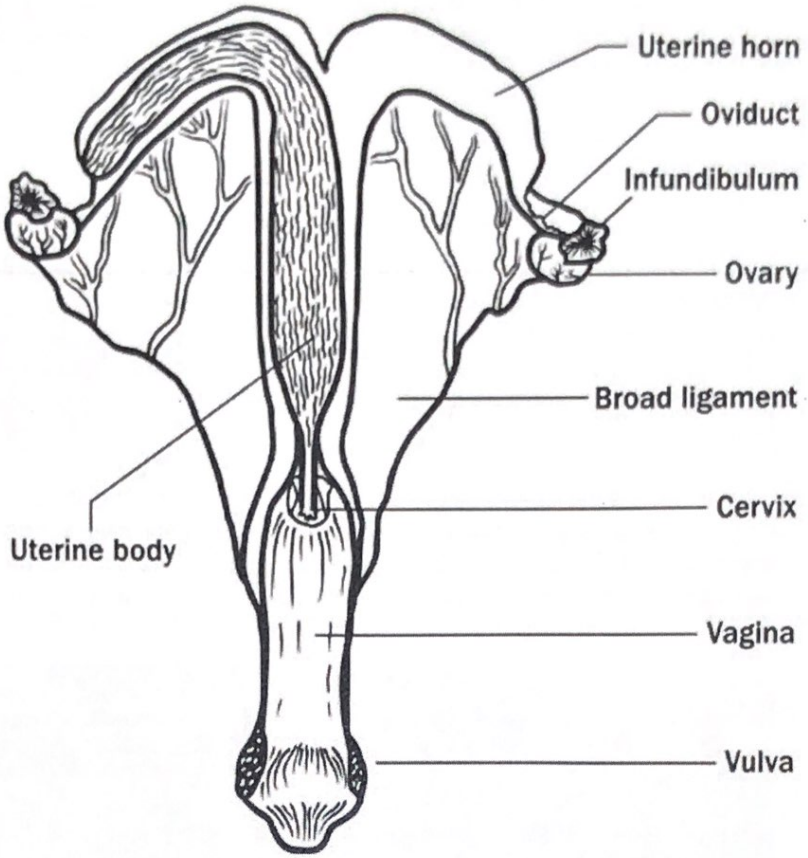


Figure 60.0: Vulva of a cow; Vulva incision to aid parturition (episiotomy)

The whole productive tract in the female are attached into the body by the broad ligament, this structure has the following anatomical parts

a. Mesovarium which supports the ovary.
b. Mesosalpinx which supports the oviduct.
C. Mesometrium which supports the uterus.



MARE REPRODUCTIVE ORGANS

CHAPTER NINE

ENDOCRINOLOGY

Body functions are regulated in two major ways

1. Through the **nervous system** which is fast and short acting and
2. Through the **endocrine system** which is slow and long acting.

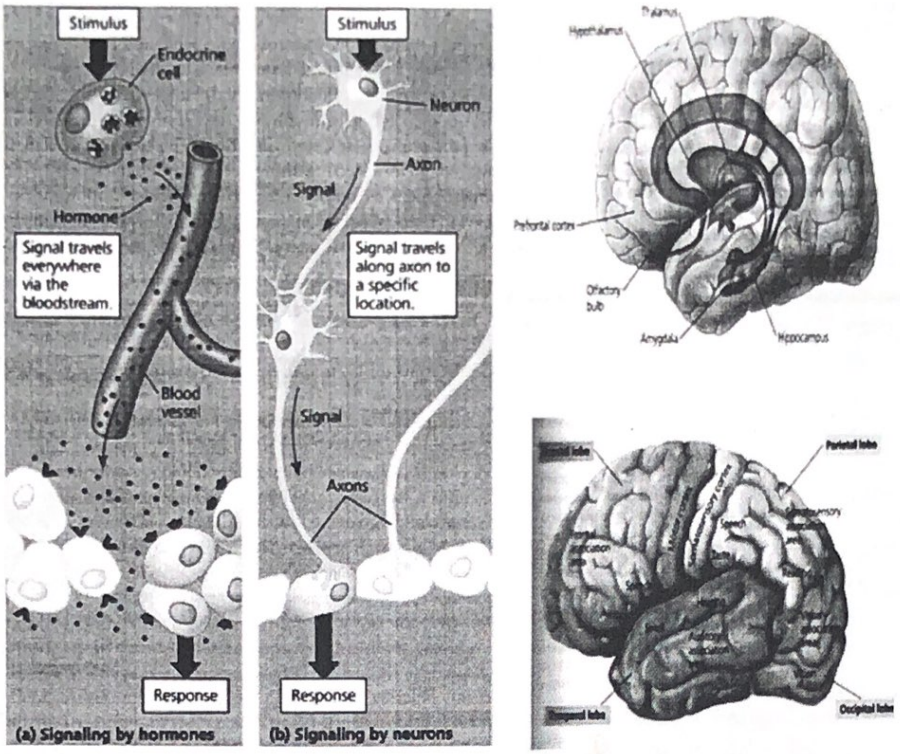


Figure 61.0: Regulation of body functions by hormones and neurons; and the limbic brain.

The study of endocrine gland was started by Starling in 1905 who applied the word "hormone" to secretin, a chemical, secreted into the blood stream which regulated the flow of pancreatic fluid. Thus an endocrine gland was defined as a normal tissue that is ductless and secretes chemical messengers (hormones) directly into the blood. As a result of this definition, some tissues which secrete hormones e.g. alimentary canal, were omitted from being considered as endocrine glands.

A new definition was therefore given by Grossman in 1979 which defined hormones as chemical principles, released by regulator cells directly into the blood system to regulate the activity of specific target cells that do the actual work. The cells that produce the hormones are called regulator cells. They sense specific changes in their extracellular environment and respond by sending chemical messages that alter the activity of specific target cells. Such messages are sent in three modes, viz: through nerves (neurocrine), blood borne (endocrine) and diffusion through extracellular spaces (paracrine).

Hormones can be broadly divided into two local and general hormones (Fig. 62.0). Local hormones such as **acetylcholine** released at the para-sympathetic/ skeletal nerve endings and **secretin** released by the duodenal wall and transported in the blood to the pancreas. General hormones are secreted by specific endocrine glands and transported in the blood to cause physiological actions at distant point in the body.

HORMONES OF REPRODUCTION: The ductless glands that comprise the endocrine system influence many vital functions of an animal throughout the life span. The hormone secretions of these glands (hypothalamus, pituitary, ovary, uterus, testis, accessory glands, thyroid, and adrenal gland) can dramatically alter patterns of reproduction, metabolism and growth. That these physiologic functions are of paramount importance in livestock production is widely recognized.
Hormones are chemical agents synthesized and released from specialized ductless (endocrine) glands and carried in the blood to target tissues to evoke adjustment.

The importance of hormone can be broadly categorized to include the following:

a. Development of life (morphogenesis): Hormones such as oestrogen causes uterine growth while testosterone causes the growth of the accessory organs. The presence of a particular hormone helps in sex differentiation in the embryonic stage and subsequently in life.
b. Coordination of physiological events: Hormones assists in the coordination of specific life events such as puberty, oestrus, libido, pregnancy, parturition, lactation, etc. Female animals become sexually receptive during oestrus immediately before or after ovulation with resultant fertilization after mating. The ovarian hormones simultaneously act on the oviduct and uterus as the above events occur to enable fertilization and implantation. Pregnancy cannot occur without proper synchronization of these events.
c. Maintenance of homeostasis: Life functions within a particular threshold and any abnormal variation may result in death. Constancy of the internal environment is partly due to the endocrine system, through the action of hormones like insulin, glucagon, epinephrine and adrenocorticoids.

Gland	Hormone	Chemical Class	Actions	Regulated By
Hypothalamus	Hormones released from the posterior pituitary and hormones that regulate the anterior pituitary (see below)			
Posterior pituitary gland (releases neurohormones made in hypothalamus)	Oxytocin	Peptide	Stimulates contraction of uterus and mammary gland cells	Nervous system
	Antidiuretic hormone (ADH)	Peptide	Promotes retention of water by kidneys	Water/salt balance
Anterior pituitary gland	Growth hormone (GH)	Protein	Stimulates growth (especially bones) and metabolic functions	Hypothalamic hormones
	Prolactin (PRL)	Protein	Stimulates milk production and secretion	Hypothalamic hormones
	Follicle-stimulating hormone (FSH)	Glycoprotein	Stimulates production of ova and sperm	Hypothalamic hormones
	Luteinizing hormone (LH)	Glycoprotein	Stimulates ovaries and testes	Hypothalamic hormones
	Thyroid-stimulating hormone (TSH)	Glycoprotein	Stimulates thyroid gland	Hypothalamic hormones
	Adrenocorticotropic hormone (ACTH)	Peptide	Stimulates adrenal cortex to secrete glucocorticoids	Hypothalamic hormones
Thyroid gland	Triiodothyronine (T_3) and thyroxine (T_4)	Amine	Stimulate and maintain metabolic processes	TSH
	Calcitonin	Peptide	Lowers blood calcium level	Calcium in blood
Parathyroid glands	Parathyroid hormone (PTH)	Peptide	Raises blood calcium level	Calcium in blood
Pancreas	Insulin	Protein	Lowers blood glucose level	Glucose in blood
	Glucagon	Protein	Raises blood glucose level	Glucose in blood
Adrenal glands				
Adrenal medulla	Epinephrine and norepinephrine	Amines	Raise blood glucose level; increase metabolic activities; constrict certain blood vessels	Nervous system
Adrenal cortex	Glucocorticoids	Steroid	Raise blood glucose level	ACTH
	Mineralocorticoids	Steroid	Promote reabsorption of Na^+ and excretion of K^+ in kidneys	K^+ in blood; angiotensin II
Gonads				
Testes	Androgens	Steroid	Support sperm formation; promote development and maintenance of male secondary sex characteristics	FSH and LH
Ovaries	Estrogens	Steroid	Stimulate uterine lining growth; promote development and maintenance of female secondary sex characteristics	FSH and LH
	Progestins	Steroid	Promote uterine lining growth	FSH and LH
Pineal gland	Melatonin	Amine	Involved in biological rhythms	Light/dark cycles

Figure 62.0: Major endocrine glands

CLASSIFICATION OF HORMONES

Hormones can be classified on the basis of

- Effects on reproduction i.e. direct or indirect effects
- Site of origin or production e.g. gonadal hormone, hypophysial hormone, etc
- Chemical types or properties e.g. steroid hormone, protein or peptide hormones
- Types of responses evoked in target organs e.g. growth hormone, reproductive hormone, lactation hormone, parturition hormone, etc.

1. Classification on the basis of effects on reproduction

a. Primary or type I hormone: These are hormones with direct effects on the various aspect of reproduction such as spermatogenesis, oogenesis, ovulation, sexual behaviour (oestrus, libido, etc), fertilization, implantation, gestation, parturition and lactation. This deals with information and transferring processes. These hormones are peptide in nature, fast acting, effective for short duration and effective through the cyclic AMP (adenosine monophosphate). These hormones include follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, oxytocin, testosterone, inhibin, oestrogen, progesterone, relaxin, pregnant Mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), prostaglandin (PGF2Ü) and all hypothalamic releasing hormones such as gonadotropin releasing hormone (GnRH), thyrotropin releasing hormone (TRH), corticotrophin releasing hormone (CRH), growth hormone releasing factor (GH-RF), prolactin releasing hormone (PRH) and prolactin inhibiting hormone (PIH). Other type-1 hormones include epinephrine, glucagon, insulin, gastrin, secretin, parathormone, thyrocalcitonin, etc.

b. Secondary or type II hormones: These are hormones necessary for the developmental and maintenance function of the animal such as growth, development, metabolism, etc without which an efficient reproduction cannot occur. Such hormones are slow acting, have persistent effects for long duration, have permissive effects and act through protein synthesis. These hormones include growth hormone (GH), somatotropic hormone (STH), thyroid stimulating hormone (TSH), thyroxine, adrenocorticotropic hormone (ACTH), antidiuretic hormone (ADH), insulin, etc.

2. Classification on the basis of the site of origin:

a. Hypothalamic releasing hormones these are produced in the hypothalamus and control the synthesis and release of hormones from the anterior pituitary (adenohypophysis) e.g. GnRH, CRH, PRH and PIH.

B. Hypophysial hormones these are hormones produced in the pituitary (hypophysis) e.g. FSH, LH, prolactin, adrenocorticotropic hormone (ACTH), oxytocin, vasopressin, growth hormone (GH) and thyroid stimulating hormone (TSH).

c. Sex steroid hormones these are produced in the gonads (ovary and testis) e.g. oestrogen, progesterone, relaxin, inhibin, androgen or testosterone.
d. Adrenal hormone these are produced in the adrenal cortex of the adrenal gland above the kidney e.g. Glucocorticoid
e. Pancreatic hormones:these are produced in the islet cells of the pancreas e.g. Insulin
f. Placental hormones these are produced in the cells of the placenta and function in the same way as FSH and act as supplementary corpus luteum e.g. HCG and PMSG. Also produced by the placental are oestrogen, progestins and relaxin.
g. Uterine hormone these are hormones produced by the endometrial cells of the uterus e.g. Prostaglandin

3. Classification on the basis of chemical type/ properties of hormones

The common thing about all the hormones is that they are secreted in gland cells and passed directly into the blood and are transported in free or bound form to target organ or tissues where they controls certain systems. Chemically there could be peptides/ protein, glycoprotein, steroids and fatty acids hormone. Peptides/ protein hormones include adrenocorticotropic hormone (ACTH), oxytocin, hypothalamic releasing hormone, FSH, LH and prolactin while steroid hormones include oestrogen, progesterone, relaxin, inhibin, androgen, glucocorticoid, HCG, PMSG, prostaglandin, etc.

4. Classification on the basis of the types of responses evoked

a. Growth hormone
b. Reproductive hormone
c. Pregnancy hormone
d. Parturition hormone
e. Lactation hormone
f. Libido hormone

Functions of Hormones

1. Releasing hormones: The releasing hormones could be called primary sex hormones. They are produced in the hypothalamus and they control the synthesis and release of the anterior pituitary hormone. There are 4types of releasing hormones.

Gonadotropins releasing hormones- hormones of the hypothalamus producing
- Gn- RH which causes or stimulates the release of FSH and LH by the adenohypophysis (anterior pituitary).
- PIH (prolactin inhibiting hormone)
- PRH (prolactin releasing hormone)
- CRH (corticotropic releasing hormone) - causes the release of ACTH (Adreno corticotropic hormones).

All these hormones are peptides controlling the release of the adenohypophysial (anterior pituitary) hormones.

2. Anterior pituitary hormones: These are
   - FSH (follicular stimulating hormones)
   - LH (luteinizing hormone)

   These are called gonadotropins involved in follicular growth, estrogen release, ovulation and corpus inteum formation in the female. In male FSH also known as ICSH (interstitial cell stimulating hormone) is involved in spermatogenesis and testosterone release.
- Prolactin: Secreted by the anterior pituitary involved in milk synthesis.
- ACTH (Adreno corticotropic hormone) is involved in the release of Gluco corticoid while FSH, LH and prolactin are proteins. ACTH is a polypeptide.

3. Posterior pituitary hormones: this is known as oxytocin. It is a peptide hormone involved in parturition and milk ejection.
4. Sex steroids: the first group of the sex steroids are the ovarian hormones
   - Oestrogen (oestradiol) it is the steroid that is listed and functions in mating behavior, secondary sex characteristics, maintenance of female duct system and mammary gland development.
   - Progesterone Is another ovarian steroid mainly for pregnancy and mammary gland development.
   - Relaxin: This is a polypeptide, functions in pelvic expansion during parturition and cervical dilatation.
   - Inhibin: is a protein functioning in preventing FSH release when not required.

   Testicular hormones are referred to as Androgen and the most common one is testosterone. Others include androstenedione, dehydroepiandrosterone (DHEA), dihydrotestosterone and anabolic steroids.
   - Testosterone: is a steroid functions in male mating behavior, spermatogenesis, maintenance of the male duct system and maintenance of the accessory sex glands.
   - Inhibin is also produced in the testis and function to control the release of the FSH.

5. Adrenal steroids: Produced from the adrenal cortex produces glucocorticoid which is mainly steroids and functions in milk synthesis and parturition.
6. Placental hormones :

a) The first one is the human chorionic gonadotropins (HCG): this is a protein and functions as FSH.

b) Pregnant Mare serum Gonadotropins (PMSG): is a protein, functions as FSH and acts as supplementary corporal luteum in mares

   Also produced by placental are:
   - Estrogen
   - Progestin
   - and Relaxins

7. Uterine hormones: the uterus produces prostaglandins and the popular one in prostaglandin F2(PGF2α) It is a lipid functions in corpus luteum regression and also in parturition.

Differences between anterior and posterior pituitary

The posterior pituitary is also known as neurohypophysis. It originates from the embryonic brain tissue as an extension of the hypothalamus. It is made up of the neural lobe, the infundibular process and the median eminence. The anterior pituitary on the other hand also known as adenohypophysis originates from the embryonic gut tissue. It is made up of the *pars distalis*, *pars tuberalis* and *pars intermedia*(Fig. 63.0 and Fig. 64.0). Histologically three types of cells can be differentiated in the adenohypophysis using standard staining techniques chromophobes (50%), acidophils or eosinophils (35%) and basophils (15%). Tissues of the neurohypophysis are made up of non-medullated nerve fibres, blood vessels, neuroglia and pituicytes.

The hypothalamus forms the floor and the 3rd lateral wall of the ventricle of the brain. The hypothalamus receives signals from almost all possible sources in the nervous system e.g. pain-depressing or exciting-thought, olfactory stimuli, concentrations of nutrients electrolytes, water and various hormones in the blood excite or inhibit various portions of the hypothalamus. The hypothalamus thus appears to be a collecting centre for information about the body. The neurohypophysis is richly innervated by the hypothalamo-hypophysial tract from the hypothalamus. These fibres originate from the supraoptic and paraventricular nuclei in the hypothalamus. These are fine unmyelinated fibres. The hypothalamus is connected to the adenohypophysis by the hypophysial blood system with very scanty innervations, if any at all. The *pars distalis* possesses a systemic arterial supply, a portal supply and a venous drainage. The portal-hypophysial vessels form a direct vascular link between the hypothalamus and the adenohypophysis.

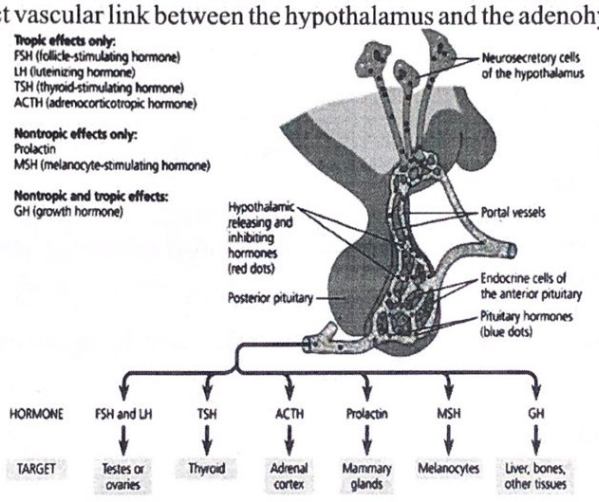


Figure 63.0: Production and release of adenohypophysial (anterior pituitary) hormone

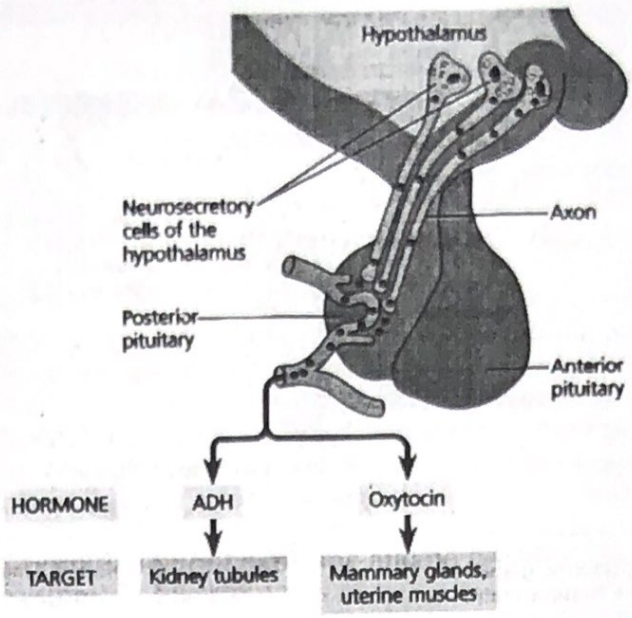


Figure 64.0: Production and release of neurohypophysial (posterior pituitary) hormone

Luteinizing hormone (LH) is a glycoprotein produced by the anterior pituitary. It is produced by the basophil cells.

Functions

1. Stimulates the development of the interstitial or Leydig cells of the testis and secretion of androgen.
2. Causes ovulation alone or in combination with FSH
3. Acts synergistically with FSH to promote oestrogen secretion and increased ovarian weight.
4. Stimulates progesterone secretion in luteal cells.
5. In hypophysectomized female animal, it causes repair of the interstitial tissue of the ovary.

Follicle stimulating hormone (FSH) is a glycoprotein produced by the anterior pituitary.

Functions:

1. Stimulates sertoli cells.
2. Acts synergistically with LH to promote oestrogen secretion and increased ovarian weight.

3. It causes enlargement and ripening of the follicles.
4. It causes an increase in ovarian weight.
5. In hypophysectomized male rat, it results in development of testicular tubules and spermatogenesis.

Human Chorionic Gonadotrophin (HCG): Found in the blood and urine of pregnant women. It prevents involution of the corpus luteum and its action closely parallels those of the luteinizing hormone.

Pregnant Mare Serum Gonadotrophin (PMSG): Found in the blood of pregnant Mares. It originates from cells in the endometrial cups of the placenta. It has a follicle stimulating effect.

Menopause or Castration Urinary Gonadotrophin: e.g. Human Menopausal Gonadotrophin (HMG). First found in the blood and urine of menopausal women and later in the urine of both sexes after castration. It is probably of pituitary origin and very largely follicle stimulating in nature.

Prolactin (PRL) is a protein produced by the acidophil cells of the pars distalis of the anterior pituitary. Other names are lactogenic or mammotrophic or luteotrophic hormone.

Functions

1. It has a stimulatory effect on mammary gland development and milk synthesis.
2. It promotes ovulatory surges of luteinizing hormone (LH).
3. It stimulates the LH responses of corpus luteal cells. This leads to decreased progesterone secretion short luteal phase and amenorrhoea (absence of menses).
4. In female, prolactin is implicated in progesterone secretion and maintenance of corpus luteum.
5. It stimulates the LH response of Leydig cells causing decreased testosterone secretion, decreased libido, erectile impotence and infertility.
6. Acts synergistically with androgens to stimulate male accessory sex organs like the prostate and vesicular gland, and play a role in testosterone secretion.
7. It also reduces conversion of testosterone to its active metabolite, dihydrotestosterone.
8. It causes increased renal retention of sodium, potassium and water, leads to fluid retention which causes premenstrual oedema.
9. It increases conversion of 25-hydroxycholecalciferol to 1, 25-dihydroxycholecalciferol in the renal tubules.
10. It reduces glucose tolerance and increases insulin secretion.
11. It increases PGE secretion which is reduced in schizophrenia.
12. It increases the growth of breast cancer.

Regulation of Prolactin secretion

Prolactin secretion is stimulated by oestrogen, TRF, PRF, enkephalins, exercise, stress, suckling, sleep and hypoglycaemia. It is inhibited by bromocriptine and dopamine.

At the pituitary level prolactin probably inhibits its own secretion by enhancing effects of dopamine on prolactin-secreting cells. At hypothalamic level, prolactin stimulates secretion of dopamine.

Growth hormone (GH) or Somatotrophic hormone (STH): It's a protein and is specie specific, synthesized in the acidophilic cells and stored in the intracellular granules. It is released into the blood by exocytosis. Rate of secretion in the adult is variable during each 24 hours with a normal daily output of about 1.4mg. Its concentration in adult primate is 3ng/milliliter while in the child it is 5ng/milliliter. The rate of secretion depends on the individuals' nutritional state or stress. Various stimuli such as emotional, febrile and surgical stresses affect the secretion through the activation of an adrenergic system. Secretion is increased in starvation, hypoglycemia, exercise and excitement. Other factors that stimulate GH secretion include glucagon, androgen (at puberty), oestrogen, prostaglandins, thyrotropin releasing factor, phase IV sleep protein-calorie malnutrition, anaesthesia and haemorrhage. Factors that inhibit its secretion include cortisol and somatostatin.

Functions:

1. GH is necessary for the growth of all body tissues and is required throughout the reproductive life of an animal.
2. Promotes growth in the soft tissues, cartilage and bone as a result of stimulation of protein synthesis.
3. Promotes increased size of the cells and increased mitosis with development of increased numbers of cells.
4. It increases amino acid uptake and stimulates protein synthesis in all cells of the body by:
    a. Increasing the transport of amino-acids across cell membranes,
    b. Increase ribosomal RNA content,
    c. Increase nitrogen, phosphorus, calcium, sodium and potassium in cells,
    d. Decrease protein catabolism.
5. It increases mobilization of free fatty acid from the depots and its utilization for energy while decreasing carbohydrate utilization.
6. It increases blood glucose level (diabetogenic action) by:
    a. Increasing liver glucose output,
    b. Decreasing glucose transport into the cells (anti-insulin action),
    c. Decreasing insulin binding on membranes,
    d. Decreasing glucose catabolism, especially in muscles.aldosterone.

7. It has a lactogenic effect.

The actions of growth hormone on skeletal growth and probably some of the metabolic actions are mediated by plasma factors known as **somatomedins**, synthesized mainly in the liver and the kidney. Somatomedins are polypeptides and are of three types, viz somatomedin- A, somatomedin- B and somatomedin- C. Somatomedins increase protein synthesis, glucose oxidation and decrease free fatty acid mobilization. It has a negative feedback effect on GH secretion.

Growth hormone - Insulin relationship

Growth hormone stimulates the cells of the pancreas to secrete insulin but in chronic doses it causes degenerative changes in the cells thereby decreasing insulin production. This leads to a condition known as metahypophysial diabetes. In acromegalic diabetes, the level of plasma insulin is raised and the binding of insulin peripherally is reduced.

Adrenocorticotrophic hormone (ACTH): This is a polypeptide formed from a large precursor hormone in the basophil cells of the par distalis.

Functions

1. Primarily stimulates the two innermost zones of the cortex of the adrenal gland the zonae fasciculata and reticularis to produce steroid hormones.
2. It sensitizes the outermost zone of the adrenal cortex (zona glomerulosa) to other stimuli which induce the release of aldosterone.
3. It has a melanophore stimulating effect.
4. It possesses a fat- mobilizing activity.
5. It has a salt and water retaining property and a virilizing manifestation.

ACTH secretion is controlled by two factors - a. feedback control of the anterior pituitary; b. influence from the central nervous system through the hypothalamus, this is the most effective e.g. pain from stress is transmitted to the portion of the hypothalamus secreting the corticotropin releasing hormone (CRH) causing the release of adrenocorticotrophic hormone (ACTH).

secretion is controlled by two factors a. feedback control of the anterior pituitary; b. influence from the central nervous system through the hypothalamus, this is the most effective e.g. pain from stress is transmitted to the portion of the hypothalamus secreting the corticotropin releasing hormone (CRH) causing the release of adrenocorticotrophic hormone (ACTH).

Melanocyte stimulating hormone (MSH): MSH has the same chemical structure as the first 13 amino acid of ACTH. It occurs in two forms Ü and â forms. It is not present in circulating blood in man. In some lower animals it is secreted by the pars intermedia.

Function

1. The only known function in man is to cause pigmentation of skin by increasing melanin synthesis in the melanocytes. Melanin appears to be able to move out of these cells in human and is then dispersed in the surrounding dermal cells. It is independently controlled by the hypothalamus in respect to the amount of light to which an animal is exposed or in response to other environmental factors.

Thyroid stimulating hormone (TSH) or Thyrotrophin: This is a glycoprotein secreted by the basophil cells of the pars distalis

Functions

1. Its primary function is to exert a powerful controlling influence over the activity of the thyroid gland.
2. It increases the secretion of thyroxine (T_4) and triiodothyronine (T_3) which play a role in nitrogen and energy metabolism by the thyroid gland.
3. It increases the activity of the iodide pump thereby increasing the amount of iodine available for the elaboration of the hormone.
4. It increases the iodination of tyrosine and coupling to form the thyroid hormone.
5. It increases the size of the thyroid gland.

Abnormally low thyroxine levels delay onset of puberty, interfere with normal pregnancy and may affect spermatogenesis in the adult.

Hormones of the Gonads

There are 2 hormones of the gonad in the female - oestrogens and progestogens, and one hormone of the gonad in male - androgen.

Oestrogen are groups of steroids with similar physiological activities, they are produced by the theca interna cells of the graafian follicle within the ovary, corpus luteum and the placenta. It circulates bound to globulin (70%) with varying levels depending on the phase follicular phase (90-150mg/day), luteal phase (200-250mg/day) and adult male (40mg/day). Oestrogens are called female sex hormones- stimulates the development of the udder/ breast; they are luteolytic (breakdown corpus luteum, CL) in the cow and luteotropic (build-up or maintain CL) in the sow. The most physiologically important oestrogen is oestradiol. Others include oestrone, oestriol.

Functions

1. Increases the sensitivity of granulosa cells to follicle stimulating hormone.
2. Influences the manifestation of mating behaviour induce behaviouraloestrus.
3. Increases mitotic activity of germinal epithelium.

4. Increases mitotic activity of the cells of the vagina.
5. It is involved in cyclic changes in the female tract stimulates increased growth (cornification) of vaginal epithelium.
6. Increases uterine blood flow
7. Stimulate increased growth of the uterus (endometrium and myometrium).
8. Stimulate uterine contraction (increase the frequency/ amplitude of contraction).
9. Cause increased growth/ muscular activity of the oviduct.
10. Influences duct development in the mammary gland.
11. Involved in development of secondary sex characteristics stimulate development of female contours, cause maturation of epiphysial cartilage while inhibiting growth of long bones.
12. Stimulates water retention and mineral metabolism

Progestogen: is another group of steroids, the most important being progesterone produced by the lutein cells of the corpus luteum, and the placenta in pregnant animals. It circulates largely bound to albumin and some specific globulin. The blood level varies preovulatory phase (0.1-0.3mg/100ml), after ovulation (1.5mg/100ml), shortly before menstruation (0.2-0.4mg/100ml) and during pregnancy (14mg/100ml). It acts synergistically with oestrogens to cause oestrusbehaviour and is necessary for the maintenance of pregnancy hence the name pregnancy hormone.

Functions

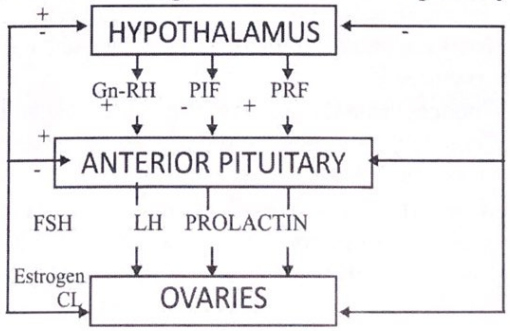
1. Inhibition of sexual behaviour i.e. it stops animal from standing to be mounted.
2. Causes differentiation of the endometrium.
3. Causes appearance of maternal behaviour in some species.
4. Ordinarily inhibits ovulation via decreased luteinizing hormone secretion.
5. Maintains pregnancy by inhibiting uterine contraction and promoting glandular development in the endometrium. It synergises with oestrogen to prepare the uterus for pregnancy and the mammary gland for lactation.
6. Acts synergistically with oestrogens to induce behaviouraloestrus.
7. Acts synergistically with oestrogen to cause the growth/ development of the lobule alveolar system of the mammary gland.
8. Inhibit spontaneous uterine contraction and the response of the myometrium to oxytocin.
9. Induces the mucification of the vaginal epithelium.
10. Inhibits the ovulatory surge of LH when present at high level (basis for most oestrus synchronization).
11. Induces the formation of secretory endometrium in an oestrogen-primed uterus which is necessary for implantation of the blastocyst. Such endometrium is characterized by increased mucosal thickness, increased coiling of the glands, oedema of the stroma and the presence of glycogen- droplets in glandular cells.

Both oestrogen and progestogen are involved in feedback mechanism of the hypothalamic pituitary.

Testosterone: LH in the male stimulates the leydig cells of the testis and the adrenal cortex to produce androgen which are another group of steroid hormones as well as very little amount of female sex hormones and adrenocortical hormones. The main androgen produced is testosterone and it is called male sex hormone. The amount of testosterone secreted in the male is 4-9mg/day while 0.2-0.5mg/day is secreted in the female.

Functions

1. Stimulates the development of male secondary sexual characteristics such as body configuration, distribution of pubic, chest, abdominal hair/ beard in primates, and the cocks comb.
2. Maintenance of the male duct system.
3. Involved in the expression of male sexual behaviour i.e. sex drive or libido.
4. Stimulates the growth, development and secretory activity of the accessory sex glands/ organs (prostate gland, cowper's gland, vesicular gland, penis and scrotum).
5. Involved in the functions of the tunicaldartos muscle in the scrotum.
6. Stimulates spermatogenesis in hypophysectomized animal and also hasten the onset of spermatogenesis in seasonal breeders.
7. Conditions the hypothalamus to produce gonadotropins at constant rate at puberty.
8. Is responsible for the development of male characters in utero.
9. Induce nitrogen retention which helps in muscle laydown, hence it is better to keep intact males for fattening than castrates except where male odour is a problem.
10. Stimulates the synthesis of erythropoietin in the kidney causes low density lipoprotein which is associated with a greater risk of vascular disease in male.
11. Involves in feedback mechanisms to regulate the hypothalamic-pituitary-axis thus regulating reproductive behaviour in males. **Inhibin** is important in the control mechanism because of its inhibiting action on the anterior pituitary to stop the release of FSH.



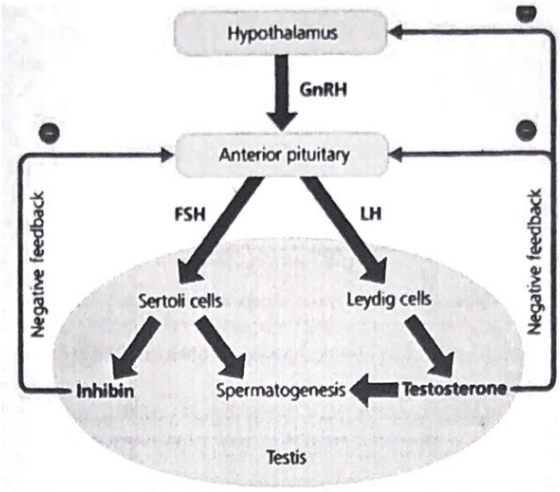


Figure 65.0: Schematic diagram of Hormone regulation of reproduction

HORMONES OF THE NEUROHYPOPHYSIS

Two hormones are secreted by the neurohypophysis - Oxytocin and Vasopressin (antidiuretic hormone). The major site of origin is the hypothalamus, but the posterior pituitary or neurohypophysis acts as a storage site. In the hypothalamus two nuclei - supraoptic and paraventricular are responsible for the synthesis, from where the hormones bound by neurophysin travel down numerous axons and are stored in the neurohypophysis from where they are released into the circulation.

Oxytocin: This is an octapepetide

Functions

1. It facilitates milk ejection (milk let-down) by stimulating the contraction of the myoepithelial cells in the mammary gland.
2. In the male it enhances ejaculation of semen.
3. It increases sperm and ova transport in the uterus/ oviduct by stimulating smooth muscle contraction of both tissues.
4. It assists in foetal expulsion during parturition by stimulating the contraction of the smooth muscle of the uterus. This is only observed during the late stages of pregnancy when the uterus is dominated by oestrogen.

Factors influencing oxytocin secretion

Suckling reflex: Suckling initiates integrated neuro-endocrine reflexes mediated via the hypothalamus and pituitary. The result is that oxytocin secretion leads to milk ejection and uterine contraction.

Anti-diuretic hormone (ADH) or vasopressin: Also an octapeptide. Vasopressin was first thought to cause an increase in blood pressure when the pituitary extract was first discovered and tested; hence the name vasopressin was given to it. It is now known that this is a pharmacological effect. The physiological effect is that of anti-diuresis (reducing the volume of urine), hence it is now called Antidiuretic hormone.

There two forms of vasopressin **arginine vasopressin**, AVP (contains the amino acid arginine) and **lysine vasopressin**, LVP (which contains the amino-acid lysine). They both have identical structures which include the amino acid phenylalanine. Oxytocin differs from the two vasopressins by having the amino acid leucine instead of either arginine or lysine and isoleucine instead of phenylalanine. AVP is found in most mammals including man while LVP is present in pig and hippopotamus.

Functions

1. The primary function is on the kidney causing increased free water resorption from the distal renal tubules and collecting ducts.
2. It increases intracellular water in muscles with a loss of sodium and potassium thereby causing low serum sodium. This effect is modulated by aldosterone (mineralocorticoid) i.e controls water and electrolyte balance in the body with the aid of the mineralocorticoids.
3. It causes vasoconstriction of renal arterioles and this increases water resorption.
4. In large doses it causes vasoconstriction of uterine smooth muscle and myoepithelial cells of the breast.

Factors influencing ADH secretion

The principal mechanism involved in the release is a variation of plasma osmolarity.

1. Plasma osmolarity: An increase in plasma osmolarity results in an increased release of ADH from the neurohypophysis. The change in plasma osmolarity is detected by the osmoreceptors found in the anterior hypothalamus. These osmoreceptors are sensitive to changes in effective osmotic pressure of the plasma as small as 2%. Increase in the effective osmotic pressure leads to the shrinkage of the osmoreceptors which then increase their rate of discharge. This stimulates the supraoptic nucleus (SON) and causes the release of ADH.
2. Changes in blood volume: There are some volume receptors located in the great veins, atria and pulmonary vessels which respond to changes in the blood volume. Decreased blood volume leads to decreased stretch of the receptors. This causes a fall in vagal afferent which allows the SON to discharge which result in the release of ADH into the blood. This mechanism is very important in haemorrhage. A fall of blood volume results in a decrease in the number of action potentials from the pressure receptors.

This decreases the tonic inhibitory effect normally induced by the volume and pressure receptors. The result is an increase in the secretion of ADH.

3. Other factors: Other factors such as emotional stimuli, stress, trauma, pain, surgery, nicotine, barbiturates, angiotensin, some anaesthetics, increased temperature and chlorpropamide also increase ADH secretion. Some these factors like emotional and surgical stresses act through the higher centres. Alcohol, tranquilisers and some emotional states decrease ADH secretion by altering the balance between facilitatory (cholinergic) and inhibitory (adrenergic) influences.

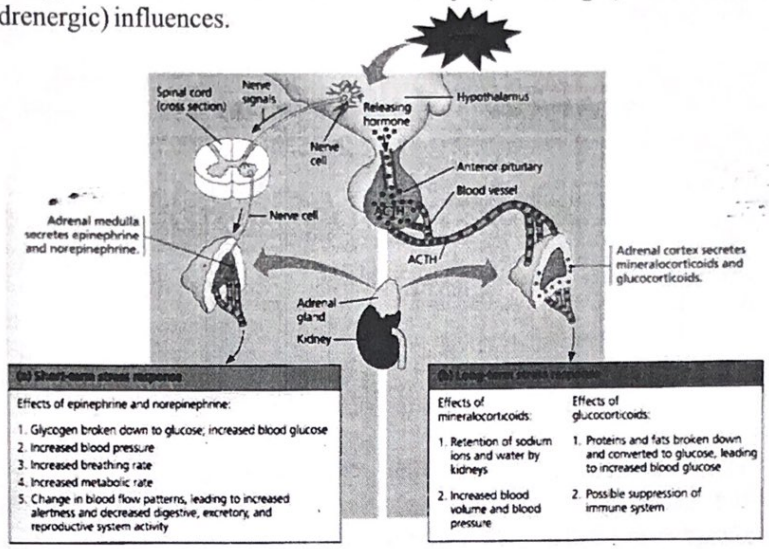


Figure 66.0: Stress and the adrenal gland hormone

Role of prostaglandins in Reproduction

Prostaglandin is a group of biologically active lipids. They have arachidonic acid (a twenty carbon unsaturated fatty acid) as their precursor. There are two classes prostaglandin E compound and prostaglandin F compounds. They have hormone like actions but are not produced by any specific tissue but are produced by cells all over the body. They are produced by cells in the uterus and vesicular glands in the male. Among the prostaglandin, PGF**2** have received most attention in reproductive physiology.

PGF**2**cause regression of corpus luteum (CL) and have stimulating effect on smooth muscle. It has therefore received attention in the control of oestrous cycle and has also been proposed or used in promoting ovule transport, sperm transport and parturition. Its use in the control of oestrous cycle is known as **oestrus synchronization.**

PGE**2** has not received much attention but appears to have anti- PGF**2** α

PINEAL GLAND

It is located above the hypothalamus, arising from the roof of the 3rd ventricle. It is large in young animals/ infant. It begins to involute before puberty. Very little is known about the gland but melatonin has been isolated from it. The pineal body is believed by Descartes to be the seat of the soul. It is the vestigial organ which is homologous to the 3rd eye found in reptiles. It has been suggested that the gland plays a role in breeding season in seasonal breeders such as Goat, Sheep, and Mare.

Functions

1. It produces a growth inhibiting factor.
2. It is a source of a compound that cures schizophrenia.
3. It secretes a hormone that antagonizes the secretion or effects of adrenocorticotrophic hormone.
4. It is a source of a factor that regulates the secretion of aldosterone.
5. It is a source of melatonin.

MECHANISM OF ACTION OF HORMONES

Hormones function by either of two mechanisms viz

1. Activation of the cyclic AMP system of the cells
2. Activation of the genes of the cells which cause the formation of intracellular proteins that initiate specific cellular functions

Hormones that act through the first mechanism are called the first messenger while the second messenger being the cyclic AMP. The first messenger binds to the receptor within the cell membrane leading to the activation of an enzyme (adenyl cyclase) which catalyses the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (AMP). The cyclic AMP itself is destroyed by an enzyme, phosphodiesterase. Such first messenger may also stimulate cyclic AMP formation by inhibiting the formation of phosphodiesterase.

This mechanism of action involves these basic steps

- The hormone passes through the cell membrane of the target cell.
- It binds with a specific receptor in the cytoplasm.
- The receptor bound hormone travels into the nucleus and binds to another specific receptor on the chromatin.
- The hormone-receptor complex calls for the production of messenger RNA (mRNA) molecules which code for the production of proteins.

While cyclic AMP acts as a stimulator of actions, in many instances prostaglandins seem to act as inhibitors. The prostaglandins acts in ways unrelated to intracellular hormonal mediation, a few microgram cause maximal effect since they are very potent substances.

Hormones which act by the second mechanism cause formation of proteins in target cells. The hormone enters the cytoplasm of the cell and binds to a receptor. The complex then enters the nucleus leading to the formation of messenger RNA which diffuses back into the cytoplasm to effect the processes leading to the formation of new protein. Hormones that use this process include the steroids.

CONTROL OF HORMONAL SECRETION

The basic function of a hormone is to regulate the activity of its target cells in a specific direction. In order to maintain this function the endocrine gland receives constant information or signals (feedback) on the state of the systems regulated from a variety of different sources. The rate of secretion of the hormones is therefore determined by the integration of these different signals. There are two types of feedback- positive feedback and negative feedback (direct, indirect or short negative feedback).

a. Positive feedback: the change caused by the action of an hormone alters the rate of release of that hormone so that its effect is further enhanced e.g. the release of oestrogen from the ovarian follicle, controlled by the FSH from the anterior pituitary. At a particular plasma concentration, oestrogen stimulates the release of FSH and LH from the anterior pituitary so that more oestrogen is secreted.

b. Negative feedback

1. Direct negative feedback: this relates the rate of release of the hormone to the blood concentration of the chemical substance which it controls or to the chemical product of the metabolic process which it regulates e.g. the relationship of insulin and the blood glucose concentration. Insulin causes its target cells to take up blood glucose thereby decreasing the blood glucose levels; a change in this level in turn alters the rate of insulin secretion.
2. Indirect negative feedback: this when the central nervous system (CNS), particularly the hypothalamus is indirectly involved in the regulation of hormone secretion from an endocrine gland by controlling the release of the appropriate hormones from the anterior pituitary gland. This is particularly important in the control of the hormones of the thyroid, adrenal cortex and the gonads.
3. Short negative feedback: the hypothalamus controls the release of hormones by the anterior pituitary by secreting releasing or release inhibiting factors. Some of the anterior pituitary hormones have a feedback effect on the release of these factors. This is an example of the short negative feedback.

OTHER HORMONE PRODUCING ORGAN

Thyroid gland: The thyroid is a bilobed ductless gland located on each side of the trachea near the junction with the larynx i.e. below the larynx on either side of (and anterior to) the trachea. It is highly vascularised with profuse blood flow (3.5 6ml/g/tissue/min or 5litres/hr/gland).

The follicles of this gland secrete the hormones triiodothyronine (T3) and thyroxine (T4) from iodine and thyrocalcitonin. Removal of the gland in young animal results in stunting of the physical, mental, and sexual development so that the animal remains infantile and undeveloped. In older animal's removal of the gland is less dramatic but results in thickening of the skin, loss of hair, and a sluggish physical and mental state in different degrees. Administration of the hormones or feeding of thyroid extracts has been demonstrated to compensate for loss of the gland. The changes noted are evidence that function of the thyroid is essential to normal development and continues to be essential in maintaining or regulating the rate of metabolism necessary for an individual to cope with the environmental changes that it may encounter. Thyroid weight has been observed to decrease relative to body weight.

Functions

1. Calorigenic effects it increase oxygen consumption in some metabolic organs.
2. Growth and differentiation results in early metamorphosis in frog.
3. Affect metabolic processes increase basal metabolic rate, keeps blood lipid/ cholesterol at normal level and increase absorption of carbohydrate from the gut.
4. It is essential for adequate myelination of nerves and brain development.
5. It is concerned with osmoregulation in poikilotherms.
6. Stimulate almost all aspects of carbohydrate metabolism.
7. Stimulate the synthesis, mobilization and degradation of lipids.

Thyrocalcitonin (calcitonin): produced by the C- cells or parafollicular cells of the thyroid gland. It is also found in the thymus. It decreases calcium levels in three ways - by decreasing the activity of the osteoclasts, by increasing the activity of the osteoblasts, and by preventing the formation of new osteoclasts from osteoprogenitor cells. Calcitonin is secreted at the rate of 0.5mg/day and it is not secreted until calcium plasma level reaches 9.5- 11mg/100ml plasma. Calcitonin helps maintains the normal level of calcium in the body. Calcitonin secretion is increased by high calcium in the plasma and by gastrin. Calcium absorption from the gut is inhibited by acidic gut pH, excessive amount of phosphate/oxalate, flour (this contains phytic acid), calcitonin, prostaglandin E, glucocorticoids, growth hormone, thyroid hormone and bile salts. Calcium absorption from the gut is stimulated by vitamin D metabolite (1, 25 - dihydroxycholecalciferol) and parathyroid hormone.

Parathyroid gland: the gland arises from the 3rd and 4th branchial arches and they are four in number. They contain two types of cells - chief cells (secrete parathyroid hormone or parathormone) and the oxyphil cells which appear before puberty and whose function is unknown.

Function

1. It increases the blood calcium level by increasing the calcium and phosphate absorption from bone.
2. It decreases the blood phosphate level by increasing its excretion by the kidney.
3. It causes proliferation of the osteoclasts, followed by increased osteoclastic reabsorption of the bone itself.

Conditions which tend to lower plasma calcium such as low calcium diet, vitamin D deficiency and low calcium ion concentration in the extracellular fluids, cause the gland to increase the rate of secretion. Parathyroid glands are enlarged in rickets, during pregnancy and lactation. Its secretion is decreased when there is excess calcium in diet, increased vitamin D in diet and in bone absorption caused by factors other than parathyroid hormone e.g. disuse of bone.

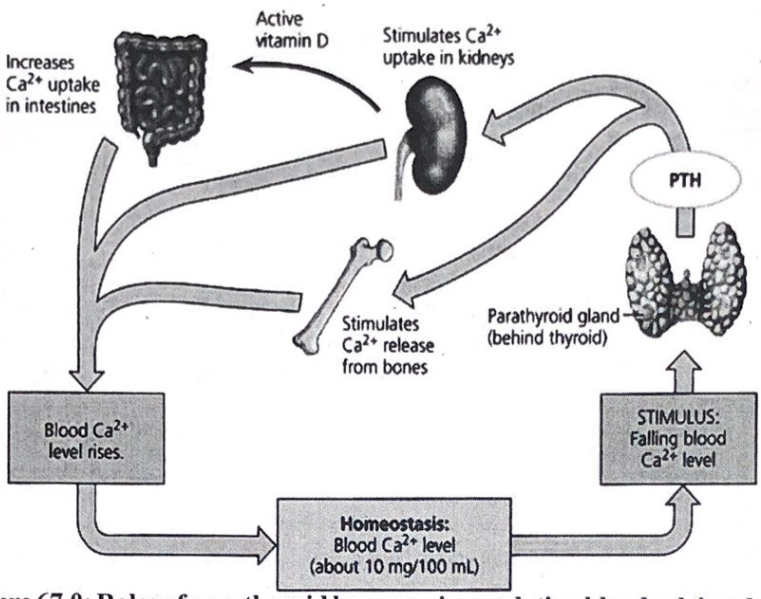


Figure 67.0: Roles of parathyroid hormone in regulating blood calcium level

Adrenal glands: The adrenal glands are located near the kidneys. The right and left glands differ in shape, location, and orientation with reference to the kidneys. The right adrenal is triangular in shape and about 6cm long by 3cm thick in the mature animal. Its location is between the kidney and the vena cava near the liver. The left adrenal is near the back of the kidney between the rumen and the renal vein in ruminants. It is flat and triangular, being about 6cm long and 2cm thick in the mature animal.

The glands have an inner portion (the **medulla**) and an outer portion (the **cortex**). The adrenal medulla is a functional part of the nervous system and serves as a bridge between the nervous and endocrine systems in that various stimuli can trigger the release of the hormones epinephrine (adrenaline) and norepinephrine (noradrenaline), which is involved in regulation of body functions through various cardiovascular, respiratory, metabolic, muscular, and other reactions. The adrenal cortex does not have a functional nerve supply and it operates normally even if transplanted to a position remote from its normal site. It is known to produce more than 50 steroids, some of which are essential for life. The steroid hormones influence carbohydrate and protein metabolism, influence the water/ electrolyte balance, and produce effects similar to those produced by the sex hormones. Adrenal cortex consists of sheets of cells separated by vascular sinusoids but three basic layers are recognized the outer zone (**zona glomerulosa**), the inner zone (**zona fasciculata**), and the innermost zone (**zona reticularis**).

Adrenocortical hormones can be divided into three groups, viz

1. **Mineralocorticoids** which are primarily concerned with salt and water metabolism.
2. **Glucocorticoids** which deals with carbohydrate, fat and protein metabolism.
3. **Androgens** which deals with sexual characteristics.

The three groups are not rigidly separate and most corticoids show some characteristics of all three.

The zona glomerulosa secretes mineralocorticoids, the zona fasciculata secretes glucocorticoids and the zona reticularis secretes androgens.

Functions of mineralocorticoid (a naturally occurring mineralocorticoid is **aldosterone**)

1. Influence the renal tubular cell (and other salt transporting cells) to re-absorb sodium and to lose potassium.
2. It stimulates intracellular synthesis of an aldosterone-induced protein (AIP), the operant substance in achieving the aldosterone effects.
3. It stimulates excretion of potassium.

Factors controlling the release of Mineralocorticoids

a. Changes in plasma concentration of Sodium and Potassium 10% decrease of plasma Na and 10% increase in plasma potassium stimulate synthesis/ release of aldosterone.
b. Adrenocorticotrophic hormone stimulates aldosterone secretion. This effect is more on the glucocorticoids.
c. Renin- Angiotensin system Renin is an enzyme synthesized in the juxta-glomerular cells of the kidney. The stimulus for the release of renin is a decrease in serum Na or low renal arteriolar blood pressure as well as reduction of blood flow to the kidney.

Increased sympathetic activity may result in a direct stimulation of the juxta-glomerular cells via the adrenergic renal sympathetic nerve fibres. Renin starts the angiotensin-aldosterone response. A plasma protein (angiotensinogen) is activated by renin to produce angiotensin I which is later converted to angiotensin II which is a potent vasoconstrictor. Ag II also stimulates the zona glomerulosa to secrete aldosterone.

Functions of Glucocorticoid

1. Carbohydrate metabolism stimulates hepatic gluconeogenesis.
2. Antagonises the peripheral action of insulin on glucose uptake.
3. Protein metabolism in excess it causes severe wasting of the muscle, it also inhibits amino acid uptake by the tissue.
4. Fat metabolism in large amount it causes redistribution of fat.
5. Permissive effect for glucagon and catecholamine in exerting their calorigenic action.
6. Vascular reactivity causes vascular smooth muscle to become responsive to catecholamines.
7. Skeletal muscle prevents fatigue in skeletal muscle.
8. Blood cells and lymphatic organs increases eosinophils and basophils, decreases neutrophils, platelets and red blood cells.

Excess Glucocorticoid

1. Decreases growth hormone
2. Antagonizes the effect of vitamin D and calcium absorption.
3. Increases excretion of calcium from the kidney.
4. Inhibits normal inflammatory process.
5. Inhibits normal immune response.
6. Inhibits synthesis and release of histamine.

Glucocorticoid secretion can be controlled by negative feedback mechanism and ACTH causes increased secretion.

Functions of Adrenal Androgens

1. They are essential for the normal development of male external genitalia in utero.
2. They stimulate the growth of auxiliary/pubic hair at puberty. The hair can grow even in the absence of gonads.
3. They stimulate the production of grease in the skin.
4. They stimulate protein synthesis hence growth.
5. At puberty the androgens act synergistically with the thyroid hormones.

Hormones of the Medulla Adrenaline and Noradrenaline

Functions of Adrenaline

1. It has pronounced pressor action by decreasing peripheral resistance, constricts the arterioles of kidney and capillaries of skin, and dilates the vessels of skeletal muscles, liver and coronary system.
2. It stimulates the myocardium and increases oxygen consumption of the heart. It also dilates the coronary vessels and increases coronary flow.
3. Increases respiration after a short period of apnea.
4. It inhibits muscle of the stomach, intestine, bronchioles and urinary bladder.
5. Excites the muscle of the splenic capsule, ureter, trigone and the sphincters.
6. It causes hyperglycaemia and glycosuria.
7. It reduces clotting time.
8. It increases muscle tone and may cause tremors or muscular twitching.

Other effect include

- Secretion of saliva
- Contraction of the spleen
- Increased metabolism
- Fall in blood potassium
- Sweating in some animal
- Increased flow of urine
- Causes arousal and anxiety

Functions of Noradrenaline

1. It increases total peripheral resistance.
2. It dilates coronary vessels and increases coronary flow.
3. In man it causes bradycardia i.e. slow rate of heart contraction, resulting in a pulse rate less than 60 beats per minute.
4. Antagonizes insulin relieving hypoglycaemic convulsion to a less degree than adrenaline.
5. Mobilizes fat from adipose tissue
6. It constricts the cutaneous vessels
7. It causes reduction in blood flow through the brain.

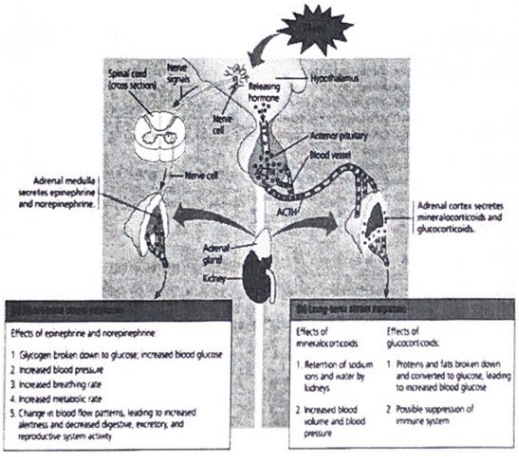


Figure 68.0: Adrenal gland regulation of the body

The Pancreas: is a tongue-shaped glandular organ lying below and behind the stomach. Its head is encircled by the duodenum and its tail touches the spleen. It is about 18cm long and weighs about 100g. It secretes the hormones insulin and glucagon, and also alkaline pancreatic juice which contains digestive enzymes involved in the digestion of fats, carbohydrates and proteins in the small intestine. The endocrine pancreas is limited to the **islets of Langerhans** and human pancreas contains 1,000 000 islets. The islets of Langerhans contains four types of cells A_1 cells, A_2 cells, B cells and D cells which makes up about 3% of the pancreas.

A_1 cells produce gastrin

A_2 cells produce glucagon (<25%)

B cells produce insulin (75%)

D cells produce somatostatin, GH - RIH (1%)

Glucagon: is a polypeptide with a half-life of 10minutes and plasma concentration of 50 150ng/litre. It is also secreted by the stomach mucosa and the upper small intestine. It appears to act only intracellularly by stimulating the catabolism of all the three major classes of organic material.

Functions

1. Stimulates the breakdown of glycogen, proteins and glycerides.
2. Stimulates glyconeogenesis by the liver from the products of glycolysis and proteolysis.

Insulin: is a polypeptide hormone produced by the beta cells of the pancreas. Its secretion is regulated by blood glucose level and it opposes the action of glucagon. It has an effect on the metabolism of carbohydrate, protein and fat by stimulating the transport of glucose into cells.

Functions

1. It lowers blood glucose concentration by lowering tissue threshold thereby increasing the rate of glucose uptake by the peripheral tissues; and also by decreasing glucose output by the liver.
2. It lowers the free fatty acid (nonesterified fatty acid) content of the blood by increasing the permeability of cell membranes; inhibits the breakdown of triglycerides; and indirectly supplies intracellular glucose whose oxidation can provide energy for triglycerides synthesis.
3. It lowers the free amino acid content of the blood by increasing the permeability of cell membranes; and by increasing the formation of ribose nucleic acid.

Factors controlling Insulin secretion

1. The main factor for insulin release is blood glucose.
2. Sympathetic nervous system plays a role in continuously modulating the basal insulin secretion.
3. Stimulation of sympathetic receptors (by noradrenaline) inhibits release of insulin induced by a rise in blood glucose.

4. Stimulation of sympathetic β-receptors (by adrenaline) increases insulin secretion.
5. Insulin release is also stimulated by amino acid, fatty acid (very slightly in man), glucagon, gastrin, secretin, pancreozymin, growth hormone, glucocorticoids, progesterone and oestrogen.

The secretion is inhibited by stress.

Somatostatin: This is a tetradecapeptide containing a disulphide bridge. It functions as a growth hormone, inhibiting hypophysiotropic hormone in the hypothalamus where it was first isolated.

Functions

1. It inhibits the secretion of insulin, glucagon and pancreatic polypeptide (another hormone believed to be secreted by the pancreas).
2. It is involved in the regulation of gastro-intestinal function.
3. It acts as a synaptic transmitter in the spinal cord and other parts of the nervous system.
4. It acts locally within the pancreatic islets.
5. It suppresses the cyclic AMP levels and calcium influx.

Kidney: it secretes renin and erythropoietin

Renin a proteolytic enzyme. It is also found in pregnant uterus. It catalyses the formation of angiotensin I from angiotensinogen.

Erythropoietin a circulating glycoprotein, formed by the action of renal erythropoietic factor on a globulin in the plasma. Production of erythropoietin is increased by hypoxia, cobalt salts and androgens. Secretion is also increased by catecholamines and facilitated by alkalosis developed in high altitude. Erythropoietin causes certain stem cells in the bone marrow to be converted to proerythroblasts.

CHAPTER TEN

PUBERTY AND GAMETOGENESIS IN LIVESTOCK

PUBERTY is a physiological phenomenon occurring in the life of an animal between sexual immaturity and maturity. **It is the period of postnatal development (adolescence) when a male or female is first able to release gametes**. It marks an age in the life of an animal when the animal becomes sexually matured, in females it is marked by the occurrence of first oestrus with ovulation. It should not be considered as a stage of sexual maturity as animal bred during the first oestrus often as difficult parturition. The physiological events leading to puberty are not quite understood but it seems that puberty occurs when gonadotropins are produced by the anterior pituitary in concentration high enough to stimulate follicular growth and ovulation. Follicle growth can be detected several months before puberty but until ovulation occurs puberty cannot be detected. Age and body weight at puberty can be affected by genetic and a number of environmental factors. Generally any factor that is capable of slowing down growth rate would delay puberty. Factors which have been identified include plane of nutrition, high environmental temperature, and poor health like helminthiasis, ectoparasitism and other disease problems. Average age at puberty in pig is 7-10 months, 8-11months in ewe-lamb, 8-11months in European Cow and 15-24months in Mares. Body weight also varies with species/ breed e.g. in the Jersey cow it is 160kg, Holstein 270kg but in Vom, Jos Nigeria, it has been reported to attain 240-250kg body weight, in gilts 68-90kg and in Ewe 27-34kg.

Puberty in male: Time of puberty in male is more difficult to determine than in the female. This is because the first differentiations of spermatozonia precede the release of first spermatozoa from the seminiferous tubules by about one month, and sperm transport from the testis to the vas deferens needs at least 2weeks. As in the female, age at puberty in the male could be influenced by a number of factors such as:

a. Genetic factors e.g. age at puberty differs with breed of animal.
b. Climatic factors e.g. high ambient temperature will lead to delayed puberty and modify age at puberty.
c. Nutritional factor: there is a correlation between body weight and testicular weight or age at first oestrus. With normal nutrition puberty occurs when the animal attains 60% of its adult bodyweight in sheep and 45% in cattle, a similar relation does not exist in pig e.g. if growth is accelerated by overfeeding, body weight at the time of puberty is higher than normal and the animal reaches maturity at a young age. The reverse is for poor nutrition.
d. Social factors such as exposure to opposite sex - the presence of individuals

of the opposite sex generally quicken puberty while same sex delays onset of Puberty.

The two pre-requisite necessary for attainment of puberty in both sexes are -

1. Differentiation of the organs of reproduction: This occurs during foetal life when the gonad and the steroid target organs such as the hypothalamus, sexual organs and the liver becomes either masculine or feminine in type.
2. Maturation of gonad and the sensitization of the hypothalamus by steroid hormones which are progressively attained thus leading to sexual activity at puberty.

Growth and development of the reproductive system continues beyond puberty to reach peak performance during which the animal is said to have attained **sexual maturity.** It occurs several weeks, months or even years after puberty, depending on the species.

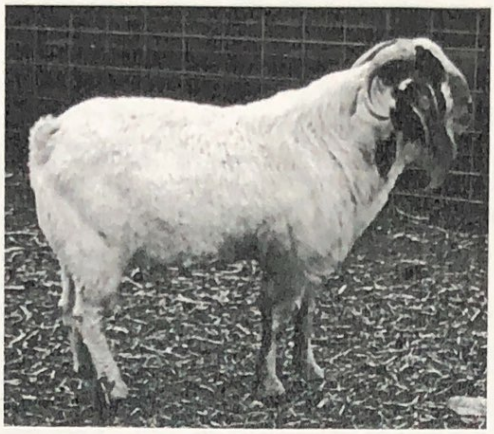


Figure 71.0: A mature male goat

GAMETOGENESIS

From sexual differentiation to onset of puberty the testis remain unchanged, supportive cells lines seminiferous tissues while undifferentiated germ cells or gonocytes occupies the centre of tubules.

A slow increase in the relative number of germ cells which has been shown to be independent of gonadotropin occur in ram-lambs. At the onset of puberty gonocytes migrate to the periphery of the tubules and produce spermatogonia while supporting cells produce sertoli cells. A lumen becomes visible at the centre of the seminiferous tubule while the first meiotic prophase stage appears. Two types of spermatogonia - $A_s$spermatogonia or stem cell and $A_o$spermatogonic or reserved sperm cell.The A_s gives rise to A_1spermatogonia which inturn forms spermatocyte. The A_o cell keeps contributing to the population of $A_s$spermatogonia between puberty and adulthood. This stem cell multiplication leads to threefold increase in testicular weight between puberty and up to 5years in the bull.

Spermatogonia differentiation and transformation of spermatid to spermatozoa are gonadotropin dependent.

Sperm Capacitation

After the formation of spermatozoa in the seminiferous tubule two maturation processes occur before they can effect fertilization. The first maturation process occurs in the epididymis, this comprise of the sperm:

a) Gaining the ability to be motile.
b) Gaining the ability to be fertile
C) Dropping or losing the cytoplasmic droplet.

The second maturation process is capacitation. The process of this is not quite understood but it is known that it takes place within the female reproduction tract and may involve the removal of the pliable protein layer from the surface of the spermatozoa to permit the release of enzymes (hyaluronidase,corona penetrating enzyme and trypsin-like enzyme) from the spermatozoa (acrosome) that are required for the penetration of the outer surface of the ovum known as zona pellucida, as well as corona radiata and cumulus oophorus. An experiment in rabbit shows that the sperm must have been in the uterus or oviduct 4hours before it could effect fertilization. That the immersion of capacitated spermatozoa into seminal plasma leads to loss of capacitation and the ability to effect ovule penetration and thus fertilization. This shows that decapacitation factor (DF) is present in the seminal plasma of the rabbit, bull, boar, stallion, man and monkey, but is absent in dog. The exact nature of the factor is not known but may have a molecular weight as low as 500. DF can be spun (centrifuged) out of the rabbit seminal plasma at 100,000 rpm. The sperm has to undergo some physiological process before it can fertilized i.e. has to be capacitated in the female tract.

FORMATION, DEVELOPMENT AND MATURATION OF FEMALE GAMETES

Oogenesis is the process of oocytes or egg formation in the ovary. It involves the proliferation, growth and maturation of the female germ cells. **Oocyte** is an immature ovum. The ovary, unlike the testis remains in the abdominal cavity throughout the female life. It is made up of two portions an outer portion (**cortex**) and an inner portion (**medulla**). A layer of cuboidal cells (**germinal epithelium**) covers the free surface of the ovary. The medulla consists of fibroelastic connective tissue, nerve and blood vessels. It's a bridge between the nervous and endocrine systems in that various stimuli that trigger the release of the hormones oestrogen and progesterone are passed through it. The cortex contains ovarian follicles and it's the site of egg/ hormone production. Oogonia and oocytes are formed during the first half of foetal life in the ewe and the cow.

In the Sow the process is slower with oogenesis completed during the first day of life i.e. immediately after birth. A single layer of somatic cells forms around the oocyte from which arise the three layers of the follicle (**theca externa**, **theca interna** and **granulosa**). Unlike in the male the formation of oogonia is not gonadotropin dependent. The three stages of development of the oocytes is identical in all species **oogonial mitotic divisions**, **meiotic prophase** and somatic cell organization around diplotene oocytes leading to **primordial follicle formation.**

Oogenesis includes all processes from oogonial multiplication, formation of oocytes/ primordial follicles, folliculogenesis, growth/ maturation of oocytes, and ovulation. There is a great difference between species in the length of germ cell proliferation e.g. mitosis rarely occur after the first meiotic prophase in rabbit, rat and mouse while it persist after the first meiotic prophase and long after the appearance of the first primordial follicles. Oocytes that are necessary for the entire sexual life of the female are all formed during foetal development but degeneration of many of them occurs during oogenesis causing rapid decrease in oocyte number. Very well before puberty and immediately after birth in the ewe and cow circuit of follicle growth occurs leading to constant formation of graafian follicle which normally disappears by atresia, thus the stock of oocytes which will be used during the entire sexual life is formed during foetal life. This stock diminishes by atresia as soon as it is formed. Thus a cow foetus having about 2,600,000 oocytes at the 110day of gestation only has about 68,000 oocytes at birth. Also in man, there is a peak of oocytes population of about 7,000 000 at the 5th month of gestation, this decreased to between 500,000 - 2, 000 000 just before birth. At the pre-pubertal period there is a further rapid reduction to about 200, 000. At birth the follicles are in the primary oocyte stage in which the first meiotic division is arrested. Soon after birth the primary oocytes are surrounded by a single layer of flattened granulosa cells, thus forming the primordial follicles which are held in reserve in the ovary with no further increase in number.

Folliculogenesisis the process of formation, growth and development of follicles from the primordial to the mature stage.The primary oocyte which has diploid chromosomes grows in size and undergoes two maturation divisions leading to the reduction of the chromosomes to the haploid number. In the first phase of folliculogenesis, the oocyte grows in size relative to the follicle to reach its maximum size while at the same time the multiplication of the follicular cells occurs. In the second phase, there is slow increase in size of the oocyte with rapid follicular growth with increased secretions which started in the first phase. Intercellular spaces develop which increase during follicular growth. In cow, the size of the antrum increases linearly with the size of the whole follicle. The oocyte and granulose cells have a functional interaction between them. The oocyte prevents luteinization of granulosa/ theca cells while the granulosa inhibits the resumption of oocyte meiosis.

This interaction helps in the maintenance of the dictyate stage of prophase till after puberty. Growth of a follicle up to ovulation is accompanied by disappearance of other follicles in adult animals. Follicular growth is dependent on FSH or PMSG in follicles of all sizes. PMSG or PMSG and FSH induce antral follicle formation and increase cellular multiplication. LH and HCG enhance FSH effect on follicular growth. All cell types in the ovary have the capacity for steroid hormone production but the type of steroid hormone produced depends on the stage of the oestrous cycle. Oestrogen is mainly produced by the theca cells of the follicle while after ovulation the luteinized granulosa cells secrete large amounts of progesterone. LH acts along with FSH to promote oestrogen secretion.

Ovulation is the release of a mature ovum or oocyte from a graafian (matured) follicle on the ovary. At ovulation, the secondary oocyte is discharged from the follicle, surrounded by follicular cells. It is believed to be caused by a sudden outflow of luteinizing hormone from the pituitary. The mechanism of ovulation is obscure but the simplest explanation indicates that luteinizing hormone stimulates oestrogen and progesterone output which act on the follicle to cause follicular rupture. Ovulation is accompanied by a rise in body temperature of between 0.3-0.6°C and maintained throughout the post-ovulatory phase. The ovum lives for approximately 72 hours after its extrusion from the follicle, sperms apparently survive in the female tract for not more than 48 hours.

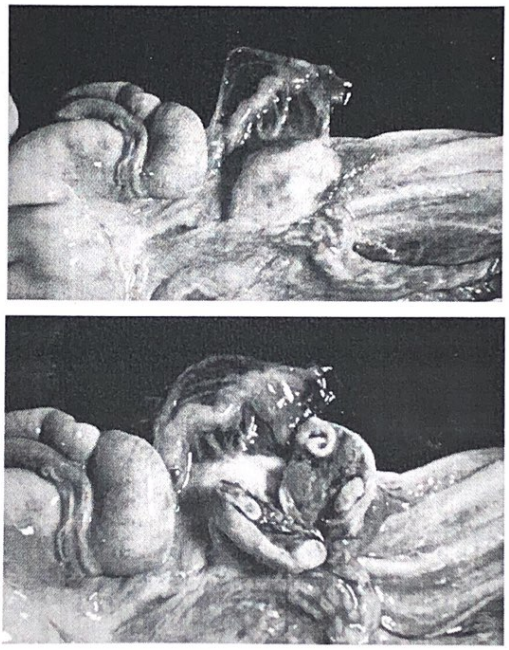


Figure 69.0: Caprine ovary with two corporal albican

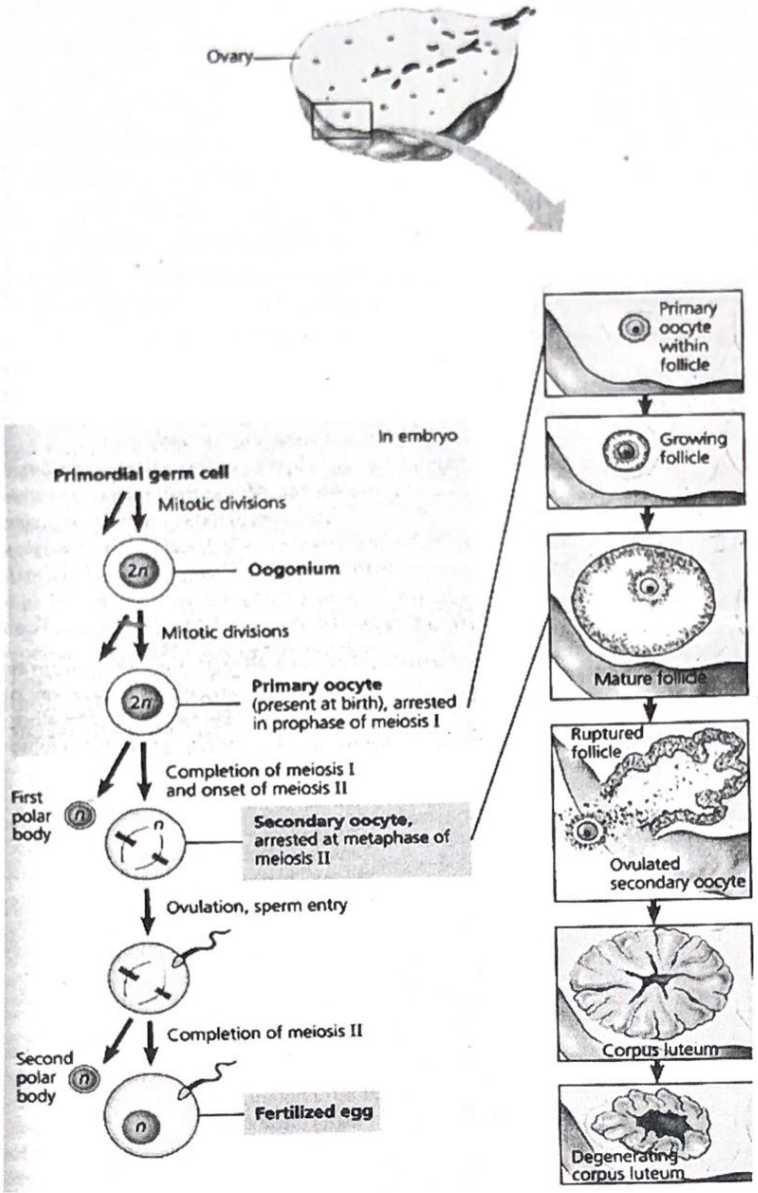
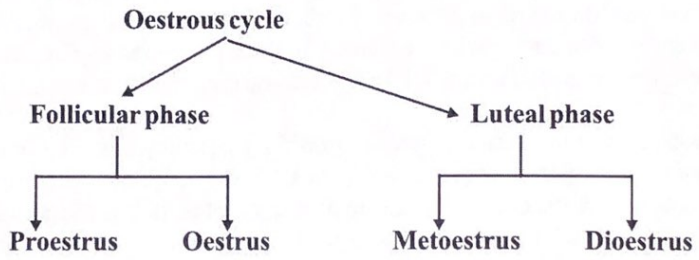


Figure 70.0: Development of the female germ cell and folliculogenesis

FEMALE REPRODUCTIVE CYCLES

After the attainment of puberty the sequence of reproductive event in the female is cyclic in nature unlike in the male where early secretion of foetal androgen regulates the hypothalamus rendering the sex centre acyclic. The length of the cycle depends on the occurrence of conception and the presence or absence of a definite breeding season e.g. sheep. Light appears to affect mainly females while temperature affects males. Ewes in temperate areas have a distinct breeding season while rams are continuous breeders. Seasonal breeders go through a non-breeding (anoestrus) period while sexual cycles are continuously repeated throughout the year in continuous breeders e.g. cattle. Ovarian cycle refers to the cyclic physiological and morphological changes occurring in the ovary. Oestrous cycle is a concomitant of the ovarian cycle which include follicular development, ovulation, corpus luteum formation and luteal regression which results in changes in the lower tract like uterus, cervix and vagina.

Oestrous cycle is the time between one period of oestrus and the next. **It is the interval from the first sign of sexual receptivity at oestrus, to the start of the next oestrus.** The length of oestrous cycle ranges from 16-25days in domestic mammals and varies between species as well as within individuals of the same species (Table 5). In the ewe it is 17days, 21days in cow, 20days in sow and 22days in mare. The range in the cow is 17-24days, 19-25days in mare. It is divided into two stages based on the ovarian cycle **follicular** and **luteal phase** and four phases based on the behavioural changes observed **proestrus, oestrus, metoestrus**and**dioestrus**. These periods are cyclical and sequential except in the period of anoestrus i.e. the absence of an oestrus which occurs in seasonal breeders or during pregnancy or any post-partum anoestrus. It could also be caused by a disease state known as cystic ovary which occurs in dairy cow, poor health condition can also induce anoestrus.



Proestrus: This is the period of preparation for oestrus during which there is increased follicular growth, regression of corpus luteum (CL) and the drop in progesterone. It is characterized by some behavioural signs which indicate the approach of oestrus such as increased frequency of urination,

attempt to mount other cows and moving away when mounted and extends to the beginning of oestrus. This period lasts for 2days. Physiological events during this period includes

i. Rapid follicular growth in the ovary,
ii. Increase in oestrogen secretion,
iii. Behavioural symptoms of approaching oestrus

Oestrus: This is the period during which the female is receptive to the male and would stand for mating. It is the period of sexual desire or heat. It is the only visually definable phase of the oestrous cycle. It is a behavioural state characterized by willingness of the female to stand to be mounted. The duration of oestrus varies with species of animal, in the ewe it is 24-36 hours, 48-72hours in sow, 12-18hours in cow but much shorter in tropical cow, 4-8days in Mare and 7-9 days in dog. During the period of oestrus, **ovulation i.e. the rupture of follicle and shedding of ovum**, may occur at 10-12hours after the end of oestrus in cow, middle to late oestrus in ewe, mid-oestrus in sow, and 1-2days before the end of oestrus in mare.

Metoestrus: This is the period of reorganization. It is the stage after the end of oestrus when the follicle matures, ovulates and the CL starts to develop. It begins with oestrusceasation and last about 3 days after oestrus, it is the period of corpus luteum formation. It is also the period of ovulation in the cow. In the cow there is a phenomenon called metoestrus bleeding which occurs in 90% of heifer and about 45% of cow. This bleeding is has a result of the withdrawer of oestrogen which increases the vascularity of the endometrium during proestrus. This vascularity peak at about 1 day after the end of the oestrus. The bleeding is not an indication of pregnancy or failure to conceive neither is it the same as menstrual bleeding in human. The early part of it is characterized by behavioural signs very similar to those observed during proestrus except that while in proestrus the distance between cows is decreasing with the passage of time, it is increasing in metoestrus. If synchronization is needed, artificial progesterone is applied to keep CL in live.

Dioestrus: This is the period of preparation for pregnancy. It's the stage of the oestrous cycle following metoestrus when the CL is the dominant structure and the genitalia (as well as the entire animal) are quiescent (at rest). It is the period of the cycle that the corpus luteum is fully functional. The duration is 15days. In the cow it begins from day 5 and end on the 16-17. When the CL regresses in the sow it begins from day 4 to end at either day 13, 14 or 15. In mare it begins on the day 8 and last to the day 19 or 20. In humans it begins around day 16 to 17 and could last to around 27 and 28.

Table 14: Oestrous cycle length, Oestrus duration and time of ovulation in some mammals

Species	Oestrous-cycle length	Oestrus duration	Time of ovulation
Cow	21-22days	18-19hours	10-11hours after end of oestrus
Ewe	16-17days	24-36hours	24-30hours from start of oestrus
Goat	21days	32-40hours	30-36hours from start of oestrus
Sow	19-20days	48-72hours	35-45hours from start of oestrus
Rabbit (reflex ovulator)	-	-	10-11hours after copulation
Mare	19-25days	4-8days	1-2days before end of oestrus
Dog	-	7-9days	1-3days after start of oestrus
Cat (reflex ovulator)	-	4days	24hours after mating
Cat	15-21days	9-10days	If no mating occurs
Mouse	4days	10hours	2-3hours after start of oestrus
Rat	4-5days	13-15hours	8-10hours after start of oestrus
Woman*	28days	continuous	Days 12-15 of cycle

*Menstrual cycle

Source: Osinowo, 2006.

Behavioural and other symptoms of oestrus: Oestrus or heat is the period the female animal is receptive to the male, this knowledge is important to farm animal managers for natural mating or artificial insemination. High levels of oestrogen in the blood have been associated with behavioural signs of oestrus. However evidence exists indicating the importance of the sense of smell, sound, sight and touch. Pheromones can cause profound shifts in the neuroendocrine control mechanisms. The smell of the boar or boar's grunting sound provided through recordings has been shown to elicit stronger oestral responses in Sow. In ewe oestrus is shown only in the presence of the ram. A combination of the senses of sight, smell and sound may be involved in eliciting stronger oestralbehaviour. Generally, female animals in oestrus are more restless, irritable and excitable during oestrus. Interests in the male become more apparent when the male of the species is around. Indication of oestrus is usually seen at proestrus. The psychological manifestation and increased activity around the time of oestrus is caused by oestrogen. Administration of exogenous oestrogen causes oestrus almost at any time during the oestrous cycle but it should be noted that oestrus can occur without ovulation (**anovulatory oestrus**). Full manifestation of oestrus requires progesterone priming. Sow in oestrus show significant changes in bioelectric potential measured externally over the ovarian region of the body (23.2mV in sow on oestrus and 6.6mV in non-oestrus sow). Vaginal pH is more alkaline in dioestrus and more acidic during oestrus.

Depending on the phase of the oestrous cycle, vaginal epithelium fluctuates between stratified squamous and low cuboidal types, mostly under the influence of oestrogen and progesterone. Oestrogen causes cornification of vaginal epithelium during early and late oestrus which later starts to break down as oestrogen levels drop after ovulation. After ovulation due to progesterone, thick mucus is secreted and the epithelium proliferates and becomes infiltrated with leucocytes. Vaginal histology can accurately monitor ovarian changes using the vagina smear technique in animals with short oestrous cycle like mice and rats but not very reliable in species with longer cycle due to a lag of one to several days in the reflection of ovarian changes in vaginal histology. The cervix does not undergo any cyclical changes but the mucus becomes thinner and more alkaline as a result of increased oestrogen, thus favouring the passage of sperm. It is thinnest at the time of ovulation. Increased progesterone after ovulation or during pregnancy makes the cervical mucus thick and more acidic. In women, ovulation is preceeded by a drop in basal body temperature but it is difficult to monitor in livestock due to difficulty in obtaining basal body temperature. The volume of genital (vagina, cervix, uterus and oviduct) secretion increases at oestrus due to the effect of oestrogen because they are all steroid target organs. In the follicular phase which is under oestrogenic influence uterine glands are simple/ straight with few branching but during the luteal phase under progesterone influence, uterine endometrium increases in thickness as the uterine glands increase in length/diameter and becomes extremely branched and convoluted. Sexual stimulation reduces length of oestrus in the cow, ewe and sow, and decreases variability of ovulation time in the ewe.

The knowledge of the individuality of the animal in question are important in oestrus detection, it is helpful to check for oestrus while the animal in quiet. The best time to check is at dawn and dusk. The different species have their peculiar characteristic oestralbehaviour.

Cow: They display strong homosexual behaviour, even when bulls are not present. Females mount each other and they stand for mounting. They smell the vulva of other cows. They frequently raise or twitch their tail. They walk along the fence-line in search of the bull. Physiological signs include: congested vulva i.e. enlarged and reddened inside but black outside. Clear mucus discharge will be noticeable. Only cows on heat will stand to be mounted.

Ewe: Oestrusbehaviour contrasts that of the cow. Ewe display no sign except in the presence of a ram when it will start rubbing her body against that of the ram and rubbing her chin on the rams neck, it will roam around the ram while smelling its genitalia, it will also be shaking its tail. The vulva is not congested and there is no mucus discharge. If artificial insemination (AI) is to be used in ewe breeding, a vasectomised or aproned (rubber with smooth surfaces coated with Vaseline) ram is used in heat detection.

Sow: In sow, the first noticeable sign is swollen vulva. There is no mucus discharge. The sow will assume a mating stand when pressure is applied to its backside. Sow needs no restraint for artificial insemination.



Mare: Mare will allow the stallion to smell and bite; she will extend her hind leg, lift her tail to the side and lower her rump. The vulva will be swollen and elongated. The libia will be seen partly inverted with the erect clitoris frequently exposed by contraction of the libia, a phenomenon known as winking. The mare should be teased with the stallion for accurate detect of heat.

An attempt by mare to fight stallion indicate mare is not on heat.
Heat detect in farm animals constitute a major problem; therefore the knowledge of the expected behaviour for each animal is very important for accurate heat detection.

Abnormal oestrus includes anovulatory, quiet or silent and split oestrus.
Anovulatoryoestrus - this is the occurrence of oestrus without subsequent ovulation. FSH level is normal but LH is insufficient. It may be as a result of climatic or nutritional stress and may also occur in the post-partum period immediately after parturition. Breastfeeding creates anovulatoryoestrus in women which varies with individuals.
Quiet or silent oestrus this is the occurrence of ovulation without behaviouraloestrus. LH level is normal but FSH level is low and oestrogen level may also be low. Occur in the post-partum period in sheep. It is also common in cow and mare.
Split oestrus this is an oestrus that is interrupted by period of non-receptivity lasting few hours to 2 days. Post-partum oestrus Oestrus occurs shortly after parturition in several species e.g. in rats, post-partum oestrus are accompanied by ovulation within 48hours after Parturition, though no further oestrus occur after this in suckled rats till after weaning, but mating at first post-partum oestrus may result in pregnancy.

Post-partum oestrus occurs within a few days in sow but without ovulation, hence no pregnancy if mated, although the removal of litter results in oestrus and ovulation a few days later. Post-partum oestrus occurs between 30 60days with low fertility in cows. It may occur 0 - 2days after parturition in ewe without pregnancy until about 30days post-partum.

Deranged cycles

Whitten effect shift in proportion of females showing heat daily caused by odour of male or ingestion of male faeces. The original experiment was conducted with mice.

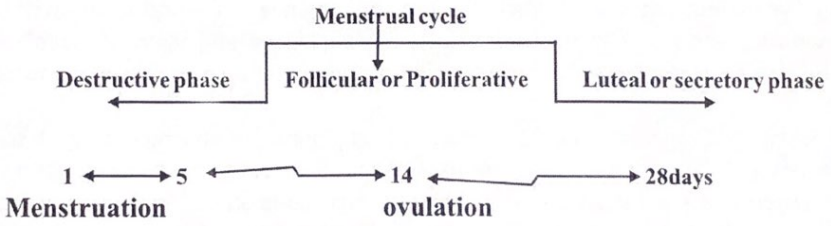
Bruce effect female mice mated by male of one strain conceive to mating by male of different strain. Introduction of male of new strain probably caused CL degeneration and ovulation of a new crop of follicles.

Sudden introduction of rams in ewe flock may have synchronizing effect on oestrus in ewes.

Transportation of mature sow from one location to another tends to synchronize their sexual activity (heat and ovulation) in relation to the moving day. Transportation also advances onset of puberty in gilt.

Psychosomatic modification - of menstrual cycle is common in women.

Menstrual cycle This is the period of regular cyclic changes in preparation for fertilization and pregnancy with a featured periodic vaginal bleeding/ shedding of uterine mucosa (menstruation) and the beginning of another. It is the interval between one menstruation and the next. A series of cyclic changes that occur in the ovary during a normal menstrual cycle is the ovarian cycle. The beginning of the menstrual flow marks the first day of the menstrual cycle. It is divided into three stages destructive (menstruation) phase (days 1-4), proliferative (follicular) phase (days 5-14) and the secretory (luteal or progestational) phase (days 15-28).



Destructive phase during the early part of the cycle FSH and LH levels are rising. This increase in the level of FSH recruits a population of stage 5 follicles for maturation.

The rise in the level of FSH/LH results from the fall of oestrogen/progesterone level due to the regression of the CL from the previous cycle, resulting in a further increase in oestrogen secretion by the theca granulosa cells. The eleveatedoestrogen

- Stimulates the proliferative growth phase of the endometrium,
- Depresses the FSH level,
- Serves as a positive feed-back signal to trigger the LH-FSH surge which leads to ovulation.

Follicular phase The uterus is supplied by two types of arteries namely the straight and superficial arteries. At the end of the destructive phase all but the deep layers of the endometrium have sloughed and the endometrium increase rapidly in width, the uterine glands increase in length but do not secrete and the superficial layer becomes more vascular and the deeper parts become dilated under the influence of the developing follicle. During the period of menstrual bleeding several primordial follicles enlarge. After a few days, about the 6th day, one of the follicles begins to grow more rapidly than the others such that the others regress and become atretic. The ovum which has outgrown the others in development undergoes meiosis. The volume of fluid within the follicle rises and the tissue between the follicle and ovarian surface becomes thinner. Eventually at about the 14th day of the cycle, the distended follicle ruptures and the ovum is extruded into the abdominal cavity. This is called **ovulation**, which is caused by a sudden outflow of LH from the pituitary to stimulate oestrogen/progesterone output which act on the follicle to cause

follicular rupture. The ovum is picked up by the fimbriated ends of the fallopian tube and transported to the uterus and unless fertilization occurs, on out through the vagina. In a regular 28-day cycle ovulation can occur between the 12th and 17th day of the cycle. In an irregular cycle ovulation occurs between the 13th and 15th day before the beginning of the next bleeding.

At ovulation the body temperature rises by 0.3 - 0.6°C due to the production of progesterone and this is maintained all through the postovulatory phase until just before menstruation when it drops. Ovum lives for approximately 72hours after its extrusion from the follicle while sperm survives in the female tract for not more than 48hours. Increased oestrogen during the follicular phase makes cervical mucus thinner and more alkaline thus favouring the passage of sperm, it thinnest at the time of ovulation. Increased progesterone after ovulation or during pregnancy makes the cervical and vagina mucus thick/ more acidic, vagina epithelium proliferates and becomes infiltrated with leucocytes.

Luteal phase - After ovulation the follicle which ruptures at the time of ovulation promptly becomes filled with blood forming the **corpus haemorrhagicum**.

The minor bleeding from the follicle into the abdominal cavity may cause peritoneal irritation and fleeting lower abdominal pain. The granulosa and theca cells proliferate and the clotted blood is replaced with yellowish lipid-rich luteal cells forming the corpus luteum. If pregnancy occurs the CL persists until the 4th month when the placenta would have been fully developed, otherwise it degenerates after menstruation and becomes a whitish scar known as **corpus albicans**. Under the influence of oestrogen and progesterone from the CL, the endometrium becomes slightly oedematous and the actively secreting glands become tightly coiled and folded. Spiral arteries become tortuous. At the end of the phase, the CL regresses and the hormonal support of the endometrium is withdrawn. The spiral arteries constrict and the part of the endometrium which they supply become ischemic. The spiral arteries dilate and the necrotic walls rupture producing haemorrhage which accompanies the sloughing and the menstrual flow. The sloughing is facilitated by prostaglandins liberated in the endometrium. Menstrual bleeding is predominantly arterial with only 25% being of venous origin. Unless the bleeding is excessive it does not produce a clot. The bleeding ends when the spiral arteries constrict and a new endometrium regenerate from the basal layer. The total volume of blood flow is between 30 70ml with flow duration of between 4 5days.

Other effects: These varies with individuals, it includes irritability, lack of concentration to less efficiency before menstruation. In most female the breasts become enlarged and the tissues become loaded with salt and water. Progesterone has been implicated for the central effects.

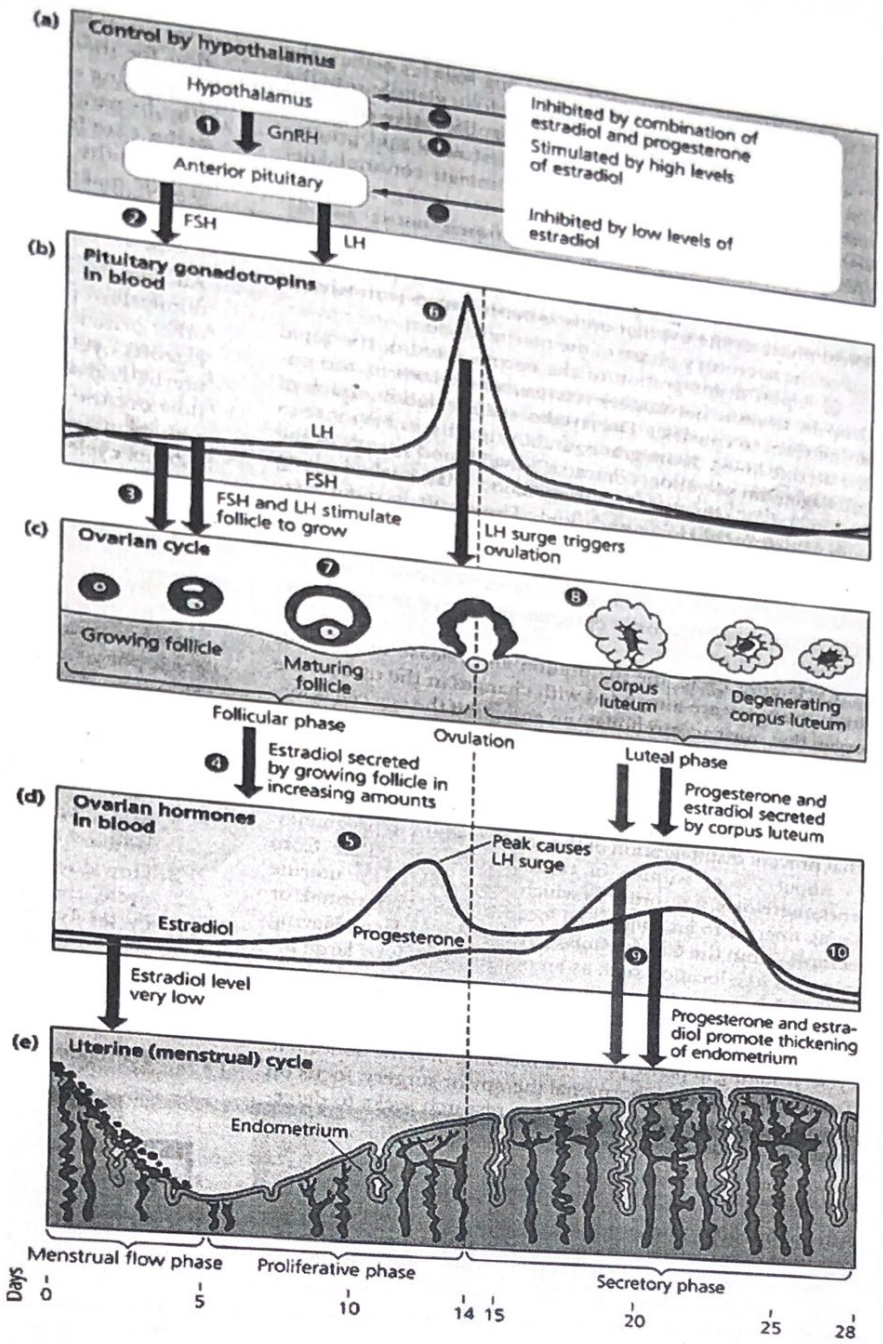


Figure 72.0: Reproductive cycle in primates

Table 15: Comparative study of oestrous and menstrual cycles

Oestrous cycle	Menstrual cycle
1. Occurs in non-primates (cow, sow, ewe, etc).	Occurs mainly in primates (monkey, woman).
2. Shows physiological/ morphological changes in ovaries, vagina and uterus with periodic peaks of sexual receptivity (oestrus).	Shows physiological/morphological changes in ovaries, vagina and uterus without observable periodic peaks.
3. Sloughing off of endometrium is not accompanied by bleeding.	Sloughing off of endometrium is accompanied by bleeding.
4. Has short follicular phase (2-5 days).	Has longer follicular phase (up to 2 weeks).
5. Luteal phase is of similar duration.	Luteal phase is of similar duration.
6. It is divided into 4 phases – proestrus, oestrus, metoestrus and dioestrus	It is divided into 3 phases – destructive, proliferative and secretory phase.

The effect of bilateral castration and removal of the ovaries depends on the period of castration or ovaries removal before puberty or after puberty.

Table 16: Effect of testis removal on the male reproduction

Ovary removal before puberty	Ovary removal after puberty
1. Permanent puberty	1. Prostate gland/ seminal vesicle atrophy.
2. Infertile internal/external genitalia	2. Penis usually shows little regression/ capable of erection and ejaculation of accessory gland fluid.
3. Failure of facial/axillary hair to grow and hairline remain typical of childhood.	3. Permanent sterility.
4. Pubic hair is female in type i.e. not extending towards the umbilicus.	4. No effect on the vocal cord.
5. Laryngeal growth is arrested and the voice fails to deepen.	5. Baldness never occurs in those who are prone to baldness.
6. Fat is deposited in the buttocks hips, pubic and breast regions.	6. Administration of pure testosterone can reverse all the changes associated with adult castration except sterility.

9. Muscles remain weak and the normal male aggressiveness is absent.
7. Epiphysial fusion is delayed which may give rise to tallness.
8. Skin is soft and feminine.

Table 17: Effect of Ovary removal on the female reproduction

Ovary removal before puberty	Ovary removal after puberty
1. Lack cyclic ovarian activity/ ovulation. 2. The beginning of menstruation is blocked. 3. Lack of growth of the breast and the rounding of the body contours. 4. Psychological changes associated with puberty are absent. 5. The growth of pubic/ axillary hair is absent.	1. Permanent sterility. 2. Caseation of ovulation/ menstrual cycle. 3. Give all symptoms accompanying menopause

HORMONAL REGULATION OF THE OESTROUS CYCLE

The oestrous cycle is regulated by the reciprocal balance between the ovarian steroid and the gonadotropic hormone of the anterior pituitary.

The anterior pituitary itself is regulated by the hypothalamus although the precise nature of the regulation is not clear; however PGF2α is known to cause the regression of the corpus luteum during the oestrous cycle. The PGF2α is released from the uterus and the mechanism of release is unknown. However in the sheep a path by which prostaglandin produce in the progesterone prime the uterus get to the ovary to cause the CL regression has been proposed.

By radio immuno assay study or technique the relative changes of the blood level of the male reproductive hormones have been determined.

Hormonal patterns during the oestrous cycle one similar in all species. It is clear that progesterone has dominant effect on the regulation of oestrous cycle. At dioestrus when progesterone concentration is high in the blood the concentration of other hormones such as FSH, LH and total oestrogen are relatively low. Some follicular growth can be detected due to small mid-oestrus surges of FSH, LH and oestrogen in some species.

This is not the same rapid follicular growth which occur 2-3days before ovulation. Also during pregnancy high progesterone concentration suppresses the gonadotropins released from the anterior pituitary through a negative feedback control, thus, suppressing oestrualbehaviour. At the end of dioestrus the CL is caused to regress by PGF2α from the uterus. The regression of the CL leads to a sharp drop in the plasma progesterone which in turns serves as a stimulus to the hypothalamus to release Gn-RH which will stimulate the anterior pituitary to release FSH, LH and prolactin.

A surge of oestrogen seen during proestrus in the sow, ewe and cow and near the start of oestrus in the mare drops near the end of oestrus. Within 24hours before ovulation surges of FSH/ LH lasting 8-10hours are seen. A surge of prolactin also occurs near the start of oestrus. This surge remains high during the oestrus period. FSH stimulates the release or surge of oestrogen but it has not been detected in the peripheral blood plasma during this period. This phenomenon has not been fully investigated or explained. It is felt that the release of FSH causes increases in binding of FSH to receptor sites in the granulosa cells, for example in cow it has been shown that FSH from the anterior pituitary becomes depleted 2 days before the LH surge. The rapid surges of FSH and LH during oestrus appear necessary for the final growth and maturation of the graafian follicle preceeding ovulation. The functional significance of increased FSH during metoestrus in the cow has not been determined. As follicles grow a surge of oestrogen occurs produced by the theca interna cells.

The oestrogen causes the behavioural signs of oestrus and triggers the release of LH through a positive feedback control. After ovulation CL forms at each ovulatory site, the formation of which occurs very rapidly within 45days after ovulation. During this period increased levels of progesterone are detected in the peripheral blood plasma. LH is the dominant hormone that controls the development of the CL. It is luteotropic and synergizes with prolactin to maintain the function of the CL in farm animals. The LH appears to perform this function by increasing the blood supply to the CL. Conversely PGF2α towards the end of the CL life cut off blood supply to CL thus depriving it of metabolite needed for the synthesis of progestin, thus causing it to regress.

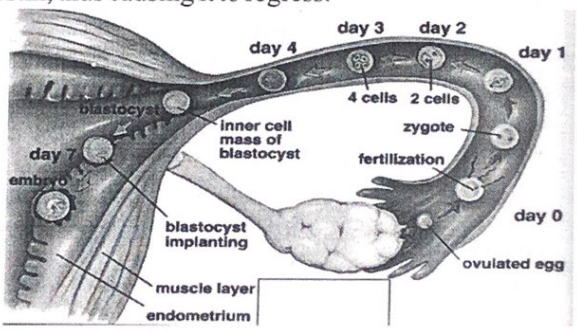


Figure 73.0: The Fertilization process in the oviduct

SENESCENCE

Few indication exist in farm animal to show the mechanism of sexual ageing, however sexual ageing has been well studied and documented in the rat, mouse and man. In aged female mammal only a slight decrease occurs in ovulating and fertilization rate, however high embryonic mortality, still-birth and post-partum losses occurs due to rapid ageing of the uterus and insufficient milk production.

In the sow numbers of litter varies from 1 to 26 with maximal litter size occurring during the 3rd, 4th and 5th parity. In cattle reduction in fertility begins at 7-8years of age; however cows that are fertile for as long as 30years has been observed. In the ram little information is available. In the bull testosterone secretion begins to decline with age after the maximum is reached at 5years but fertility seems not to be affected.

FERTILIZATION

It is the fusion of the egg and spermatozoa to form a zygote. The following represent the sequential step in the process of fertilization:

1) Spermatozoon penetrates the cumulus and the corona radiata cell and sticks to the zona pellucida. Two enzymes hyaluronidase and corona penetrating enzyme aid the penetration. Both enzymes are associated with the sperm head. The release of this enzymes are made possible by capacitation and acrosome reaction. Acrosome reaction can only occur in capacitated sperm.
2) The spermatozoon then penetrates the zona pellucida and fuses with the vitelline membrane. The zona reaction is initiated as the cortical granules disappear.The cumulus oophorus and corona radiata are shed before fertilization in some species. The zona reaction prevents the entrying of any other sperm into the ovum. Acrosin, a trypsin-like enzyme which is associated with the sperm head and the penetration with a slit left behind in the zona pellucida at the entrying point.
3) The spermatozoon is then engulfed by the cytoplasm of the oocyte or egg. Then the vitelline block is evoked.
4) The cytoplasm shrinks; the second polar body is extruded into the perivitelline space. The male and the female pronuclei is formed, syngamy occurs, the zygote forms and fertilization is completed.

Although hundreds of millions of spermatozoa are ejaculated, less than 1,000 (0.01%) reach the ampulla. During mating semen is deposited in the vagina (ewe and cow) or relaxed cervix (mare) or uterine-body (sow) or high up in the vagina (woman) by passive transport, sperm motility, aid of ciliated epithelium or uterine contraction (aided by oxytocin) which is increased by orgasm (heights of sexual excitement). The heights of sexual excitement results in orgasm during which oxytocin is released from the posterior pituitary gland. That is why milk is ejected from the breast of a lactating woman during coitus.

Orgasm is desirable during coitus but it is not essential for reproduction. Spermatozoa undergo some necessary changes (capacitation) in the female genital tract before they are capable of fertilization. Sperm are transported to the oviduct within five minutes of ejaculation in cow. Ova are flushed in follicular fluid into the infundibulum during ovulation and transported within the oviduct by muscular contraction of myometrium and the beating of the kinocilia.

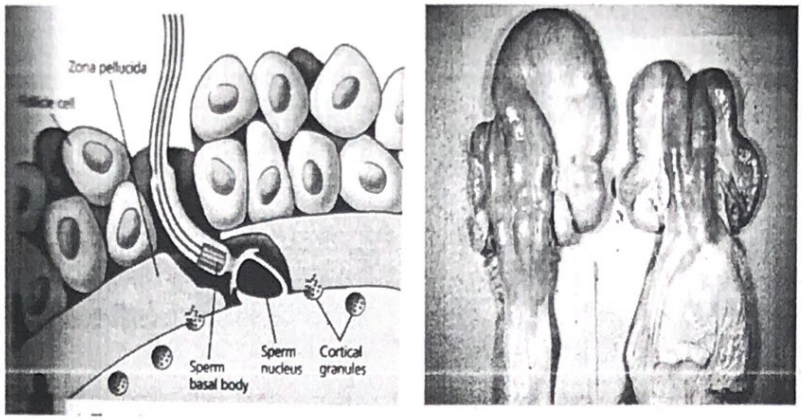


Figure 74.0: Fertilization process in mammal; uterus showing pregnant and non-pregnant uterus

Poly-spermy

This is a term describing the fertilization by more than one sperm leading to a 3n zygote. Such embryo develops up to a point and degenerate, they don't survive. Conditions causing poly-spermy are

(i) Aged oocyte

(ii) Heated oocytes, therefore the condition is more common when there is increase temperature in the female due to fever or high temperature. Polyspermy might be responsible for reduced conception rate under tropical condition.

Ageing of gamete

Reproductive efficiency may be influenced by the age of the gamete e.g. estimated fertile life of the sperm and ova are stated below.

Species	Fertile life in hours	
	spermatozoa	ova
Cattle	24-48hrs	8-12hrs
Swine	24-48	8-12
Sheep	30-48	16-24
Horses	72-120	6-8

To ensure optimum fertility therefore, managers should be diligent in the management of reproductive breeding season, heat should be regularly detected and mating should be properly timed.

GESTATION PERIOD

Gestation begins with fertilization and ends with parturition. It varies in species. It takes 114 days in Sow (3months, 3weeks, 3days), 281days in Cow (a little over 9months), 148 days in Ewe (about 5months), 156 days in Goat and 337 days in Mare (about 11months). Factors influencing gestation period could be classified as:

1. Maternal factor e.g. age of the mother, in ewe it has been observed that gestation period could be extended by 2days in 8years old ewe and above. In cattle, heifers has shorter gestation period than cow.
2. Foetal factor: In polytocous species, the litter size affects gestation period. The larger the litter sizes the shorter the gestation period e.g. twin calves are carried 3-6 days less than single calf. The sex of the foetus in monotocous species is also important. Male calves or foal are carried one to two days longer than female. Size of the foetus tends to hasten parturition. Foetal endocrine function tends to affect parturition. Prolonged gestation has been linked with pituitary adrenal hypo-function (functioning below normal).
3. Genetic factor: Breed variation occurs in gestation period in most farm species e.g. in the cattle species there are variation in gestation in Holstein compared to Jersey or Brown Swiss.
4. Physical environment: There is some evidence that a winter bred horse has prolonged gestation and it is known that seasonal fluctuation in hormonal profile affects gestation period.
5. Plane of nutrition also affects gestation, in rodents evidence exist that high temperature causes prolonged gestation.

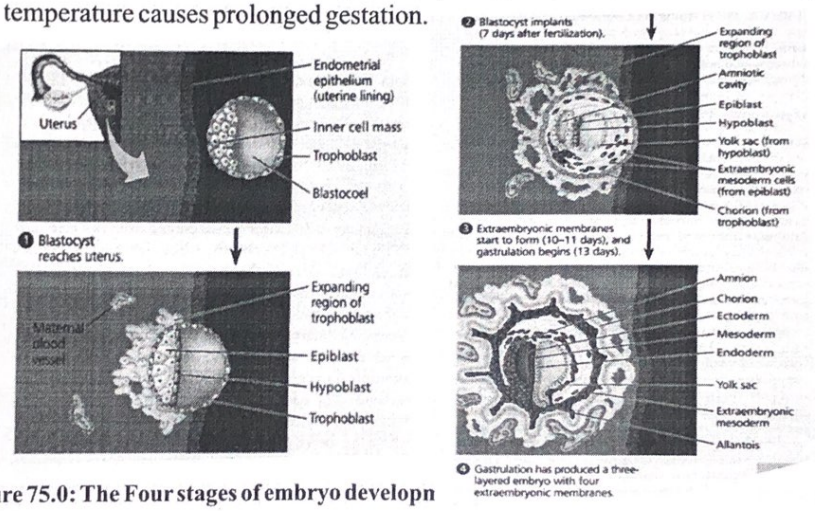


Figure 75.0: The Four stages of embryo developn

Table 18: Estimates of fertile life of sperm/ova and the tempo of embryonic development in mammals

Species	Fertile life (hours)		Days after ovulation for				
	Sperm	Ovum	2 - cell	8 - cell	Entry into uterus	Blastocyst	Gestation
Cattle	30 - 48	8 - 12	1	3	3 - 3.5	7 - 8	275-290
Sheep	30 - 48	16 - 24	1	2.5	3	6 - 7	145-155
Swine	24 - 48	8 - 10	14-16hrs	2	1.75 - 2	5 - 6	112-115
Rabbit	30 - 36	6 - 8	1	2.5	3	4	30-32
Horse	72-120	6 - 8	1	3	4 - 5	6	335-345
Man	28 - 48	6 - 24	1.5	2.5	2 - 3	4	252-274

Source: Adapted from Hafez and Hafez, 2000

Table 19: Time spent by the embryo in the oviduct and subsequent degree of development in some mammals

Species	Days spent in oviduct	Stage of development on arrival in uterus
Ungulates	3-4	4-16 cells
Rat, mouse, rabbit	3	Morula or blastula
Cat	8	Blastocyst
Dog	10	Blastocyst
Primates	4	16 cells

The ectoderm gives rise to the central nervous system, sense organs, mammary glands, skin (epidermis), hair and hooves.
Mesoderm gives rise to circulatory system, skeletal system, muscles, reproductive system, kidney and urinary system.

Endoderm gives rise to the digestive system, liver, thyroid gland, lung and pancreas.

Formation of most organs and body parts takes place between the second and sixth week of gestation in cattle. Heartbeat and blood circulation commence on day 21 of pregnancy in cattle, day 16 in the sow. Absolute growth of foetus is exponential up to parturition but relative growth declines around mid-gestation. More than 50% of the increase in foetal weight occurs during the last two months of gestation.
As the blastocyst increases in size, it can no longer absorb enough nutrients by diffusion as in the early stages, so a trasition from embryotrophic to haemotrophic nutrition occurs after implantation with the development of the placenta. Shortly after implantation and the development of placental membranes, extra-embyonic

membranes (chorion, amnion and allantois) are formed. Two extra-embryonic membranes are formed. The third which is the yolk sac is formed earlier but later disappears. The yolk sac contains the early nutrients for the developing embryo. A portion of the yolk sac folds over the embryo to form the primitive gut.

The amnion is the inner extra embryonic membrane. It forms as the trophoderm around the embryo. The amnion contains the amniotic fluid in which the embryo is suspended. The amnion is palpable via the rectum in cow between 30-45days and it is used in pregnancy diagnosis. The amniotic fluid serves as a cushion and it baths the embryo throughout gestation.

The second extra embryonic membrane is the chorion. It is the outer extra membrane which forms at the same time with amnion. It is the outermost membrane and in contact with the endometrium.

The third membrane, allantois, is located between the amnion and chorion. It is continuous with the anterior extremity of the bladder by way of the urachus which passes through the umbilical cord. The inner layer of the allantois is fused to the amnion while the outer layer is fused to the chorion, forming the allantochorion (fusion of the allantois and the chorion). By this fusion, the foetal vessels in the allantois come in close apposition to the umbilical arteries/veins located in the connective tissue between the allantois and chorion (Campbell *et al.*, 2008). The allantois is a pouch from the hind-duct near the yolk sac along the umbilical cord. It connects the embryonic bladder and contains waste product. The allantochorion becomes attached to the endometrium during placentation to form the placental.

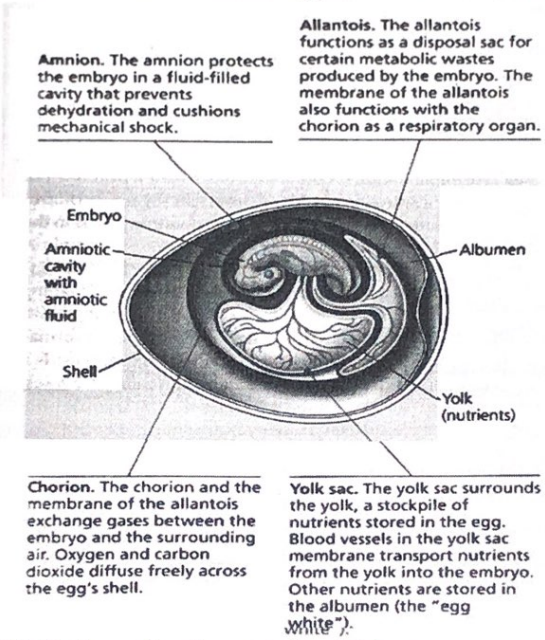


Figure 79.0: Embryo development in chicken

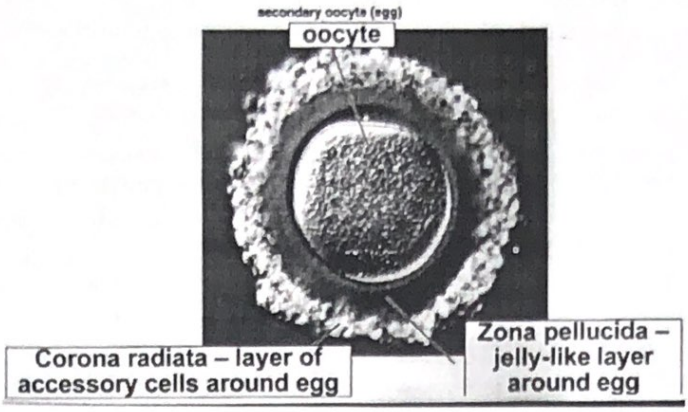
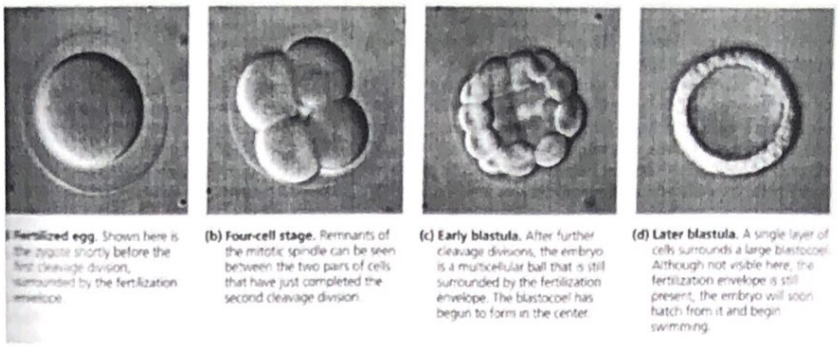


Figure 76.0: An ovum before fertilization

Embyology (Perinatal period): this involves the study of the developing fertilized ova or oocyte and it involves the following

1. Ovum before fertilization
2. Zygote
3. Morula (mass of cell with no form)
4. Blastula (formed the blastocyst)
5. Embryo
6. Gastrula

Fertilization is the fusion of two cells (male and female gametes) to form a single cell called zygote. The fusion of the male and female nuclei is known as **syngamy**and is the central process of fertilization. Fertilization occurs at the lower half of the oviduct (ampullary-isthmus junction). It begins after the extrusion of the first polar body with the sperm penetrating the ovum while the second reduction division is in progress. After the passage of the first sperm through the zona pellucid of the ovum, zona reaction occurs rendering the zona pellucid uneasy to penetrate by other sperm. Extra sperm that succeed in passing through the zona pellucida into the peri-vitelline space are called supplementary sperm. The vitelline block exhibited by the vitellus also prevents the entry of more than a single sperm apart from the zona reaction. While the fertilizing sperm is engulfed by the vitellus, the vitelline surface becomes unresponsive to contact and no further sperm is engulfed. Extra sperm which succeed in entering the vitellus despite both the zona reaction and vitelline block are called supernumerary sperm and the ovum is said to show polyspermy which is low in mammals (leads to a lethal condition triploidy).



(a) Fertilized egg. Shown here is the zygote shortly before the first cleavage division, surrounded by the fertilization envelope.

(b) Four-cell stage. Remnants of the mitotic spindle can be seen between the two pairs of cells that have just completed the second cleavage division.

(c) Early blastula. After further cleavage divisions, the embryo is a multicellular ball that is still surrounded by the fertilization envelope. The blastocoel has begun to form in the center.

(d) Later blastula. A single layer of cells surrounds a large blastocoel. Although not visible here, the fertilization envelope is still present; the embryo will soon hatch from it and begin swimming.

Figure 77.0: Cleavage in an embryo

The process of cleavage (cellular division without growth) commences soon after syngamy and continues until implantation. By the 16 - 32 cell stage the cells are crowded together in a compact group within the zona pellucida and the embryo is now known as a **morula**. It is after the morula stage that total embryonic mass increases. Cell differentiation already takes place in the morula stage, the three cells nearer the one pole becoming the embryo and the other the trophoblast. The embryo pole forms the generative pole while the trophoblast becomes the vegetative pole. Fluids starts collecting in the interstitial spaces which expand to form an inner cavity (blastocoele) during which the embryo is called **blastocyst**. The inner cell mass (blastocoele) gives rise eventually to the adult organism while the cells of the trophoblast form the placenta and embryonic membranes. It is the stage at which the zona ruptures and differentiation begins. A cavity known as the blastocoele is formed during the blastula stage. It is the stage at which the zona ruptures and differentiation begins. The embryonic cells are called blastomeres during the early cleavage stages, before blastocoele formation.

Embryo stage is the stage at which differentiation begins and it develops into the gastrula stage. The embryo spends some days in the oviduct before reaching the uterus where implantation (attachment of the embryo on the uterine wall establishing physical contact with the maternal organism). Blastocyst is well established by the time implantation occurs. In ungulates and carnivores, the blastocyst elongates and enlarges until it fills a good part of the uterine cavity (central implantation). In rodents the blastocyst remains small and becomes lodged in the fold of the uterine lumen where it implants itself (eccentric implantation). In guinea pig and man, the blastocyst implants itself interstitially by passing through the uterine epithelium, becoming completely cut off from the uterine lumen (interstitial implantation). Implantation begins in ewe between days 10 and 22 of pregnancy, days 11 and 40 in cow, day 11 in sow and days 6 and 8 in man. The organization of placental membranes and the extra embryonic membranes (amnion, allantois and chorion) begins shortly after implantation.

Gastrula stage: In the gastrula stage the following shapes can be observed.

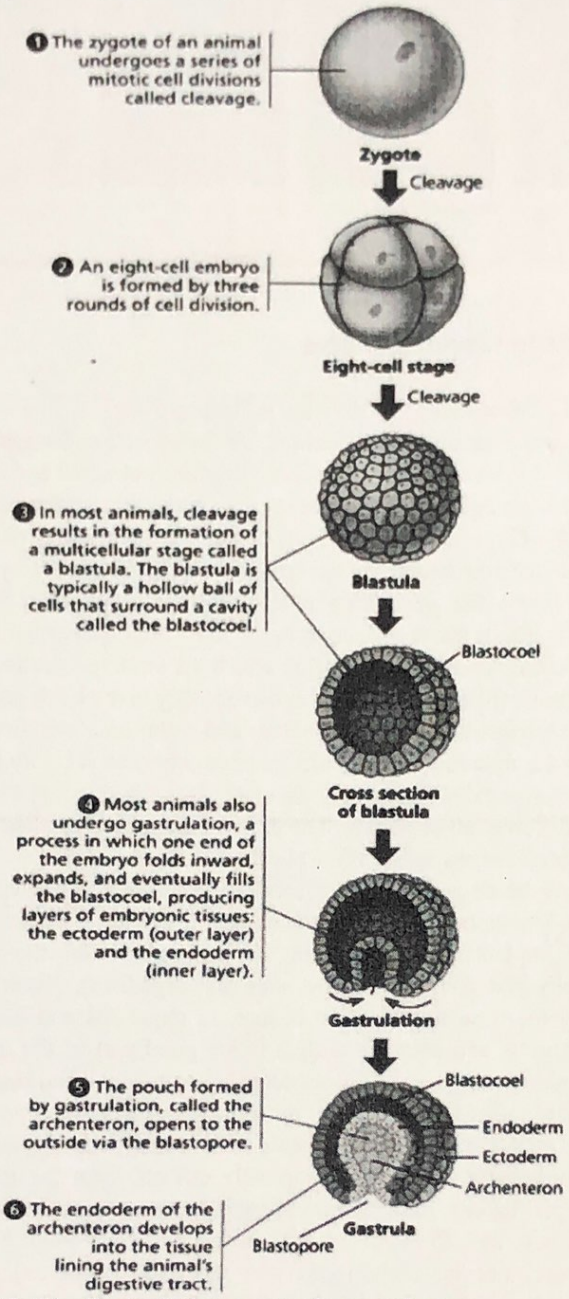


Figure 78.0: The Gastrulation process in an embryo

The outer layer of the blastocyst (trophoblast) thickens to form the ectoderm, erodes the endometrium thereby stimulating the endometrium to start the formation of the maternal part of the placenta. During implantation the blastocyst grows and develops, forming around itself a layer of cells (trophoblast) which breaks through the epithelial layer of the endometrium and invades the stromal connective tissue in primates. The trophoblast is divided into the inner layer (cytotrophoblast or Langhan's layer) and an outer layer (syncytial layer). The endometrium thereby produces numerous large polyploidy decidual cells and histamine is released. The trophoblast becomes closely associated with the outermost membrane surrounding the foetus (chorion) and continues to grow and form projections (chorionic villi) into surrounding tissues. Large cells from the endometrium invade the syncytial layer in large columns with each column developing a vascular system and villi, the mass of villi make up the placenta. The maternal blood is separated from the foetal blood by a layer of trophoblast cells, foetal connective tissue and cells lining the foetal blood vessels. With advancing pregnancy the layer of trophoblast cells become thinner. Blood from the placenta services the foetus through the umbilical arteries and veins. Blood of the mother and foetus flow in a counter current manner. The blood with least oxygen in the mother meets that of the foetus but because the partial pressure of oxygen of the mother's blood is still higher, oxygen moves from the mother's blood to the child. This applies to other materials. Gastrulation is completed by the development of these 3 layers ectoderm, mesoderm and endoderm. Cells at one pole of the blastocyst (germ-disc) give rise to three separate layers of cells (ectoderm, mesoderm and endoderm) at the early stage of differentiation.

ECTODERM	MESODERM	ENDODERM
• Epidermis of skin and its derivatives (including sweat glands, hair follicles) • Epithelial lining of mouth and anus • Cornea and lens of eye • Nervous system • Sensory receptors in epidermis • Adrenal medulla • Tooth enamel • Epithelium of pineal and pituitary glands	• Notochord • Skeletal system • Muscular system • Muscular layer of stomach and intestine • Excretory system • Circulatory and lymphatic systems • Reproductive system (except germ cells) • Dermis of skin • Lining of body cavity • Adrenal cortex	• Epithelial lining of digestive tract • Epithelial lining of respiratory system • Lining of urethra, urinary bladder, and reproductive system • Liver • Pancreas • Thymus • Thyroid and parathyroid glands

Placenta is the union of foetal and maternal tissues for physiologic exchange (Fig.80.0 and Fig. 81.0). It is a multi-functional organ performing many functions and substituting for the foetal gastrointestinal tract, liver, endocrine glands, kidney, lung, and separates maternal and foetal organisms to ensure separate development of foetus. There are different types and shapes of placental in farm animals. The types are:

1. Epitheliochorial: This type is diffuse in shape as found in mare and sow.
2. Syndesmochorial: This is mainly cotyledonary in shape and it is found cow, ewe and goat.
3. Hemochorial: it is discoid or zonary in shape and it is found in human or primates.
4. Hemoendothelial: it is discoid or spheroidal in shape and it is found in rabbit, guinea pig and rats.
5. Endotheliochorial: it is zonary or discoid in shape and it is found in dog and cats.

Physiology of the placenta

The development of the placenta is associated with viviparity. The placenta has very many functions which include:

1. Acts as the foetal gastro-intestinal tract, lung, kidney, liver and endocrine glands.
2. It separates the maternal and foetal organism, thus ensuring the separate development of the foetus.
3. It separates the blood circulation of the foetus and mother. The closeness of the two circulatory system permits the transfer of nutrients and oxygen from maternal system to foetal circulation with waste products and CO_2 from the foetal circulation to the maternal system. The exchange of material through the foetal membrane can be through simple diffusion i.e. the movement of molecules from area of high concentration to area of low concentration.
4. Active transport: Is the pumping of material against a concentration gradient. Most molecules of physiological importance are transported in this fashion (Table 20).
5. Phagocytosis and pinocytosis are also important physiological exchanges through the placenta.

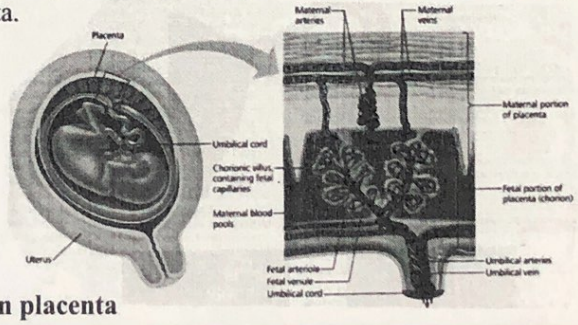
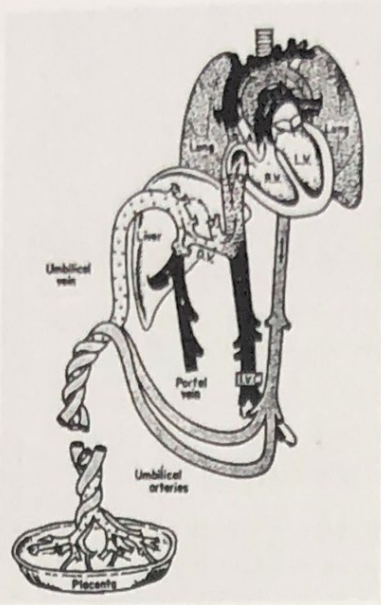
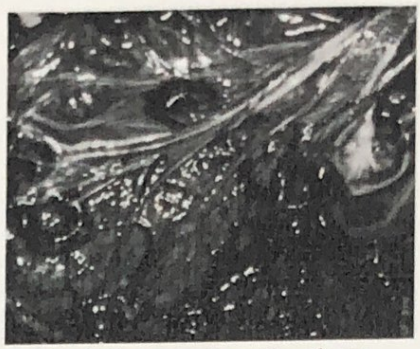


Figure 80.0: The human placenta





Placental membrane of a cow



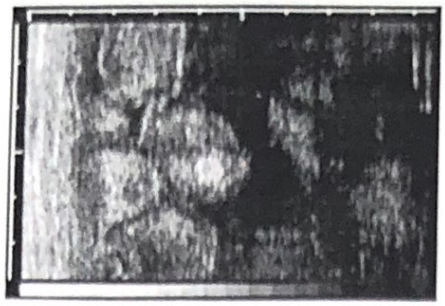
Cotyledonary placenta of ewe

Figure 81.0: Schematic representation of foetal circulation

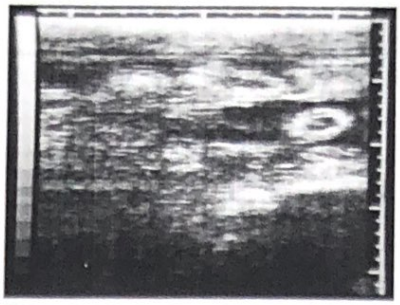
Table 20: Classification of transfer of substances across the placenta

Group	Physiologic role	Substances	Exchange mechanism
I.	Maintenance of biochemical homeostasis or protection against sudden foetal death.	Electrolytes, water and respiratory gases.	Rapid diffusion
II	Foetal nutrition	Amino acid, sugars and most water-soluble vitamins.	Predominantly by active transport systems
III	Modification of foetal growth or the maintenance of Pregnancy.	Hormones	Slow diffusion
IV	Immunologic or tonic Importance	Drugs/anaesthetics	Rapid diffusion
		Plasma proteins, Antibodies and whole cells	Pinocytosis or leakage through pores in placental membrane.

Source: Osinowo, 2006



2monthspregnancy ultrasonography showing placentom, dark-spot is foetus surrounded by fluids



Third trimester ultrasonograph showing placentom of doe

Figure 82.0: Ultrasonography in pregnancy detection

PARTURITION AND POST PARTUM RECOVERY

Parturition (birth process) is the process by which a pregnant uterus delivers the foetus and placenta from the maternal organism. It is initiated by the reversal in the oestrogen and progesterone level of the blood such that the uterus now becomes oestrogen dominated. It begins with the softening and initial dilatation of the cervix as well as the starting of the uterine contraction. The first sign in parturition is the increase in the strength of the rhythmic contractions of the uterus which may give rise to sensation of pain known as labour pains. The process ends with the expulsion of the foetus and its associated placental membrane. It is divided into three stages namely dilatation of the cervix (preparatory), the birth of the foetus (expulsive) and the expulsion of the placenta (purgatory).

Initiation of parturition: What initiate the onset of parturition is not clear, however the following theories has been proposed to explain the initiation of the parturition process.

1. We know that progesterone blocks myometrial contraction so its withdrawer induces parturition.
2. A rises in oestrogen secretion also will cause the initiation of parturition.
3. Also the release of pharmacologically active substances such as oxytocin, prostaglandin, and catecholamine has been found toward the end of pregnancy in the blood.
4. Neural mechanism has been proposed.
5. Foetal hypothalamichypophysial-adrenal axis is involved.

It is not known if any of the above plays a unique or primary role in initiating parturition. It is not clear whether the stimulus that triggers parturition is of maternal or foetal origin, however the following factors are thought to trigger parturition.

1. Mechanical factors
2. Hormonal factors
3. Pharmacologically active substances
4. Foetal factors

1. Mechanical factors: Rapid foetal growth during late gestation is thought to cause uterine distention and hence increases in sensitivity of the myometrium to oestrogen and oxytocin e.g. twins are born earlier than single, thus suggesting that the mass of the conceptus or optimal uterine stretching may play a part in the initiation of parturition. So the signal for the initiation of the process of parturition begins with the foetus.
2. Hormonal factor: Oxytocin released from the posterior pituitary plays a role in the initiation of parturition e.g. it is known that oxytocin level shows little changes at the initial stages of parturition but rises to a high level during expulsion of the foetus and falls there-after, also a fall in progesterone and a rise in oestrogen, a feature of the final stages of pregnancy could cause the release of oxytocin and thus initiate parturition.
3. Pharmacologically active substances: Prostaglandin (PGF) has been found to be important in the process of parturition in sheep and goat, also neurohumoral mediators such as endogenously released catecholamine and acetylcholine may also be important in initiating parturition. Foetal hypothalamus stimulates the foetalhypophysis to produce ACTH which stimulates the production of cortisol by the adrenals. Cortisol stimulates increased release of oestrogens and less progesterone by the placenta. Oestrogens stimulate the release of PGF2α from the endometrium and enhances oxytocin effect on the myometrium. PGF2α causes luteolysis, leading to a fall in progesterone secretion. These lead to the release of oxytocin from the neurohypophysis, uterine contraction and relaxation of the cervix. Oxytocin release following distension of vagina/cervix plays important part in the second stage of parturition. Relaxin causes expansion of the birth canal.
4. Foetal factors: The foetal hypothalamicpituitary-adrenal axis initiate parturition in the sheep (Fig. 83.0), this has been proven and the mechanism is shown thus

MATERNAL PHYSIOLOGY IN PREGNANCY

1. Vulva and vagina changes: Oedema and vascularity of these organs is highly pronounced in cattle and horses. Vagina mucosa is initially pale and dry but becomes oedematous and pliable just before parturition.
2. Cervix: Mucus plug of pregnancy is developed by the cervical crypt to seal the cervical canal. The external os of the cervix becomes closed but before parturition the plug breakdown and the os open. The tissues of the cervix which are normally tough and collagenous become soft. The mucus secreting glands become more active and keep the cervical canal continually filled with mucus.
3. Uterine changes: The uterus enlarges with the muscular wall remaining quiescence thus preventing premature expulsion of the foetus. Three phases of uterine changes occur.

i. The first is endometrial proliferation: This occurs before blastocyst attachment. It is characterized by marked progestational sensitization. This characteristic changes which include increased vascularity and growth of the endometrial gland is under progesterone.

ii. Uterine growth: This begins after implantation. It comprises of muscular hypertrophy involving extensive increases in the connective ground tissue and fibre element and collagen content of the tissue. The weight increases from 70g to 1000g (in human) with muscle fibres increasing in number by mitotic division.

iii. Uterine stretches: At this period the body growth diminishes as the uterine content is growing at accelerating rate. The volume of the content increases from zero to 5 7 litres.

4. Ovarian changes: CL forms from the ovulated egg of the graafian follicle and in most species including the cow, the CL of pregnancy persist at a maximum size between the pregnancy. In the cow it is greenish brown in colour and does not protrude above the surface of the ovary.
5. Changes in pelvic ligament and pubic symphysis: Towards the end of pregnancy the pelvic ligament gradually relaxes. This is very much marked in the cow and ewe than in the mare. The relaxation is related to high levels of oestrogen in late pregnancy and to the action of the hormone - relaxin. The pubic symphysis of the young females undergoes demineralization to permit some separation of the pubic symphysis at parturition. The ligaments holding the birth canal bones and the pelvis together become slacker and the joints are more mobile during pregnancy.
6. Endocrine system: There is evidence of increased activity of the thyroid gland which also increases in size, although the basal metabolic rate does not increase more than can be accounted for by the increase in weight. There is also an increase in the activity and size of the adrenal cortex. There is an increased output of the thyroid, parathyroid and adrenocortical hormones.

7. Increased weight: The weight increases generally by 20 -30lbs with half of the increase due to the enlarging foetus, the placenta and the amniotic fluid alone. The other half is due to increase in udder or breast size, blood volume, protein anabolism, retension of extra vascular water.
8. Cardiovascular/ respiratory system: There is an increase in blood volume which is about 30% towards the end of pregnancy. There is no corresponding increase in the red blood cell mass so that the red blood cell count and the haemoglobin concentration tend to fall. This is known as the physiological anaemia of pregnancy. It is an iron-deficiency anaemia which is corrected in human by taking folic acid and iron tablets. The cardiac output rises to a maximum at 28 - 30 weeks when it may be up to 30 40% above the non-pregnant level. The systolic arterial blood pressure is usually normal or a little below while the diastolic is usually low due to dilatation of arterioles in the forearm increasing the flow to 6 times the non-pregnant level in human. The diaphragm is pushed upwards and there is an increase in the width of the thoracic cavity. The ventilation rate increases and is due to the action of progesterone on the medulla.
9. Gut and urinary system: There is a general loss of smooth muscle tone particularly the colon. Nausea/vomiting in the morning (usually 2weeks after missed period) are not uncommon during the early stages of pregnancy in human due to loss of smooth muscle tone caused by change in hormone level) but not in livestock. Pica (craving/desire for uncommon food substance) occur at this period, though varies with individual, it is influenced by vitamin or mineral deficiencies. In early pregnancy, the kidney works over efficiently, and the bladder fills with urine more quickly leading to frequent urination (an early symptom of pregnancy). The renal blood flow (RBF) and glomerula filtration rate (GFR) alter in parallel to the cardiac output. The glucose in urine is due to the increase load presented to the tubules. This reappears at the last weeks of pregnancy due to pressure of the foetus head in the uterus on the bladder resulting in frequent urination. There is also an increase in the size of the ureters which become dilated and flaccid.

HORMONES OF PREGNANCY

Successful pregnancy is achieved through the delicate balance of certain hormones in the females' body e.g. the CL persist in all farm animal species through pregnancy except in the mare and ewe. In the cow, goat and sow the ovary is essential for maintenance of pregnancy. Some experiments have been performed to determine the importance of some of this organ to maintenance of pregnancy and the result is shown in Table 21 below.

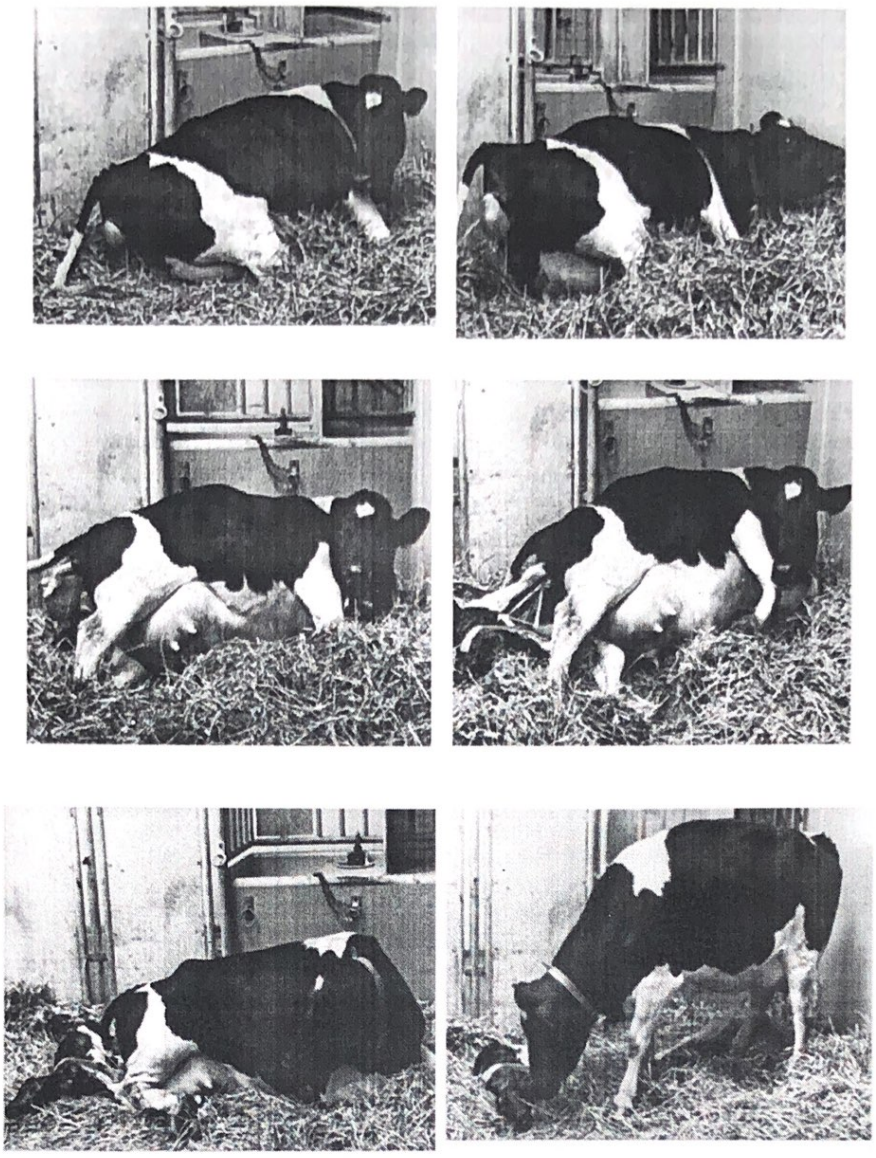


Figure 84.0: Parturition in a dairy cow

3. **Purgatory stage**: This is the expulsion of the foetal membrane. After the foetus is born the uterus continues to contract but the contractions are painless. The uterus closes down on the placenta and the movement of the wall over it detaches it and it is expelled. The uterus is cleaned out of the remaining foetal membranes and cellular debris. Normally this is done within 12hours in the ewe and cow and 5-30 minutes in human. Beyond this period the foetal membrane known as the placental is said to be retained. If the placental is retained, the ewe or cow could be left alone without any intervention. Such retained product clears on its own and the female retaining the product will still be capable of reproducing. In a lot of cases it may cause infection so initiate some treatment 48-72hours after parturition with antibiotic boluses or an antibiotic intra-uterine infusion. A volume as large as 1litre antibiotics will be infused at 24-36hrs after parturition, this method gives satisfactory result. Manual extraction is contra-indicated i.e. should not be done (Drost,

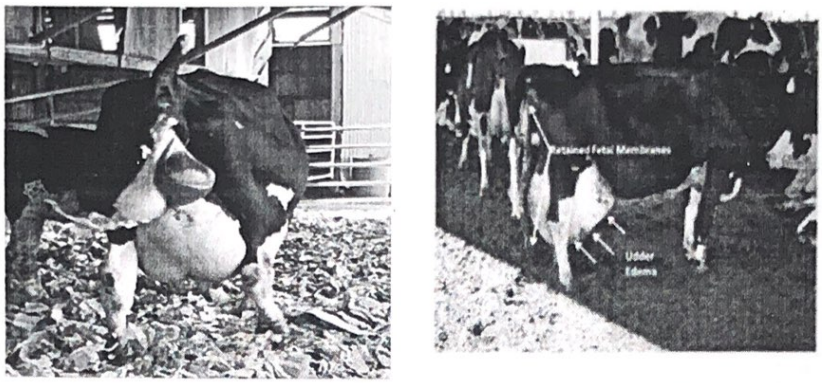


Figure 85.0: Cow with after-birth; Dairy cow with retained placenta

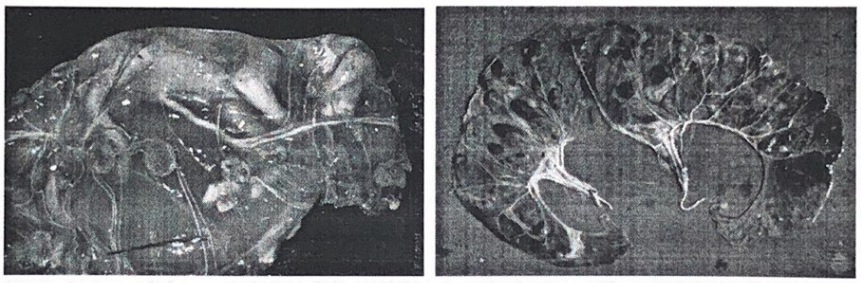


Figure 86.0: Placenta with normal conceptus in ewe; Placenta of twin birth, notice the cotyledon for attachment to the uterine caruncle

There are three presentations which accompany parturition, namely **cephalic**, **breach** or **transverse**.

Table 21: The effects of ovariectomy and hypophysectomy on gestation in mammals.

Animal Species	Length of gestation	Ovariectomy (days) 1st half of pregnancy	Ovariectomy (days) 2nd half of pregnancy	Hypophysectomy 1st half of pregnancy	Hypophysectomy 2nd half of pregnancy
Cow	282	-	±	nd	nd
Ewe	148	-	+	-	+
Goat	148	-	-	-	-
Sow	114	-	-	-	-
Mare	350	-	+	nd	nd
Woman	280	+	+	+	+
Monkey	280	+	+	+	+
Rat	22	-	±	-	+
Rabbit	29	-	-	-	-
Bitch	61	-	nd	-	±

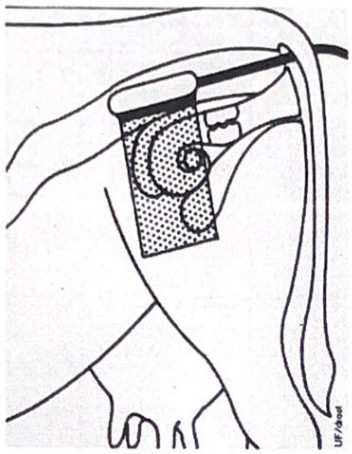
Where nd meaning not determined, ? meaning all animals aborted (ovary/hypophysis is important in gestation), + meaning no abortion occurred (ovary/hypophysis not important in gestation), ± meaning some survived while some aborted.

Maintenance of pregnancy depends on the interplay between the mothers placental and possibly the foetus. Progesterone is the dominant hormone during pregnancy; it is produced by both the CL and the placental. High levels of progesterone decrease the tone of the myometrium and inhibit uterine contraction. It does this by blocking the action of other hormones or drug on the myometrium. A high level of progesterone also prevents cyclic oestrus by preventing the release of gonadotropins. Cows, sows and goat depend on the CL progesterone for most of its gestation period. Ewe depends on placental progesterone for the last half of its pregnancy. In the Mare the CL of ovulation site remain active until day 150-180 when accessory cells formed by the stimulation of PMSG of tertiary follicle between the 35-40th day post-fertilization. This accessory CL also regresses between days 150-180. After this day progesterone for maintenance of pregnancy comes from the placental but around day 150-180 a drop in the progesterone occurs and oestrogen increase proportionately.

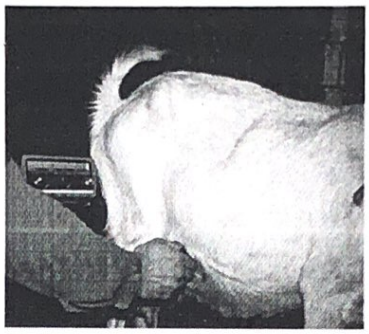
A stoppage of progesterone synthesis in any species leads to pregnancy termination. Relaxin, a polypeptide produced by the CL and placental is important during pregnancy. The concentration increases with the age of pregnancy. It appears to function in softening the connective tissues which permit the uterine muscle to stretch and accommodate the growing foetus. At early stages of pregnancy the concentration of oestrogens are low but increases with advancing pregnancy. In mare, the oestrogen concentration are high during late pregnancy, the source of the oestrogen being the placental, but the primary function appears to be its synergistic action with progesterone in preparing the mammary gland for milk secretion after parturition.

Pregnancy Tests

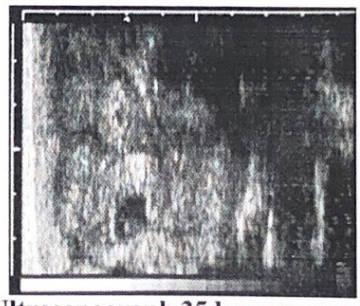
1. Immunological pregnancy tests are available which can diagnose pregnancy before clinical signs are detected. The tests rely on advanced technology made on a sample of the mother's blood or urine to measure the level of the pregnancy hormone. In this immune assay test latex particles coated with anti-HCG antibodies are precipitated by urine containing HCG. Test on urine becomes positive within three days after fertilization or when oestrus or menstrual period is missed using the pregnancy strip. The absorbent strip shows the animal is pregnant when dipped in early morning urine stream for 10 seconds and after a few minutes two lines appear in the result window. Even if one of the lines is very light, it means that the test is positive. No pregnancy hormone has been detected if after a few minutes only one line is showing in the result window. The test is only useful in the early stages of pregnancy to detect the presence of the metabolites of human chorionic gonadotropin (HCG).
2. Ultrasound scanning can also be used to diagnose pregnancy (Fig.82.0). The sound waves 'echo' off different tissues and can be translated into a picture. Ultrasound can detect the presence of the sac in which the foetus lies and the foetus itself (from the 7th week of pregnancy in human). The pregnancy detector tool makes use of ultrasound method of diagnosing a foetus filled uterus.



Cow ultrasonography



Ultrasonography in doe



Ultrasonograph 35days pregnancy

Table 22: Maternal and Foetal factors required for normal birth

Maternal factors	Foetal factors
1 .Normal physical condition good health and physical Maturity.	Normal size of foetus
2. Normal size of the soft/bony birth canal.	Normal positioning of foetus(cephalic presentation) not breach or transverse presentation.
3. Normal relaxation of birth canal.	Normal form of the foetus.
4. Normal lubrication of birth canal.	Normal number of fetuses.
5. Adequate expulsive forces uterine Contraction & abdominal pressure.	

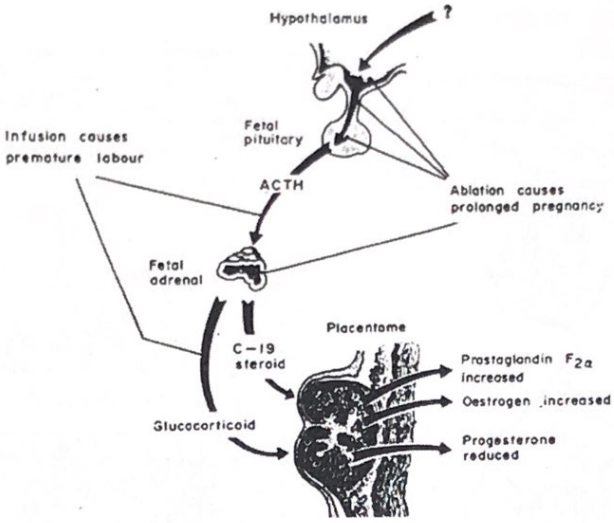


Figure 83.0: Foetal mechanism regulating parturition

Stages of parturition

There are three (3) stages of parturition as shown in Figures 84.0 and 89.0, which are:

1. **Preparatory stage** which involves the development of the necessary proteins and energy by the uterus toward the end of pregnancy. In this stage a noticeable oedematous vulva and highly developed udder with prominent milk veins is

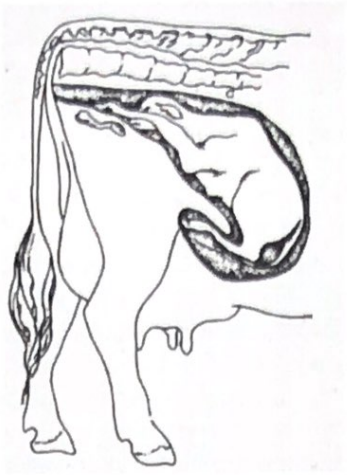
seen and the animal will be lagging behind the herd in cow/ ewe. In sow the teats are seen to be highly developed. Cow should be taken to calving pen when this signs are noticed. On receiving the initiating stimuli the uterine contraction will begin. Dilatation and relaxation of the cervix brought about by relaxin occurs with active rhythmic contractions. There are 2 types of contractions - **abdominal contraction** and **uterine contraction**.

The uterine contraction is about 90% responsible for the expulsion of foetus, oxytocin enhances this contraction, therefore it has its use during difficult contraction, so can be injected into this animal. In monotocous species contraction starts at the apex of the cornua while it begins just cranial to the foetus near the cervix in polytocous species with the remaining part of the uterus remaining quiescent. The cervix expands at the end of this stage thus allowing the uterus and vagina to become a continuous canal. The dilatation is brought about partly by

a. Foetal head pressing down on the cervix and
b. Contractions of the uterine muscle pulling the cervix up out of the way of the head.

This can last for a few hours to 1day. Symptoms of this stage are the onset of milk secretion and relaxation of the pelvic ligament.

2. **Expulsive stage**: At this stage the distended amnion with the head and the extremity of the foetus are been forced into the canal called pelvic inlet. This initiates the reflex and voluntary contraction of the diaphragm and abdominal muscle. It is characterized by increased intensity and frequency of uterine contractions. The expulsion is aided by contraction of the abdominal muscles and diaphragm and expiratory muscles accompanied by the closing of the glottis which together increase the pressure inside the abdomen. There is little resistance to the passage of the foetus by the vagina and vulva although they may be torn if the expulsion is too rapid. At this stage the foetal extremity and head within the amnion can be seen through the vulva. The passage of the foetus through the cervix into the vagina along with the rupture of one or both water bags (amnion & chorion) initiates reflex contractions which force the foetus through the canal. The amnion breaks due to pressure and the amniotic fluid lubricates the cervix/ vagina through which the foetus is delivered. In ruminants (monotocous species) foetus is expelled still attached to the foetal membrane. The last foetal cotyledons are not detached from the maternal caruncle until after the young is born. This ensures a continuous oxygensupply to the young until it is able to breathe on its own. This second stage is rapid thus preventing the foetus from being suffocated.



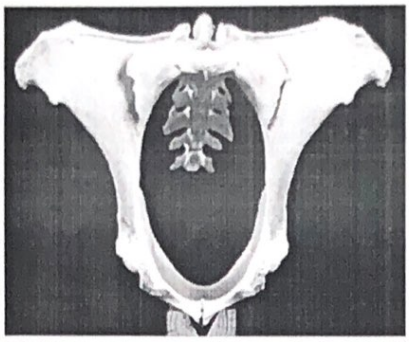




Figure 87.0: Normal position (cephalic), pelvic diameter and Breech presentation of a calf (A)

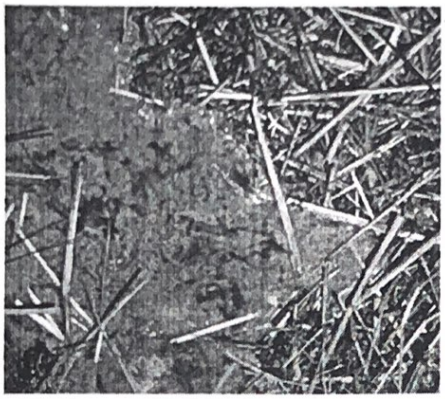
Table 23: Required time for the stages of parturition in farm animals

Species	Preparatory Stage (hours)	Expulsive Stage (hours)	Purgatory stage Stage (hours	Uterine involution (days)
Cow	2 - 6	0.5 - 1	4 - 5	45
Ewe	2 - 6	0.5 - 2	0.5 - 8	30
Sow	2 - 12	1 - 4	1 - 4	28
Mare	1- 4	0.2 - 0.5	12	13 - 25

Source: Osinowo, 2006

Post partum recovery

The important changes that take place post-partum are the regeneration of endometrium, involution of the uterus and the resumption of oestrous cycle.In the cow involution is completed by 45days post-partum i.e. the cervix and uterine mass would have undergone reduction and the endometrium returned to their non-pregnant status. In most species there is some amount of vulva discharge called **lochia**, a greyish red fluid containing blood cells and endometrial debris which gradually decreases and stops at the end of the second week postpartum. In ewe involution is completed by 30days post-partum. Intervals between parturition to first oestrus ranges from 30-72days for dairy cattle and 46-104 days for beef cattle. Interval is prolonged when cow suckle calf. In mare a foal heat is exhibited within 13days post-partum. It is routine practice to breed mare at foal heat although concept rate are low. In sow anovulatoryoestrus occur 3-5days after farrowing but they are not usually bred. In most mammals, nursing of the young delays oestrus. Nursing sow will exhibit oestrus after 40days following farrowing however weaning of piglet at any time during nursing period induces oestrus.



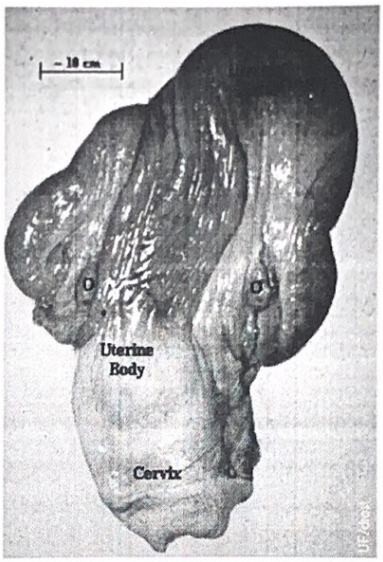


Figure 88.0: Lochia and involuted uterus

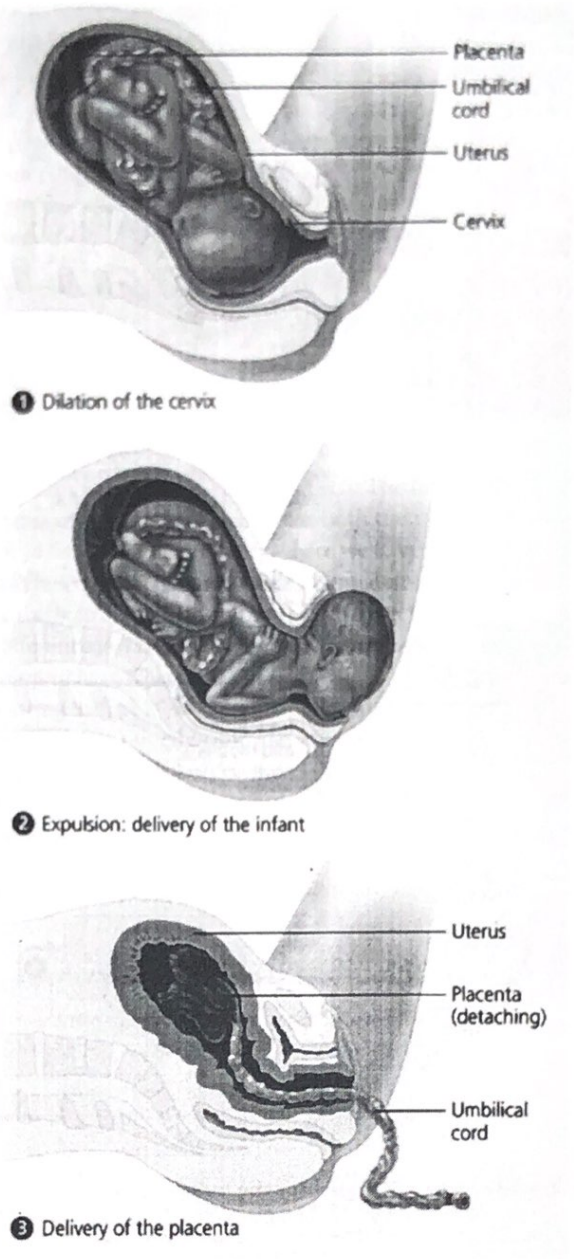


Figure 89.0: Stages of parturition in human

CHAPTER ELEVEN

PHYSIOLOGY OF LACTATION

Lactation is the process of production and release of milk from the mammary glands which is made up of two main types of tissues parenchyma or glandular tissue and stroma or supporting tissue (Fig. 90.0).It is the final phase of the reproductive cycle of mammals and the physiological state of the mammary gland is linked to the reproductive state of the animal. A failure to lactate means a failure to reproduce. Lactation is made up of three components:

- Growth of udder from the non-pregnant state
- Synthesis and secretion of milk
- Ejection of stored milk

The basic components of the mammary gland include

- The secretory cells (alveoli)
- Gland cistern
- Teat cistern
- Teat canal
- Teat

Alveoli - The alveoli are small sac-like or pear-shaped structure with walls made up of single layer of epithelial cells. It forms clusters or lobules which may be up to 200 alveoli bounded by a thin fibrous septum. Many of the alveoli within the lobule open individually into their respective terminal intralobular ducts, but groups of two or three may open together into a common duct and occasionally even large clusters may form a common opening into a terminal duct. The lobules of alveoli themselves form larger clusters or lobes bounded by thicker septa. The non-pregnant mammary gland consists of 15 - 25 lobes (like the sections of an orange) each with a main duct which dilates and empties into collecting sinuses beneath the areola around the teat (nipple). There are therefore 15 to 25 openings on the nipple in human. Going backward, each main duct has branching smaller secondary ducts, and like the branches of a tree, these ducts divide into still smaller ducts which end in alveoli surrounded by secretory cells. The entire duct system is embedded in a pad of fat which gives the breast its shape. Before pregnancy the ducts have few branches, the secretory cells are rudimentary and the udder (breast) is filled with fatty tissue. The duct system of each lobe therefore resembles a tree, the alveoli being the leaves, the small ducts the branches, and the main duct the stem. These small ducts produce high surface tension that opposes the movement of fluids so that suction is a relatively ineffective method for removal of alveolar milk. It is overcome by the covering of the alveoli with a basket-like network of myoepithelium that contracts in response to pulsatile oxytocin release by the posterior pituitary. As the ducts become confluent/ increase in diameter their epithelial lining changes from a single to a double layer of epithelial cells.

On the outer surface of the ducts the myoepithelial cells are arranged in a longitudinal manner so that on contraction they cause the ducts to shorten thereby increasing the diameter resulting in an increased flow of milk. Stimulation of the teat triggers the nerve receptors in the skin to transmit nerve impulses up the spinal cord to the hypothalamus where they cause oxytocin release from the posterior pituitary into circulation. Oxytocin is carried in the blood to the mammary gland where it causes the myoepithelial cells to contract, thereby expelling milk from the alveoli into the cisterns. In the presence of growth hormone-prolactin, thyroid hormone and corticosteroids, oestrogen from the placenta causes proliferation of the ducts while progesterone stimulates alveolar growth. As a result of placental secretion of GH-prolactin, oestrogen and progesterone, the udder (breast) grows and becomes ready for milk secretion anytime (after about 4 months in human). However no milk is secreted until after parturition which suggests that the placenta and/or the foetus have some inhibitory effects on the udder (breast) functions. At birth oestrogen and progesterone levels fall sharply. The direct progesterone block is removed and pituitary prolactin rises to replace that from the placenta.

Gland cistern - There are species differences in milk production/ storage. Human, cats, dogs and pigs store more than 80% of the milk produced by the mammary glands in the alveoli and these are ejected by **obligatory** reflex action. Ruminants on the other hand store milk in the voluminous gland cisterns (>80%) from where it can be withdrawn by suction applied to the teat (**facultative** reflex milk ejection). The cow udder consists of four mammary glands or "quarters" which are separated from one another and with separate duct system, storage cistern and teat. Internally they are separated by the double- layered medial suspensory ligament, but on the ventral surface the right and left halves are demarcated by a distinct groove on the skin. The boundary between the glands of the same side cannot be distinguished but the glandular systems are separate entity. Milk secreted in one gland cannot pass into the adjacent gland under normal circumstances. Milk ejection reflex is not essential for feeding of the young ruminant but could facilitate the process as the remaining portion of milk stored in the alveoli (20-50%) can only be removed by milk ejection. Maintenance of milk secretion depends on regular suckling stimulus on the teat (nipple) or else the udder returns to its non-pregnant size/shape (maybe smaller, stretched/droop more with less fat, wrinkled/ floppy in old age) once lactation ceased. Gland cistern receives milk from the secretory cells (alveoli) of the glandular tissue. The size/ shape of the gland cistern vary considerably. It has a multilocular appearance because of the pockets formed by the openings of the large ducts. The cistern region merges into the more solid glandular substance, the presence of numerous smaller ducts giving sections of the glandular substance a sponge-like appearance; more dorsally the glandular substance becomes dense and fleshly.

Teat cistern - This is the collecting point of milk in the teat with numerous irregular annular and longitudinal folds in the mucosal lining. It communicates at its upper end with the gland cistern through a circular opening. A double-layered epithelium lines the gland and teat cisterns and is continuous with the ducts.

Teat - This is the pointed structure on the mammary gland through which the teat or streak canal passes from the teat cistern or cavity and opens through the tip of the teat. There are four to eight radiating folds (Fürstenberg rosette) in the mucosal lining of the sinus at the point where the teat canal opens into the teat cistern. There is no sharply defined sphincter muscle surrounding the teat-canal as often alleged but the canal is kept closed by an ill-defined circular network of smooth muscle and elastic fibers. The number of teat varies with species cow (4 teats), ewe/goat (2 teats), sow (12 teats), bitch (8 teats) and primates (2 nipples).

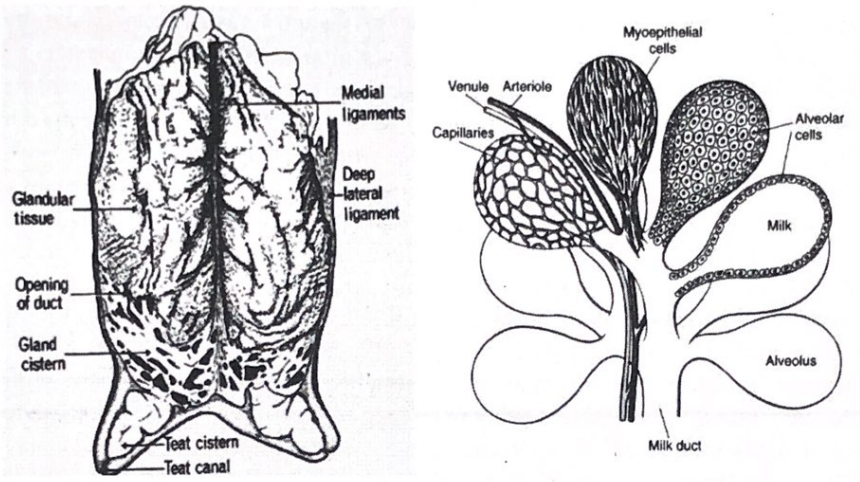


Figure 90.0: Mammary gland and alveoli of a cow

Colostrum: The first milk produced after parturition which is watery, protein-rich, lipid-poor, high in immunoglobulins and low in lactose but turns to normal milk within a few days (after the first 4days of lactation). The composition of colostrum is different from milk. Colostrum is yellowish in colour. It contains fat globules with little or no caseinogens. It has about 8% protein made up of lactalbumen and lactoglobulin. It also contains plasma proteins. The total amount of fat is less than in milk. It also differs from milk in having iron and copper several times higher than milk. It protects the young by providing antibodies and globulins which are absorbed as a whole. It also acts as a purgative for the baby.

When stimulated by oxytocin the alveoli (myoepithelial or basket cells) are compressed and milk is expelled into the larger collecting ducts (milk cistern) for removal by suckling of the young. The establishment of a nervous link between the teat (nipple) and the oxytocinergic neurons of the hypothalamus allows milk ejection from the alveolar tissue to be coordinated with the suckling reflex of the young (neuroendocrine reflex). The teat (nipple) and the skin covering the mammary gland are richly innervated with somatosensory nerve endings that respond to touch and pressure. Its sensitivity has been reported to increase around the period of parturition and at ovulation with such changes in sensory perception mediated at the receptor or within the brain. The milk ejection reflex can be conditioned like any other reflex to occur in response to visual (sight of the young or milking machine) or acoustic (sound of calf/gentle sound) stimuli which the cow has come to associate with milking. Milk ejection reflex is inhibited by adrenalin at the level of the mammary gland, by opioid peptides influencing secretion from the terminals of oxytocin producing neurons in the posterior pituitary, and by noradrenaline acting on the beta-adrenoceptors within the central nervous system.

Milk: -This is an opaque white fluid composed majorly of water, proteins, lactose, electrolytes and fat droplets. Its principal carbohydrate lactose is about 7% in man and 5% in cow. The fat is neutral triglycerides, tripalmitin and saturated fatty acids whose composition is similar in man and cow. The protein caseinogens, mainly calcium salt is 3% in man and 2 - 2.5% in cow. It also contains lactalbumen and lactoglobulin. Electrolytes present include sodium, chloride, potassium, magnesium, calcium, and biphosphate, all except chloride/magnesium are higher in cow milk than in man. Milk contains vitamins A, D, and riboflavin, but cow milk is deficient in ascorbic acid. Milk has a pH of between 6.6 - 6.8 with the rate of secretion rising rapidly at first and more slowly as the young one grows. Milk secretory rate is 1.5 - 2.0 litres a day in human. Milk composition and volume is significantly influenced by the mother's diet e.g. a fat soluble vitamins deficient is reflected in the milk composition. If given a calcium deficient feed the mother's body draw calcium from the bone which in excessive cases lead to weakening of bone particularly the pelvic bone which may interfere with subsequent pregnancies.

Lactation can be maintained for long periods at reduced level as long as the animal remains non-pregnant, receives enough food, stays healthy and is milked regularly. The sow remains anoestrous for the duration of lactation except for sterile (anovulatory) oestrus period immediately post-partum; cyclic ovarian activity is resumed after the removal of the litter.

Growth of mammary gland and lactation: The female mammary gland is well developed at birth with later changes being an increase in size. Increased reproductive hormone production at puberty and certain stimulus help to initiate this increase in size.

Hormones help to stimulate mammary tissue growth as animals treated with oestrogen alone tend to produce milk more quickly while those receiving oestrogen + progesterone come into milk production more slowly but give higher milk yield. Mammary growth and milk secretion by regular application of milking stimulus enhance production. Regular milking of animals at an early stage in the treatment is necessary when attempting to induce udder growth/ lactation in ruminants by ovarian hormone therapy. In normal pregnancy a prolactin-like hormone secreted by the placenta plays an important role in mammary growth. The onset of copious lactation occurs around parturition with some high-yielding cow but it is necessary to start regular milking process before parturition to reduce the pressure that develops in the udder. One volume of milk is produced from about 500 volumes of blood that passes through the mammary gland in high-yielding cow while it is 1volume of milk to 1000volumes of blood in low-yielding animals. Most of the blood is supplied through the large paired external pudendal arteries which leave the abdomen by way of the inguinal canal. All components of milk are derived from the blood with the major components such as protein (casein), fat and lactose formed within the epithelial cells from precursors while minor components such as vitamins, salts and immunoglobulins secreted by selective passage of materials without change of chemical form, across the epithelial cells into the lumen along with the water.

Artificial colostrums: Artificial colostrums have been crudely prepared with the following constituents;

- powdered milk 4 teaspoon full
- egg ½ an egg
- tetracycline syrup 5ml
- castor oil a few drops
- cod liver oil ½ teaspoonful
- vitamin B-complex a few drops

Osteomalacia and rickets: Osteomalacia is a condition in which the bones are insufficiently mineralized. Rickets is a name for thesame condition when it is present in prepubertalanimals. While osteoid is produced, calcium salts arenot deposited; thus the bones remain soft. This iscaused by inadequate calcium or vitamin D in the diet.Vitamin D is necessary for intestinalabsorption of Ca^{2+}. When blood calcium levels decrease due to inadequateintestinal absorption, parathyroid hormonemaintains plasma Ca^{2+} by stimulating the release ofCa^{2+} from the bone.

Parturient paresis (milk fever): As dairy cattle begin milk production, the first milkproduced (called colostrum) contains high concentrationsof Ca^{2+}. Colostrum requires approximately 3 g ofcalcium per hour to produce. When the cow cannot mobilize this amount of calcium, she can develop milk fever within 72 hrs following parturition.

Symptoms include loss of appetite, followed by muscle weakness, decreased body temperature, labored breathing, and paralysis of hind legs. If left untreated, the cow can collapse into a coma and die. To prevent milk fever, cows should be given sufficient vitamin D in the diet prior to parturition. If milk fever develops, cows are given an oral bolus of calciumcarbonate.

CHAPTER TWELVE

POULTRY REPRODUCTIVE PHYSIOLOGY

The bird is different from mammal in that it does not have a well defined oestrous cycle and the breeding season is not divided into oestrus and pregnancy phases. Instead follicular development and oviductal function continue at the same time. Modern breeds are also different from the wild species e.g. broodiness has disappeared from many breeds while some species retains their photorefactory wild state.

Peculiarities of the male reproductive system

- No pampiniform plexus
- Seminiferous tubules are not grouped into evident lobules surrounded by connective tissue but branch and anastomose freely within the tunica albuginea.
- Spermatozoa are stored mainly in the ductus deferens
- No accessory reproductive organs
- Male has no penis but an erectile phallus
- Androgen may not have any effect on spermatogenesis, except with transformation of secondary spermatocytes to spermatids. The main function of androgen is a negative feedback on gonadotropin output by pituitary.
- Testis never descends into an external scrotum.
- It is the monogametic sex

The male reproductive system of cock is made up of the testis, ductus epididymis, ductus deferens and the erectile papilla in the cloaca. The testes are suspended from the dorsal body wall, posterior to the lung and ventral to the kidney (Fig. 92.0). Blood is supplied the anterior renal artery and a variable testicular artery. The arrangement of blood vessels within the testis is much simpler than that in mammals and there is no pampiniform plexus. The seminiferous tubules are not grouped into evident lobules surrounded by connective tissue, but branch and anastomose freely within the tunica albuginea. Spermatozoa are stored mainly in ductus deferens and there is no accessory reproductive organs such as seminal vesicles, prostate, and cowpers glands. The seminiferous tubules unite with those of the rete testis and these unite with the ductuli efferentes. Thereafter the ductuli efferentes connect in several places with the short ductus epididymis, which opens into the ductus deferens, an extensive convoluted tube running the whole length of the abdomen on the surface of the kidney. At the distal end the ductus enlarges before passing through an erectile papilla extending into the cloaca; there is no comparable structure to the ampulla of mammals.

The male has no penis, but an erectile phallus (Fig.91.0 and 94.0) which makes contact with the everted vagina during copulation. Erection of the phallus is caused by its becoming engorged with blood, as are related cloacal structures (lymph folds). Presumably erection is controlled by nerves.

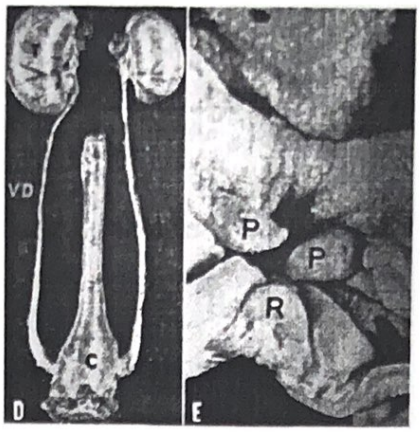


Figure 91.0:Reproductive organ of male chicken (vas deferens VD; testis- T; Cloaca- C; papillae- P; rudimentary organ- R).

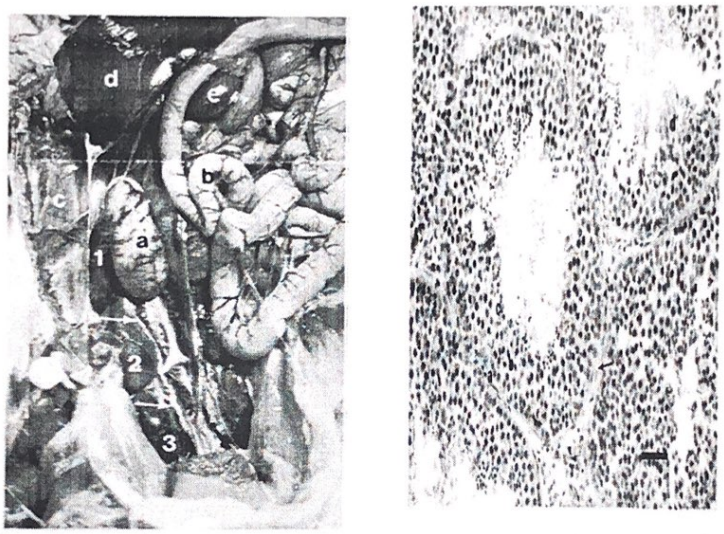


Figure 92.0: Testes of a sexually active chicken (ventral view);

Photomicrograph of the testes of adult chicken (scale 40μm). During sexual activity the bean-shaped testes (a) overlying the cranial end of the kidney triple in size and become whitein colour due to dispersal of the interstitial cells by the enlarging seminiferous tubules. The increase in size of the testes is most marked in wild birds in which the testes practically disintegrate during the non-breeding phase and have to be almost totally regenerated in time for the next breeding season. The increase in size can be as much as 300 times. The surface is highly vascular, with the epididymis lying dorsal to the testis and therefore not seen in a dorsal view. The ductus deferens (arrow) extends caudally on ventral surface of the kidney and terminates in the cloaca. The cranial (1), middle (2) and caudal (3) divisions of the kidney are visible,as well as the small intestine (b), lung (c), liver (d) and spleen (e) in this photograph. There are no accessory genital glands such as a seminal vesicle or prostate. The following could be observed in the photomicrograph of testis of adult chicken spermatogonia (a), primary/ secondary spermatocytes (b), spermatids (c) and maturing spermatozoa (d) which develop with their head attached to the sustentacular cells and their tail projecting into the lumen. The testis is enclosed by a connective tissue tunica albuginea which gives rise to the thin connective tissue septa (arrow) separating tubules. The testis has no lobules or mediastinum as in mammals. In between the tubules lies the interstitial cell which is the source of androgens.

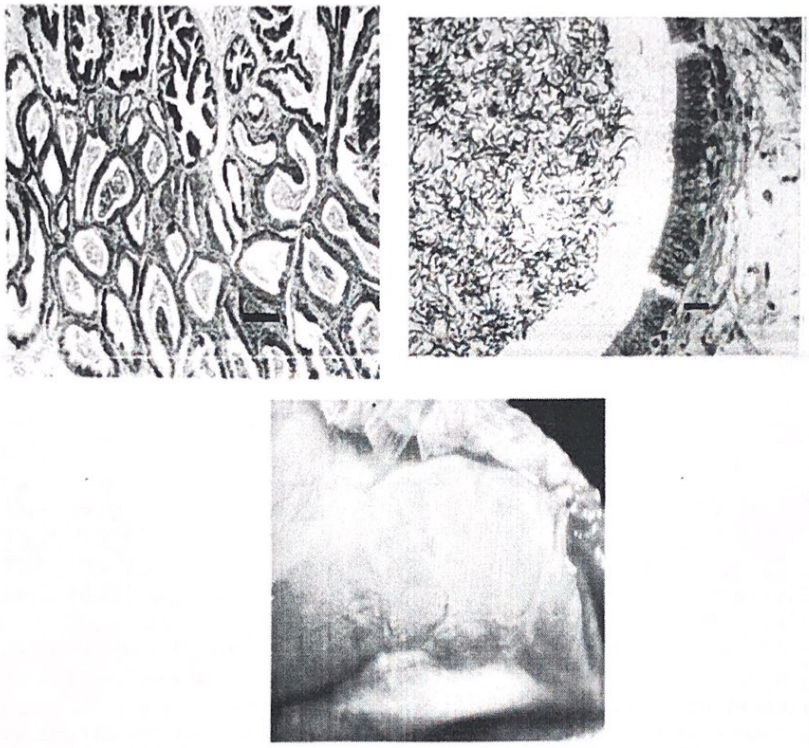


Figure 93.0: Light micrograph of Epididymis of a sexually active chicken

(H/E, scale 100µm) - coiled and anastomosing efferent ductules arise along the whole length of the epididymis so that a head, body or tail cannot be distinguished. They contain many maturing spermatozoa and open into the epididymal duct close to the surface of the epididymis; **Photograph of the Ductus deferens,** appear as a white coloured zig-zag tube due to the presence of spermatozoa, in wild birds it is relatively straight in non-breeding season and only convoluted during the breeding season; **Light micrograph of the ductus deferens (H/E, scale 10µm)** the epithelial lining is pseudostratified, spermatozoa takes 1 4days to reach the ductus deferens and only then do they become fully fertile. The lumen is packed with matured sperm.



Figure 94.0: Protruded phallus of an adult duck - Duck and other anseriforms have an intromittent type of phallus which when tumescent is about 6-8cm in length.



Figure 95.0: Kidney of a day-old chicken (ventral view, scale 2mm) aorta (a), ischiadic artery (b), common iliac vein (c), external iliac vein (d), caudal renal portal vein (e), caudal renal vein (f). Each kidney is divided into cranial (1), middle (2) and caudal (3) divisions, the boundaries between them being formed by the external iliac and ischiadic arteries. The kidney surface has a faint reticular pattern; **Ureters of a chicken (ventral view)** the collecting ducts of the kidney drain into the ureter (a) which begins within the cranial division of the kidney and runs caudally on the ventromedial border of the middle and caudal (b) divisions. The pelvic part crosses the dorsal surface of the cloacal bursa (c). and terminates in the cloaca. A bladder and urethra are absent. The peristaltic movement of the ureter moves the semi-solid urine into the cloaca. Normal excretion from the kidney consists of white or cream-coloured semi-solid urates which in the cloaca become incorporated into the faeces;

Synsacrum and right pelvic bone of an adult chicken (ventral view) the kidneys are embedded in depressions on the ventral surface of the synsacrum (a) and in the renal fossa (b) of each ilium. Because of the relatively fixed position of the kidney and the fact that large blood vessels/ nerves pass through it, there is difficulty in dissecting it intact. In poultry slaughter plants the kidneys are generally removed by suction.

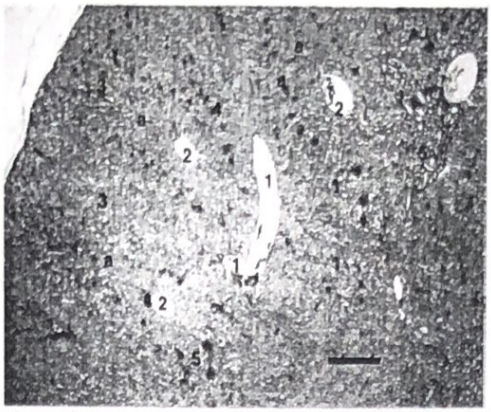


Figure 96.0: Light micrograph of kidney of a chicken (H/E, scale 600µm) ideally in histological section of the kidney a lobule appears as a pear-shaped structure lying between interlobular veins (1) with an interlobular vein (2) and artery at its centre, only bits of the lobules (a) are visible in this photograph. Lying beside the interlobular veins are perilobular collecting tubules. The broad part of the lobule is the cortical region which contains nephrons (3) and the peritubular capillary network. The renal corpuscles (4) lie approximately halfway between the peripheral interlobular veins and the central intralobular vein. The narrow part of the lobule is the medullary region which contains some nephrons and the collecting tubules (5). A cortical region drains into several medullary regions and a medullary region drains the cortical region of several lobules. Because the lobules (and lobes) lie at different depth of the kidney, there are no continuous layers of cortex and medulla as there are in mammals.

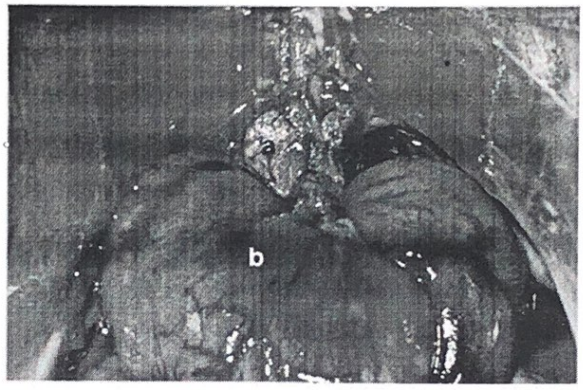


Figure 97.0: Adrenal glands of an adult male chicken (ventral view) the right and left adrenal glands (a) are yellow structures lying on either side of the mid-line at the cranial end of the kidneys and dorsal to the testes (b). They are firmly attached to the cranial extremity of the epididymis. Unlike in mammals the cortical and medullary cells do not form two distinct regions but intermingle.

Female reproductive system: The left and right gonads and oviducts are present in the avian embryo but in most species only the left ovary/ oviduct develop after hatching and become functional. The female reproductive system of the hen consists of

1. Ovary In birds as in mammals, two ovaries and oviducts (Fig. 98.0 and 100.0) are formed during embryogenesis, but there is a suppression of further development of the organs on the right side. The suppression is probably cause by early production of steroid hormones by the left ovary; removal of the left ovary results in the development of the right gonad into an ovo-testis with both male and female tissue. The functional left ovary acts in the same way as mammalian ovaries produces ova and female sex hormones. The ovary is made up of a medulla (which contains connective tissue, blood vessels/nerves) and a cortex (contains oogonia that give rise to the oocytes). The pear-shaped, immature ovary is about 15mm long by 5mm wide, lying in the body cavity, ventral to the aorta and cranial to the kidney, and close to the two adrenal glands. Although variable, the blood supply usually arises from the gonadorenal artery. Two veins drain blood from the ovary. It is extensively innervated from the sympathetic chain by way of the adrenal/ ovarian plexus.

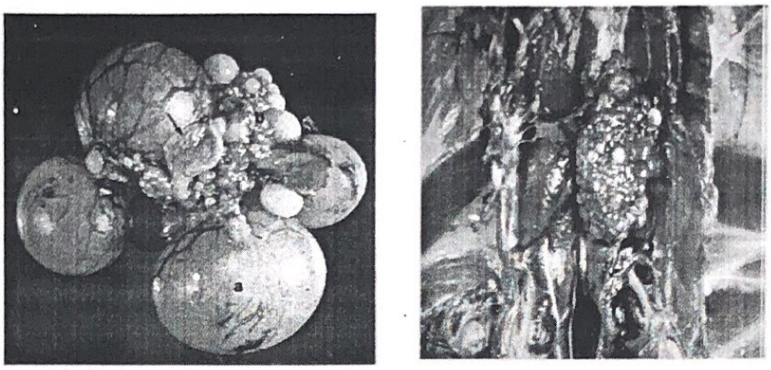


Figure 98.0: Ovary of a laying chicken and an inactive (non-breeding state) ovary of a moulting chickenresembling shrunken juvenile gonad, weighs 2-6g. Chicken come into lay at about 18-21weeks. The ovary then weighs about 60g and resembles a bunch of grapes with four or five large, mature follicles (a) and thousands of smaller immature follicles (b). The yellow colour of the immature follicle is due to the presence of yolk, the protein and lipids of which are manufactured in the liver and travel in the blood stream to the oocyte. About two hours before ovulation the primary oocyte in the follicle undergoes the first maturation division to form the secondary oocyte. Ovulation takes place under the influence of luteinizing hormone and in the chicken generally occurs about 30minutes after an egg is laid. Immediately after ovulation the follicle becomes a thin walled sac, the post-ovulatory follicle (arrow), which in the chicken regresses in about 10days. Unlike in mammals there is no corpus luteum.

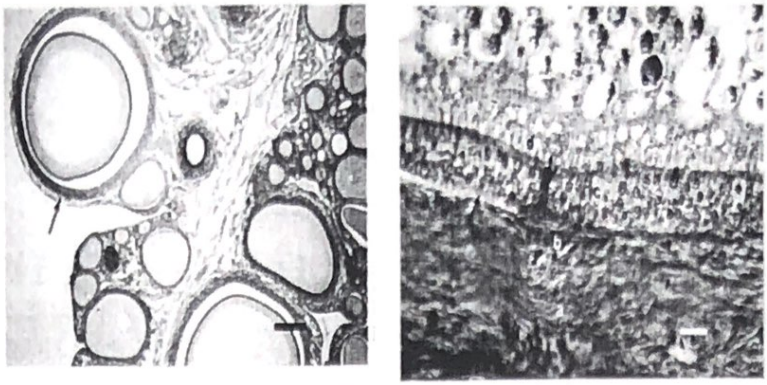


Figure 99.0: Light micrograph of chicken ovary (H/E, scale 500μm) showing follicle in different stages of development with one large follicle (arrow) lying at the surface of the ovary; **Walls of a mature chicken follicle** showing four layers of wall the relatively wide theca externa (a), much narrower theca interna (b) composed mainly of collagen fibres/ fibroblast-like cells, the stratum granulosum (c) which is the inner epithelium of the follicle and is frequently pseudostratified. At the junction (arrow) of the stratum granulosum and theca interna there is a thick basal lamina. The zona radiata (d) rests on the surface of the stratum granulosum and is a mixture of the secretions and radial processes of the granulosum cells and the radial processes of the oocyte cytolemma. Within the oocyte, yolk droplets (e) can be seen (scale 10μm).

2. Oviduct The oviduct (Fig. 100.0 and 101.0) lies in the left dorsal part of the body cavity to the left of the gut mesentery and is pink due to its great vascularity. It is suspended by the peritoneal dorsal ligament which continues around it to form the ventral ligament. The glands in the wall of the oviduct are responsible for the formation of the white and shell of the egg. It has the following functions:
    - Production of the egg components
    - Transport of developing egg
    - Gives the egg its peculiar shape

In the mature sexually active chicken the oviduct may reach a size of about 65cm but when it goes out of lay, the length of the oviduct is dramatically reduced to about 15cm. The oviduct is made up of the following components

A. Infundibulum has fimbrae on its surface which catches the released yolk (ovum) from the ovary. The chalaziferous region may contribute to the formation of the chalazae, for albumen is added, and to the perivitelline membrane. However, the formation of the perivitelline membrane and the chalazae is a continuous process that starts in the infundibulum and ends in the distal oviduct. Egg stays here for 15minutes. It is the site of fertilization.

b. Magnum albumin secreting region, it is the longest section with thicker walls and great external diameter. Average speed of egg is 2mm/ minutes; hence it stays here for 2-3hours (Fig. 102.0 and 103.0).

c. Isthmus membrane secreting region, it is narrower with thinner walls, its structure is similar to that of the magnum, although the glands appear to contain material of a keratinous nature. The shape of the egg is produced by the membranes and not by the calcified shell that is laid on an already formed shape. Egg stays here for 1-1.30hours (or 75minutes).

d. Shell gland (uterus) characterized by a pouch-like section joined to the isthmus by a short neck and extensive muscularization. The main process is calcification. During its stay in the shell gland, rotation of the egg around the polar axis leads to the completion of the formation of the chalazae, which started in the infundibulum, and the stratification of the albumin. The final task of the shell gland is the formation of the cuticle and pigmentation (porphyrins) deposited shortly before laying. The egg spends about 20 hours of its total time in the oviduct (26 hours) in the shell gland.

e. Vagina this acts as a passage from the shell gland to the cloaca and plays no part in the formation of the structure of the egg. However, it does play an important role in the processes involved in fertilization, for it contains the 'sperm-storage glands'. Sperm can survive for many days in the female oviduct (up to 32 days in chicken and 70 days in turkey).

Laying hens have a special type of bone, called medullarybone, which allows hens to store calcium necessaryfor eggshell production. Medullary bone is foundin bones possessing a good blood supply. It is absentin the humerus, metatarsus, and toes, with smallamounts found in the skull and cervical vertebrae andlarger amounts found in the femur and tibia. Foundonly when the birds are producing eggs, medullarybone grows from the inner endosteal surface of theshaft of long bones forming interlacing spicules that fi ll the marrow space. Eggshell formation occurs largely at night, a timewhen laying hens are not eating and therefore are notabsorbing dietary calcium. During the time of eggshelldeposition, osteoclasts surround the trabeculae andactively reabsorb this bone in order to supply thecalcium necessary for eggshell formation, which lastsapproximately 20 hours. During the last 15 hours ofeggshell formation, the shell gland of the hen secretescalcium at the rate of 100150 mg/hr, a rate that wouldddeplete blood calcium in 818 min. Thus, medullarybone provides an essential source of blood calciumnecessary for shell deposition. The deposition of medullarybone is induced by estrogen.

Egg-laying fatigue in birds: Similar to milk fever in cows, high-producing egg layinghens can develop weak and brittle bones. A henmust deposit as much as 810% of her total calciuminto the eggshell each day.

Since the eggshell is deposited during the night, the hen must draw upon the calcium reserves located in a specialized type of bone called medullary bone. Under the influence of estrogensand androgens secreted from the developing follicles, medullary bone is produced in hens 2weeks prior to commencement of egg laying. As blood calcium levels decrease during eggshell formation, the hen releases parathyroid hormone, which mobilizes bone calcium. If there are insuffi cient stores of calcium in the bones, the bones become weaker as they become demineralized.

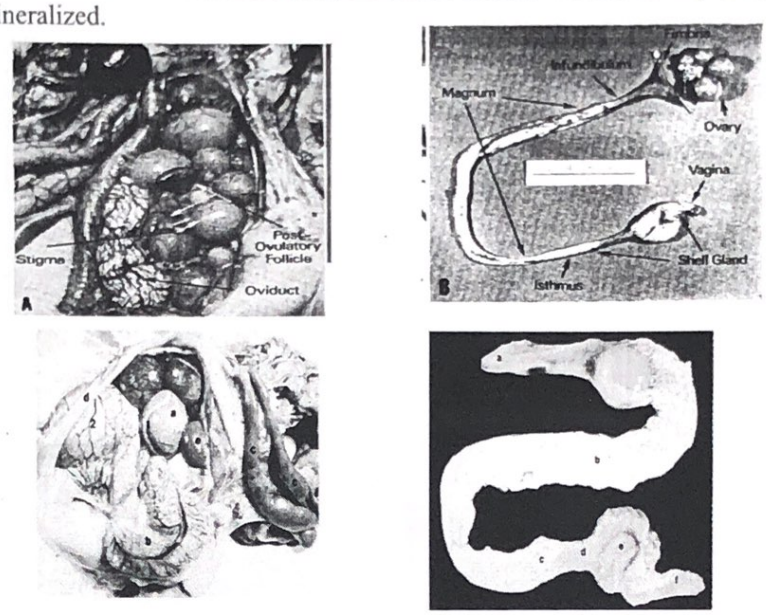


Figure 100.0: Hens ovary showing graafian follicles (A) and the reproductive tract (B); Ovary and oviduct of an in-lay chicken (Right lateral view) showing ovary (a), oviduct (b), right /left caeca (c), rectum (d), spleen (e), white avascular band called stigma (1) on the follicle surface where the wall of the follicle split at ovulation to release the oocyte, presence of egg in uterus (2); Parts of the oviduct infundibulum (a), magnum (b), isthmus (c), tubular part of uterus (d), pouch of uterus (e), and vagina (f). Presence of egg is shown in the cranial part of the magnum in this photograph.

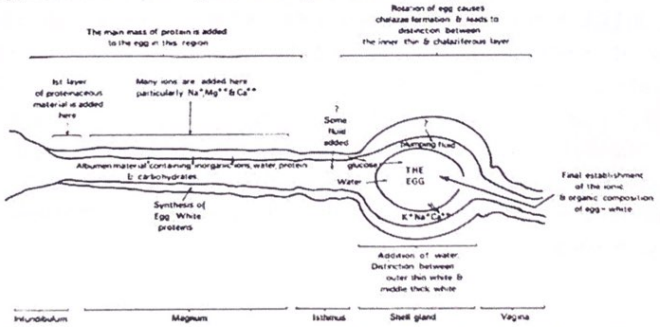


Figure 101: Diagramatic representation of the oviductal function in egg formation process

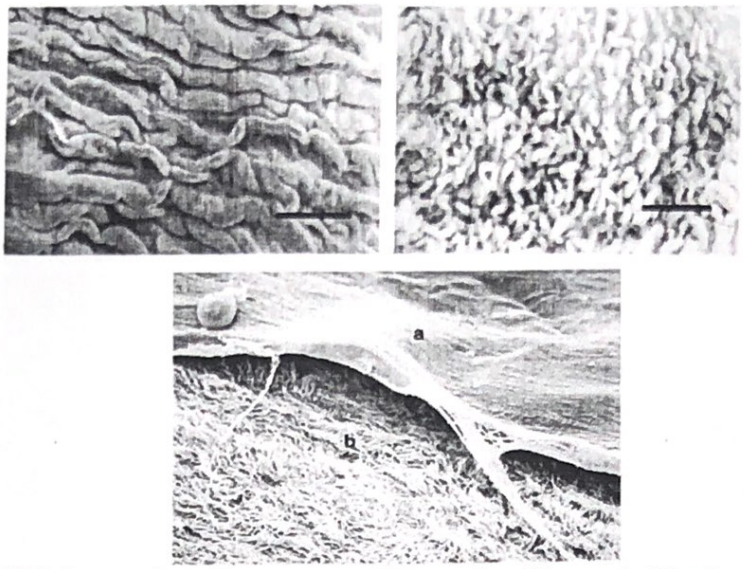


Figure 102.0: Inner surface of the magnum and inner surface of the pouch of the uterus of an adult chicken photographed under water (Scale, 10mm); **Scanning electron micrograph of the inner surface of the magnum of an adult chicken**. A continuous layer of mucus (a) covers the surface. The numerous ciliated cells (b) of the epithelium tend to obscure the goblet-like cells (x18, 000).



Figure 103.0: Light micrograph of the magnum of a non-laying hen (H/E, scale 100µm); Light micrograph of an in-lay hen (Periodic acid-Schiff, scale 20µm). Throughout the oviduct there are two types of glands unicellular glands in the epithelium, and multicellular glands in the mucosal folds. The unicellular glands are especially well-developed in the magnum and are stained red/ resemble goblet cells (2nd micrograph). Alternating with the unicellular glands are ciliated cells. The multicellular glands (a) are also massively developed in this part of the oviduct because in the sexually active bird the cytoplasm of the gland cells is packed with secretion, it is difficult to identify the lumen of the glands. Outside the glandular layer is the smooth muscle (b) which is best developed in the vagina/ uterus. In the proximal part of the vagina it forms a sphincter.

When a hen pants in hot weather to increase heat-loss by evaporation from the respiratory tract, she also causes a reduction of CO_2 and HCO_3 ? ion in the blood resulting in thin shell in hot weather. So calcium in feed should be increased to correct the defect. About 2g of calcium are deposited in each egg shell. Thus a hen has a high requirement for dietary calcium than any other domestic animals.

- The female bird is heterogametic sex
- It is photostimulatory

A characteristic feature of poultry reproduction is that each successive egg of a sequence is laid later during the day. This is brought about by LH release occurring later during the dark period and not by the length of time the egg spends in the oviduct. Because LH release occurs later in the dark period, although the reasons are unknown, there can be no ovulation and subsequent egg production (oviposition) till 24hours later (pause day). After the pause day, the following sequence is initiated by the next onset of darkness and the cycle is repeated. After ovulation, egg formation appears to be largely outside direct hormonal control, although oestrogens and progesterones are known to play important roles in the production of egg-white proteins and calcium metabolism.

It has been shown that prostaglandins can affect oviposition, though their importance in the normal functioning of the hen has not been stated. Similarly, the oxytocin-like hormones can affect oviposition, but in the normal animal other mechanisms also may operate to bring about the expulsion of the egg. Other hormones could indirectly affect reproductive performance e.g. body calcium is affected by parathyroid hormone and calcitonin but the availability of calcium for shell formation is important. Corticosteroids/ thyroxin may influence reproductive activity by their effect on general metabolic processes.

Avian endocrinology: In comparison with mammals, avian endocrinology is poorly understood and even the male is less known than the female.In thefemale reproduction can be divided into two endocrinologically distinct phases:

- Those in which the physiologic mechanisms lead to sexual maturation/ subsequent maintenance of sexual state.
- Those that result in the development of a single egg.

The difficulties in understanding ovarian endocrinology arise due to the fact that similar hormones are active in each case, and one phase is superimposed on the other. Steroid hormones are produced by the left ovary in early embryogenesis, and the oestrogens present may be responsible for the suppression of the right ovary at this time. Without the presence of the right ovary, the oviduct fails to develop further. After hatching, growth and development of the ovary/oviduct continue but only slowly. As birds approach sexual maturity they become photostimulatory which result in future sexual development with the hypothalamus altering its output of gonadotropin-releasing hormones in response to changing day length (not total daylight).

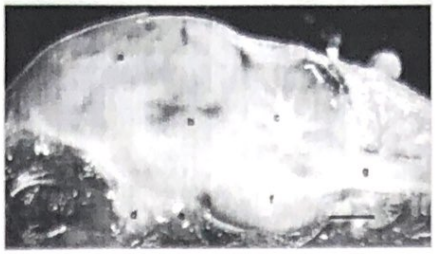


Figure 104.0: Median section of the brain of a day-old chicken (scale, 2mm) telencephalon (a), optic tectum (b), cerebellum (c), optic chiasma (d), pituitary (e), pons (f), medulla oblongata (g).

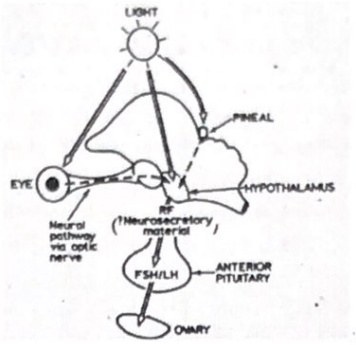


Figure 105.0: Illustration of the effect of light on oviposition

Light may affect reproductive activity through three pathways:

- The eyes
- The pineal gland
- Direct stimulation of the hypothalamus

The relative importance of these is not known, but it seems that the eye is the main pathway. FSH/LH produced by the anterior pituitary through GnRH stimulation from the hypothalamus act on the ovary causing rapid development of ovarian follicles and increased steriodogenesis of ovarian steroid producing cells. The major steroid hormones involved in reproduction are oestrogen, androgen and progesterone.

Functions of oestrogen

1. They cause oviductal occluding plate to breakdown.
2. They increase the uptake of calcium from the GIT
3. Act on the liver to produce the specific yolk lipoproteins
4. Increase deposition of body fat.
5. Cause changes in the vascular system
6. Stimulate growth of the oviduct
7. Cause the pubic bones to 'spread'
8. Play a role in the deposition of extra calcium in the bones

9. Help to bring about the typical female behavior
10. Play a feedback role in the control of gonadotropin output.

Functions of Progesterone

1. Progesterone is more specific in its effects, but it is also involved in the calcium metabolism, female behavior (broodiness) and oviductal growth and function. In contrast to oestrogen, it appears to have a positive feedback effect on gonadotropin production.

Functions of androgen

1. Androgens are also produced by the female and play a part in oviductal growth and differentiation and the development of the secondary sexual characteristics. They may affect pituitary output of the gonadotropins.

All three types of hormones are probably produced throughout the breeding season at their individual rates. It is their interaction that maintains the reproductive state. Sometimes they are complementary, as in the regulation of oviductal growth by both oestrogens and androgens, but in some instances they are antagonistic, as when androgens and oestrogens act against each other in comb development. Little is known about the cells responsible for steroid production in the ovary. It's been claimed that oestrogens are produced by the interstitial cells of both the stroma and follicles, but the evidence is based mainly on the interpretation of the activity of the steroid-dehydrogenase enzymes that are present. The source of androgens is unknown; the only work regarding their origin, which suggests that the stromal interstitial cells are responsible has not been repeated. Progesterone seems to be a product of the granulose cells of the preovulatory follicle but unlike in mammal, the granulose cells of the post-ovulatory follicles in birds may be incapable of producing it.

With the final maturation of the follicles, a daily cycle is initiated, which is super imposed on the general endocrinologic pattern. Light again plays a major role because the initial entrainment appears to be the transition from light to dark which, in some way, triggers the specific release of LH for a short time. The release of LH occurs about eight hours after the transition from light to dark, and it results in an increase in the concentration of LH in the blood, which rises to a peak value of 3- 4 ng/ml, a level that is two to three times greater than the resting value. Ovulation occurs about 4hours later.

There has been much speculation about the specific trigger for LH release which results in the evoking of oestrogen, progesterone and androgen. It has been reported that an increase in plasma LH occurs approximately at the same time as an increase in oestrogen, progesterone and androgen in birds.

Egg composition: The egg is composed basically of three components central yolk mass, the egg white or albumin and the shell.

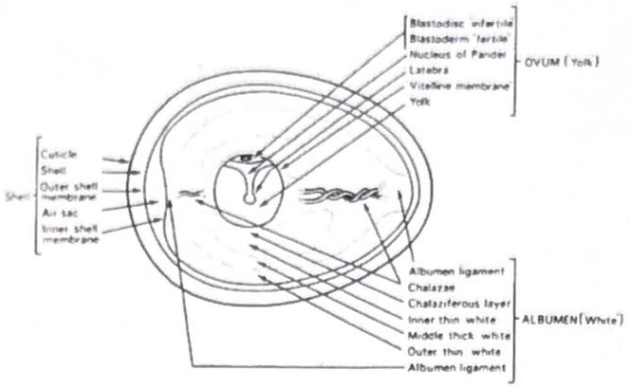
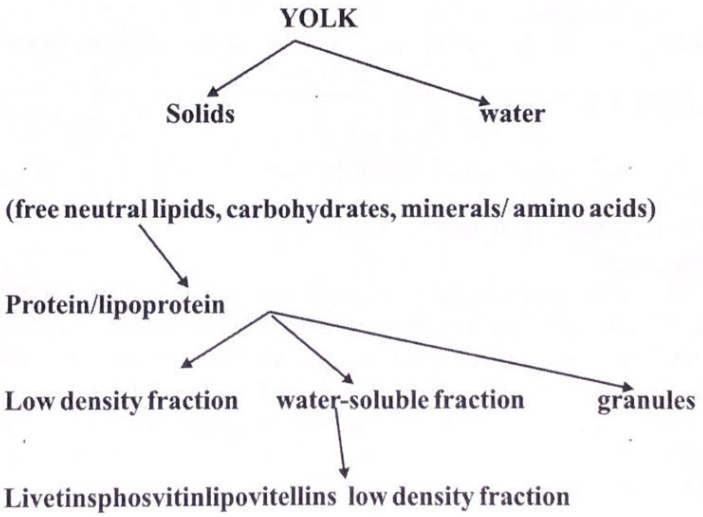


Figure 106.0: Structure of an egg.

Table 24: The major components of the egg

Common name	Specific name	Components
Egg - yolk	Oocyte	Blastodisc (infertile) or blastoderm (fertile) Nucleus of Pander Latebra (white-yolk) Yolk (yellow-yolk) Vitelline membranes: a. Primary membrane (oocyte cell membrane). b. Secondary membrane (perivitelline membrane). c. Two tertiary membranes (formed from oviduct).
Egg - white	Albumen	Albumen ligaments Chalazae Chalaziferous region Inner thin white Middle thick white Outer thin white
Egg - shell	Shell membranes	Inner membrane (air space)/ outer membrane.
	Mineralized part	Organic matrix, inorganic crystals, pigments
	Cuticle	Organic part, pigment.

1. **Central Yolk Mass:** It consists of the secondary Oocyte in non-fertile egg or the fertile zygote. It is bounded by the perivitelline membrane. The cytoplasmic part of the cell (blastodisc or blastoderm in case of fertile egg) is a grayish-white spot containing the female haploid pronucleus, which produces either a male or female embryo in the bird, unlike the mammal, the female is the heterogametic sex. In a fertile egg the area is covered by a double layer of cells at the time of laying. Occupies a small fraction of the oocyte i.e. about 3mm in diameter. The germinal disc floats on a cone of light-coloured yolk (white yolk) that extends downward to end in a ball, the latebra. The chemical composition of white yolk differs from that of the yellow as it contains a greater proportion of protein and its structure has certain peculiarities. The major part of the central yolk mass is composed of the familiar orange-yellow viscid fluid (yellow yolk) which has been described as an oil-water emulsion with the continuous phase as aqueous protein. Scattered throughout this are various droplets and yolk spheres. The central yolk mass weighs about 19g in the chicken, although its size is age-dependent with lower weights associated with pullets. Chemically, the central yolk mass is an heterogenous mass containing proteins, lipids, pigments and a variety of minor organic/ inorganic substances. The low- density fraction contains nearly all the lipid fraction as lipoproteins and much phosphorus. The water-soluble fraction (livetin), which comprises 10% of the mass, contains about 30% of the protein mainly as plasma albumin, glycoproteins and -globulin. The granular fraction contains phosvitin, phosphorus, calcium and lipovitellins. The function of the yolk is to provide the initial nutrient for embryogenesis but it is difficult to distinguish between substances essential for embryonic development and those that might be present as accidents of the mechanisms responsible for yolk function.



2. **Albumen (white):** This is the white of the egg that surrounds the central yolk mass. It has about seven major regions formed by the rotation of the egg in the oviduct. It is an almost pure aqueous protein that contains about 40 proteins. Since it surrounds the developing embryo, it forms an aqueous mantle preventing desiccation and also contributes material to embryo during later development. Other functions have been ascribed to specific proteins, since many of the proteins in-vitro has bactericidal properties, while others have enzymatic activity and some are enzyme inhibitors.

Table 25: Characteristics of egg white proteins from the hen

Components	Percentage	Molecular weight	Biological Properties
Ovalbumin	54	46,000	
Ovotransferrin	12-13	76,600-86,000	Binds Fe, Cu, Mn, Zn; may inhibit bacteria.
Ovomucoid	11	28,000	Inhibits trypsin
Globulins	8	36,000-45,000	
Lysozyme	3.4-3.5	14,300-17,000	Split specific β-(1,4)- D-glucosaminides; lyses bacteria
Ovomucin	1.5-2.9	?	Anti-viral haemagglutination
Flavoprotein	0.8	32,000-36,000	Binds riboflavin
Ovomacroglobulin	0.5	760,000-900,000	
Ovoglycoprotein	0.5-1.0	24,400	
Ovoinhibitor	0.1-1.5	44,000-49,000	Inhibits proteases, including trypsin and chymotrypsin
Avidin	0.05	68,300	Binds biotin
Papain inhibitor	0.1	12,700	Inhibits proteases, including papain and ficin

Source: *Feeney & Allison, 1969*

3. Shell: The shell is made up of three structures
   - The membranes
   - The mineralized part, and
   - the cuticle

There two shell membranes that are toughened sheets of fibrous protein both about 70μm thick which separate at one end to form the air-sac. It provides the surface on which mineralization can occur; fibers from them penetrate outward to produce the organic matrix of the shell. It helps reduce the speed of bacterial entry and allow the bactericidal properties of egg white act more effectively. The mineral of the shell is almost pure calcite (calcium carbonate), with some Mg, formed of radiating crystals. Running vertically through the shell are pores that allow gases to

The shell, apart from the pores, is impervious to most substances that might adversely affect the micro-environment of the embryo. It also provides mechanical strength and a rigid support to maintain the orientation of the heterogeneous internal components. Its strength is determined mainly by its curvature and thickness, although other factors are involved. The shell also provides calcium for the developing embryo. The outer covering of the egg, the proteinaceous cuticle reduces water loss and bacterial contamination.

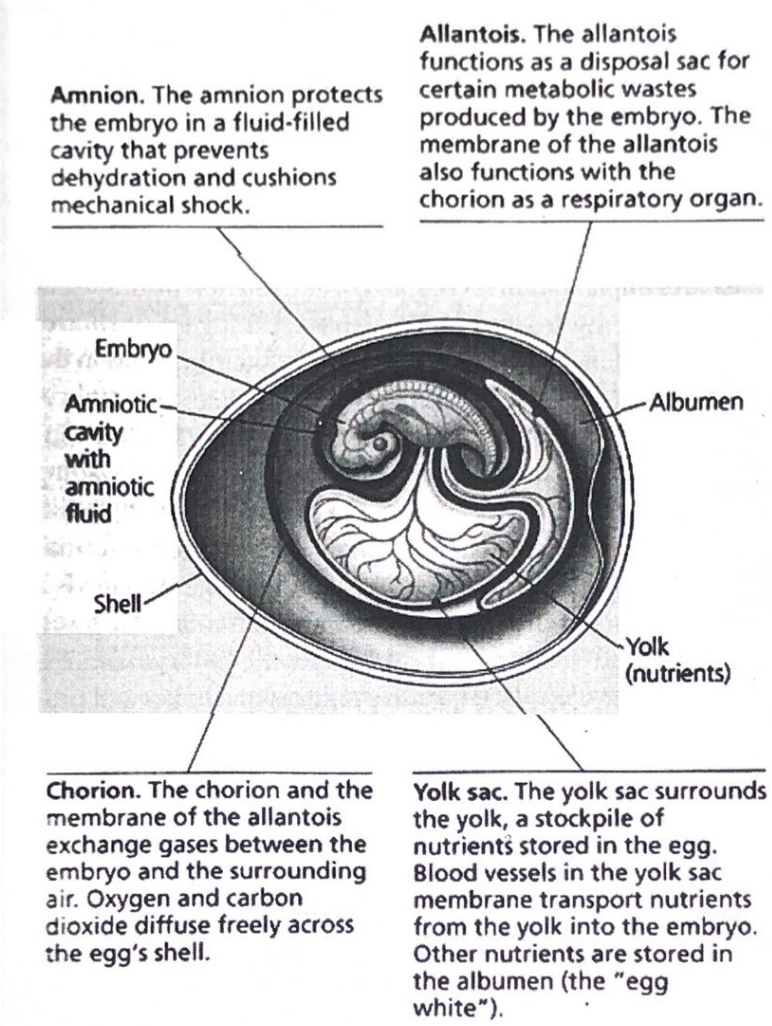


Figure 107.0: Extra-embryonic membranes of birds

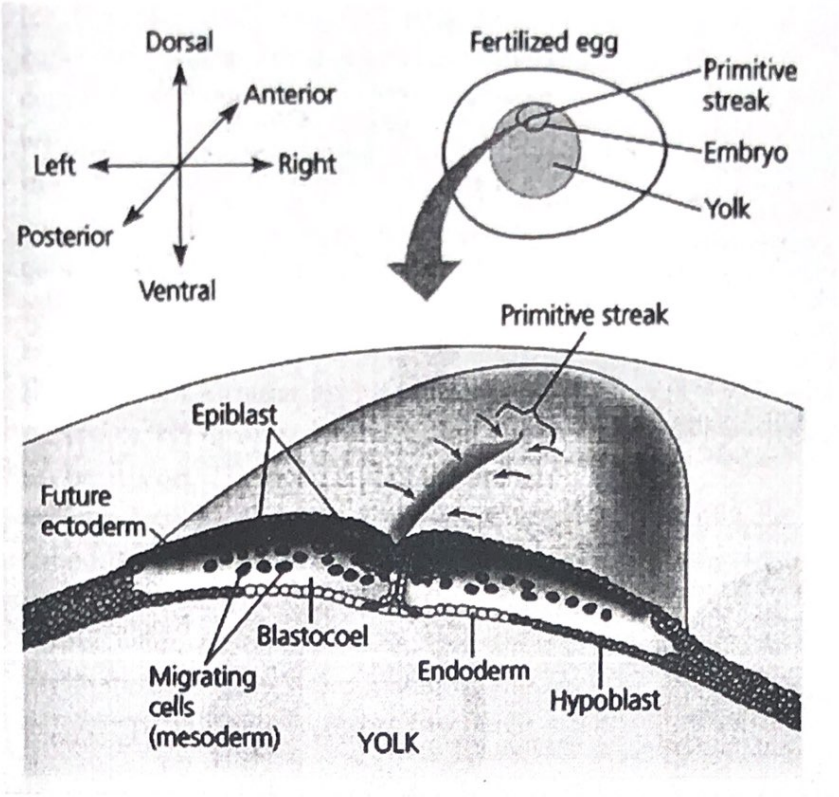


Figure 108.0: Gastrulation in chick embryo

CHAPTER THIRTEEN

ASSISTED REPRODUCTIVE TECHNOLOGIES

The knowledge of the basic physiology of the female and male reproductive systems as well as the various methods to synchronize reproductive cycles is necessary for the understanding of assisted reproductive technologies. Various techniques have been developed for improving reproductive efficiency in farm animals which include -

1. ARTIFICIAL INSEMINATION
2. OESTRUS SYNCHRONIZATION
3. SUPEROVULATION (MULTIPLE OVULATION)
4. FERTILITY CONTROL
5. EMBRYO TRANSFER
6. CLONING (NUCLEAR TRANSFER)
7. IN VITRO FERTILIZATION
8. EMBRYO OR SPERM SEXING

1. **ARTIFICIAL INSEMINATION:** This is the manual collection of semen from proven males, its processing and introduction into the female reproductive tract of an oestrus female. This is in contrast to the natural means that involve mounting, intromission and ejaculation. It involves three basic stages
   - Semen collection
   - Semen evaluation (sperm motility and sperm concentration)
   - Semen preservation or extension
   - Insemination (recto-cervical method, vagino-cervical method, speculum)

Importance of artificial insemination

1. It allows the use of outstanding sires and dissemination of valuable genetic material even to small farms.
2. It reduces the risk of the spread of sexually transmitted diseases such as Brucellosis, Leptospirosis, Trichomonas, etc
3. Semen export reduces the cost of international transport since it is lesser to import semen than the animals thereby promoting world trade in genetic resources.
4. It solves time/space constraints in animal breeding due to the possibility of preserving semen for a long period, allowing the use of dead proven sire.
5. It enables better planning of breeding/ production programme with the aid of oestrus synchronization and insemination of large number of animals with adequate record keeping.
6. Provide a research tool for investigating many aspect of male and female

7. It reduces production cost as it is much cheaper to use AI than keeping stud-bulls.
8. It allows cross-breeding to change production emphasis e.g. a change from beef to dairy animals.
9. It makes possible the introduction of new genetic material through the importation of semen.

Disadvantages of artificial insemination

1. It requires skilled technicians that are in short supply in villages.
2. It is not subject to the nomadic production system
3. It is in social conflict with the stereotyped nomadic idea, prompting the reference to AI progeny as bastards.
4. It is ineffective due to the absence of a clearly defined breeding pod.
5. Lack of adequate infrastructures such as electricity, supply of cryogens, good roads, etc in developing countries renders it ineffective.

Semen collection: Semen can be collected from the male by two methods:

a. Direct stimulation of the musculator of the male reproductive tract
b. Simulation of the influence of the vagina on the glans penis

Simulating the effect of the vagina requires the use of artificial vagina (AV) when the male mounts a teaser animal or dummy (usually a brown wooden object shaped like a sow or a steer). Stimulation of the male reproductive tract involves the use of electroejaculator.

Artificial vagina (AV) is a device consisting of a rigid cylinder with a thin rubber lining separated from its wall to create an air-tight compartment (reservoir) usually filled with warm water through a valve. The reservoir is filled with warm water (40-42°C) such that the inner lining is appropriately stretched so that the temperature and pressure of the device closely mimic the influence of the vagina. Air is often blown into the water-filled compartment till the inner thorough surface closes. The device normally carries a rubber cone at the end with an insulated calibrated test-tube into the ejaculated semen drains. The upper end through which the penis is inserted is lubricated with petroleum jelly.

A teaser is a steer or nymphomaniac cow or one maintained permanently on heat by injection of oestrogen in oil, restrained in a semen collection crate to be mounted. During semen collection the male animal is led to the teaser, allowed to snif the teaser but not allowed to mount if attempt is made (false-mounting). The animal is not allowed to mount on at least two attempts in order to heighten sexual urge which results in ejaculation of higher quantity of semen. When the male is allowed to mount, the protruding penis is deflected into the AV for ejaculation, which is signaled by a lounging movement that takes less than 30 seconds in bull/ ram. The AV is held upright to allow the semen to drain properly into the test-tube after the male dismount.

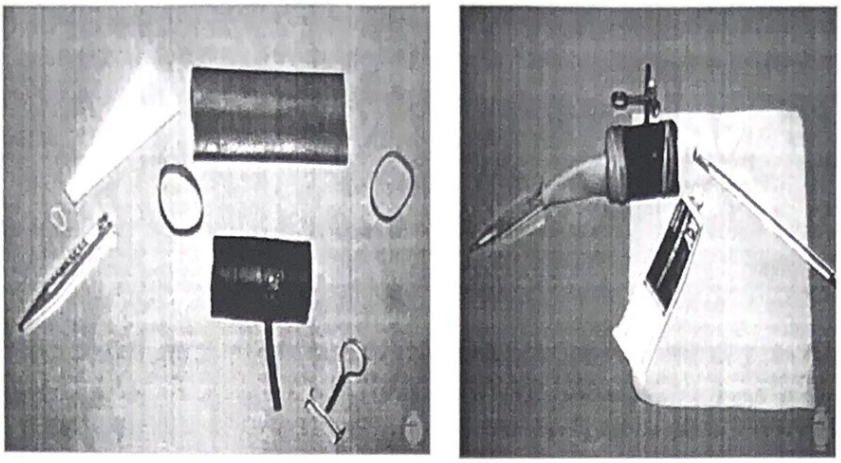


Figure 109.0: An artificial vagina

Gloved-hand technique is used in boar where the semen collector wears a rubber glove on one hand with which the screw-like end of the penis is firmly held after mounting the dummy to mimic the locking-in of the cervix. A dummy needs not bear any anatomical resemblance to the species involved but a well padded structure of appropriate dimension with three or four steel or wooden legs. The dummy should be well impregnated with odoriferous secretions such as preputial fluid, saliva and stale urine. Secretions from other males have been found to be more stimulating than the cervical or vagina mucus from oestrus female. At ejaculation which last for about 10 - 30 minutes semen is released in three distinct fractions (pre-sperm, sperm rich and post-sperm). The pre-sperm fraction is made up of preputial secretions that are low in spermatozoa while the post-sperm fraction though voluminous, is made up of gelatinous bulbourethral gland secretions with few spermatozoa. The sperm-rich fraction is a creamy-white fluid with high sperm-concentration suspended mainly in vesicular gland secretions. A flask holding three large test tubes (250ml each) is used to collect the different fraction of the ejaculated semen.



Electroejaculator is an electrical probe inserted in the male's rectum to deliver intermittent voltage surges from a battery pack for electrical stimulation of the nerves around the accessory sex glands, leading to involuntary ejaculation. The insertion of a bipolar (rectal) probe delivers low electric current by manipulation of the rheostat knob to stimulate the animal to ejaculate. Usually there is no full erection, the penis will protrude and ejaculate without full erection. Animals varies in their sensitivity to electroejaculator, however the technique has been successfully used in bulls, rams and goats but less used in boar and stallion. The method has no harmful effect on the male and the spermatozoa produced but the semen is usually more voluminous and of poorer quality than that collected by AV or natural method. The animal must be well restrained during electroejaculation to contain any violent reaction arising from electric shocks.



Figure 110.0: Electroejaculation technique in bull semen collection

Factors affecting semen output during semen collection

Age - Older males produce more spermatozoa than younger ones.

Species of animal - Boars release about 150ml of semen at one ejaculation compared to 10ml in bulls and 1-2ml in rams which is more highly concentrated.

Breed of animal - Sperm output varies with breed and this could be related to body size and scrotal size. Holstein-Friesian bulls produce more spermatozoa per ejaculate than Bunaji bulls.

Individual differences - In the same species/ breed there are individual differences in the amount of spermatozoa released during ejaculation as a result of differences in age, libido and scrotal circumference.

Degree of sexual preparation - Long sexual preparation increase semen output. There is increase in sperm output with false- mounting or teasing of male.

Method of semen collection - Semen collection by AV is usually more concentrated than those collected by electroejaculation which is usually more watery and have poor quality.

Frequency of semen collection Sperm output per ejaculate declines with increase in the frequency of ejaculation. Although total sperm output per week increases with increasing frequency of ejaculations from once a week to about 8times per week.

Semen evaluation: -Evaluation of semen has long been used in assessing male fertility on routine basis in AI laboratories. Semen to be used for AI must be evaluated after collection to determine its quality, though large variation is known to exist between technicians and laboratories in the subjective evaluation of semen characteristics. This has been reduced with the development of the computer assisted sperm-head morphometry analysis (ASMA). Evaluation criterion includes semen colour, semen volume, sperm concentration, sperm motility, live-dead ratio and sperm abnormalities. Good quality semen is creamy-white colour, translucent semen indicates low concentration while blood stained or unusual colour indicates contamination/ poor quality. Semen volume is determined from the calibration on the collection test-tube. Sperm motility can be assessed using a light or phase-contrast microscope (x 400 magnifications) fitted with a warm stage (or a water-bath with temperature similar to body temperature). A drop of semen is placed on a glass slide under cover slip and observed for progressive, stationary or backward motility. Semen with progressively motile sperm at 70 - 80% is considered good. Photomicrography can also be used to measure sperm motility, sperm velocity and time-lapse for more accuracy. Sperm concentration can be determined by haemocytometer counts of diluted semen or taking absorbance readings of diluted semen using a spectrophotometer at 600nm caliberated against haemocytometer counts. Percentage of live sperm can be determined from semen smears stained with eosin-nigrosin stain (the dead sperm will absorb the eosin stain and appear red) with the ones alive at the time of staining appearing white as they do not absorb the stain. Sperm morphology reflects the functional condition of the testes and excurrent duct system to some extent and there is considerable evidence that increased sperm cell abnormalities are associated with poor conception rates. Stained semen smears are prepared by touching a spreader slide to a drop of stain mixed for 10 seconds with an appropriate amount of semen. The spreader is then pulled slowly across the smear slide surface at a 30° angle, dried on a heated slide warmer or by passing quickly through a flame and observed using the phase-contrast microscope at x1000 magnification under oil-immersion. Abnormalities below 5% indicate good quality semen. Abnormalities observed for include primary (acrosome defect; macrocephalic or microcephalic or elongated-narrow or short-broad or double or pyriform head; double or swollen or abaxially attached or coiled - mid-piece; abaxial tail, folded tail at the connecting piece, tail thick anomalies and multiple tail), secondary (acrosomal vacuolation, detached head, proximal/ distal protoplasmic droplet, tail-tail agglutination, multiple heads, folded, coiled or bent tail) and tertiary abnormalities due to vigorous agitation, overheating, rapid cooling etc.

Primary abnormalities generally arise due to disorders of the seminiferous or germinal epithelium (faulty spermatogenesis) while secondary abnormalities occur during passage of sperms through efferent ducts, epididymis and vas deferens (malfunction of the duct system). Sperm abnormalities have been associated with early embryonic loss, lowered fertility/ embryo quality and reduced capacity to bind to the ovum. Two hundred (200) cells must be analyzed to achieve an accurate estimate of the percentage of normal spermatozoa.

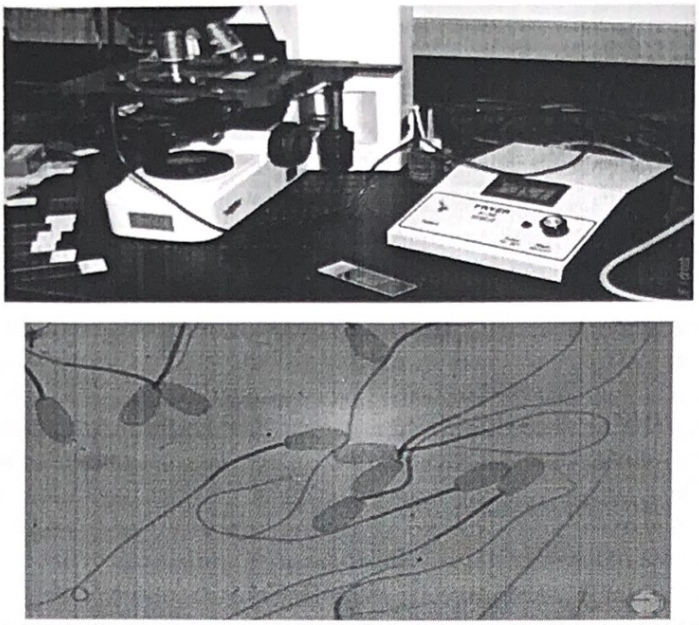


Figure 111.0: Semen evaluation equipment; epididymal spermatozoa

Semen preservation: Ejaculated spermatozoa do not survive for long as sperm lose viability with length of storage unless preserved by the addition of an extender or diluents. The preservation of semen involves the slowing down of spermatozoa metabolic rate while at the same time keeping the pH constant. Sperm metabolic rate can be slowed down by either cooling the cells or chemically inhibiting metabolism by creating an anaerobic condition by exposing it to carbon (iv) oxide gas. pH control involve the control of the production of metabolic by products through the use of buffer or dialysis as well as the provision of an energy source like glucose. If the temperature is to be reduced, there is need to maintain cell integrity by preventing cold sock during cooling or freeze-injury in case of frozen storage by the use of egg-yolk or milk and glycerol or erythritol (cryoprotective agents). Semen extenders or diluents are liquids added to semen primarily to preserve and increase the volume to obtain more insemination doses for AI from the ejaculate.

Characteristics of semen extender includes

a. It must be isotonic with the semen (have the same free ion concentration) a 2.9% sodium citrate dihydrate will provide this type of ion concentration.
b. It must provide nutrient for sperm metabolism - egg-yolk, milk, simple sugars, coconut milk etc.
c. It must protect the sperm from cold shock injury during the cooling from body temperature to 5°C lecithin or lipoprotein from egg-yolk or milk.
d. It must provide a buffer to prevent harmful shift in pH (pH of 7 or slightly higher, spermatozoa tolerate basic better than acidic conditions) as lactic acid is formed during storage isotonic sodium citrate solution.
e. It must maintain proper osmotic pressure/ electrolyte balance.
f. It must inhibit bacteria growth use antibiotics such as penicillin/ streptomycin.
g. It must protect the sperm cells from injury during freezing and thawing, so it must be cryogenic glycerol.
h. It must preserve the life of the sperm with a minimum drop in fertility combination of known/ unknown factors.
i. It must increase the volume of semen
j. It should not contain but prevent the formation of toxic substances.

Preparation of extender

In preparing extenders care must be taken to avoid the introduction of toxic substances by the use of clean equipment/ pure substances. Substances frequently used include glucose for energy, egg-yolk or milk for protection of sperm against cold shock as they are cooled from body temperature to 5°C as well as provide nutrient. Buffers such as citrate, phosphate, tris and other organic buffers for maintenance of pH must be used. Semen extenders should be formulated to be iso-osmotic with the semen. Penicillin, Streptomycin, Polymycin-B or any other antibiotics could be added to prevent the growth of micro-organisms. Glycerol (a cryogenic compound) is added to protect the sperm against the lethal effect of freezing. Dimethylsulphoxide (DMSO) and a sugar such as lactose could also be added (Table 26).

Factors that determine spermatozoa survival in storage include the following:

a. Initial semen quality before storage
b. Processing/storage method
c. Extender composition
d. Length of storage

Table 26: The composition of some semen extenders

Components	CUE	IVT	Egg-yolk glucose citrate +	Yolk citrate	Yolk citrate	Yolk citrate milk	Tris - yolk	Milk yolk	Skim milk
Sodium citrate dehydrate, g ($Na_3C_6H_5O_7.2H_2O$)	14.5	20.0	2.8	2.0	-	2.9	-	-	-
Tris*, g	-	-	-	-	-	-	3.08	-	-
Tris-sodium citrate, g	-	-	-	-	2.9	-	-	-	-
Sodium	2.1	2.1	-	-	-	-	-	-	-
Potassium chloride, g	0.4	0.4	-	-	-	-	-	-	-
Sulphanilamide, g	3.0	3.0	-	0.3	-	0.3	-	-	-
Citric acid, g	0.87	-	-	-	-	-	1.68	-	-
Glycine, g	9.37	-	-	-	-	-	-	-	-
Glucose, g	3.0	3.0	0.8	9.9	-	-	-	-	-
Fructose, g	-	-	-	-	-	-	1.25	-	12.5mg /ml
Distilled to, ml	1000	500	100	100	100	-	100	-	-
Penicillin, IU/ml	1000	1000	-	1000	100000	1000	100000	100000	500
Streptomycin, mg/ml	1000	1000	-	1000	0.1g	1000	0.1g	0.1g	500
% Buffer (by vol.)	80	90	85	-	-	15	-	-	-
%Egg-yolk(by vol)	20	10	15	15	20ml	15	20	20	10
% Milk** (by vol.)	-	-	-	-	-	70	-	100 ml	87
Glycerol, ml	-	-	-	5	-	-	-	-	3%

*2-Amino-2-(hydroxymethyl) propane-1,3-diol; **Cow or goat milk heated to 92°C for 10minutes; CUE – Cornel University Extender, IVT – Illini variable temperature extender (often flushed with CO_2 before sealing)

Semen dilution, cooling, packaging and freezing

Semen is collected at body temperature, but must be kept warm at 30°C until a diluents or extender is added. Following semen collection, samples are taken for evaluation while the remainder is mixed with the extender. Prior to extension, the number of sperm per insemination dose is determined as follows:

Example: Given an ejaculate volume of 8ml, sperm concentration of 1, 200, 000, 000 with sperm motility of 70%. The number of motile sperm required in 1ml of diluted semen as 5,000,000. Find the quantity of extender required to dilute 8ml semen volume?

1ml of semen contains 1, 200 000 000 X 70/100 = 840, 000 000 motile sperm

Since number of motile sperm required in 1ml of diluted bull semen is 5, 000 000

Therefore, dilution rate = 840, 000 000 / 5000 000 = 168ml

Then the 8ml of semen can be diluted as 8 X 168 = 1344ml

That is you'll add 168ml of extender to 8ml of semen to make the required 5, 000 000.

Dilution rate: The objective of deciding the dilution rate is to provide optimum number of motile sperm per insemination dose. It is generally accepted that 10, 000 000 progressively motile sperm per insemination dose will provide optimum conception rate when semen is used fresh, but with frozen semen in ampoules, 15, 000 000 sperm is the minimum insemination dose. In straws, about 7 8million is adequate because sperm survival is higher when frozen in straws.

Cooling: to cool the diluted semen, place the warm pre-diluted semen container in a container of water at the same temperature. Then place this in a refrigerator and cool to 5°C. The volume of water should be sufficient enough to allow cooling to 5°C in 2 - 4 hours which is the optimum cooling time. Three part of extender is added to one part of semen which is gradually cooled to 5°C (Bull) or 15°C (Boar).

Glyceration and equilibration: Glycerol is a required dilution to semen for protection during freezing/ thawing. Glycerol protect the semen by decreasing the freezing point of solution thus making the semen to freeze more gradually and it also favours less ice formation. Equilibration is allowing the semen to make the required contact with glycerol at 5°C. Equilibration time is the time the sperm is allowed to remain in contact with glycerol at 5°C. It varies from 4 18hours.

Procedure for adding glycerol:

1. Divide the extender into 2 equal parts with one part having no glycerol.
2. Add glycerol to part 2 at twice the level desired in the final mixture. This should be 14% for yolk-citrate and tris-yolk and 20-25% for milk extenders.
3. Predilute freshly collected semen with part 1 for cooling.
4. Cool to 5°C
5. Add part2 extender slowly over a period of about one hour to bring it to the final volume. It could be added at 15 minutes interval and allowed the semen to equilibrate with the glycerol at 4 - 18hours.

Semen packaging and storage: Semen is usually packaged in glass ampoules or straws. The volume of the ampoules varies from 0.5-0.8 through 1cubic centimeter while straws are 0.25-0.3cm^3. After packaging/ sealing they are then stored in liquid nitrogen until required for use.

Thawing: iced water provides the proper thawing medium for ampoules. Ice water bath are better provided by unbreakable plastic container. Straw should be thawed in a 32-35°C water-bath. The thawing process should be completed in 12-15seconds.

Insemination: This is the introduction of fresh, diluted or preserved semen into the reproductive tract of the female animal on heat using a pipette, syringe or insemination gun 24hours after oestrus detection. The site of deposition should be the body of uterus in cow, cervical os in the ewe, uterus in sow, cloaca in chicken and vagina in man.

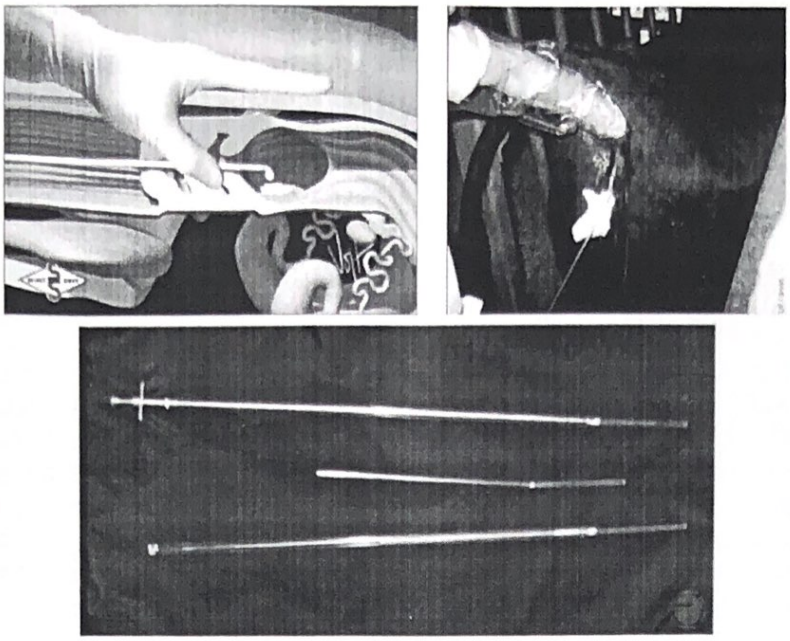


Figure 112.0: Artificial insemination in Cow; Insemination gun and

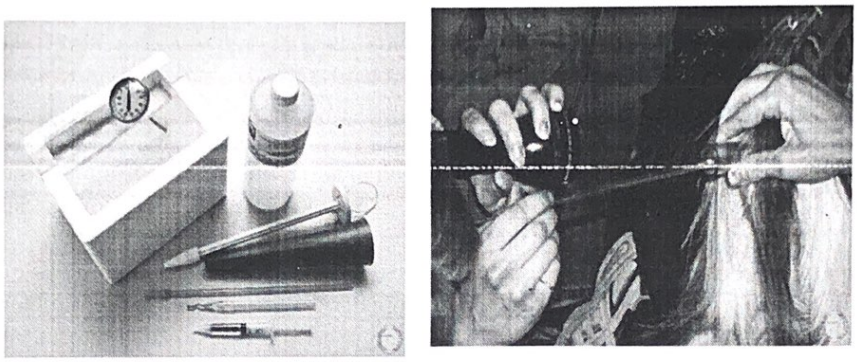
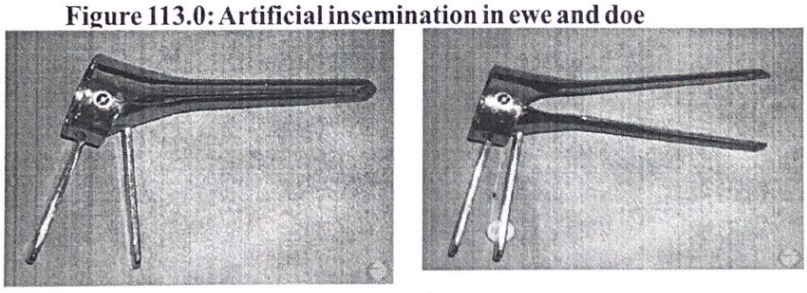


Figure 113.0: Artificial insemination in ewe and doe



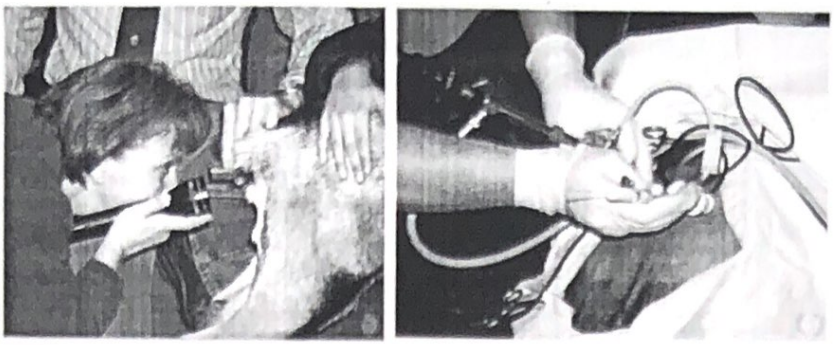


Figure 114.0: Closed and Opened Speculum; vagina-speculum (L) and Laparoscopic (R) insemination

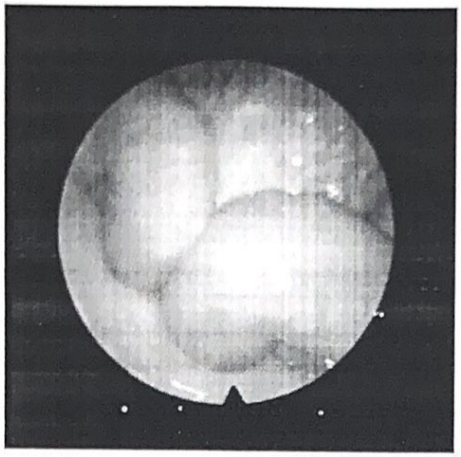


Figure 115.0: Laparoscopic view of the uterus in ewe

Insemination techniques

1. Speculum method e.g. sheep
2. Recto-cervical method e.g. cattle
3. Vagino-cervical method e.g. Horse

Insemination of cattle by the recto-cervical method

1. Get the cow or heifer properly restrained to prevent forward, backward or sideward movement. An assistant to hold the tail is advantageous.
2. Wear protective clothing (insemination gown or jumpsuit; shoulder-high rubber or disposable glove and rainboots).
3. Load the insemination pipette (liquid semen) or insemination gun or pistolette (frozen semen).
4. Lubricate gloved hand with soap and water
5. Carefully insert the lubricated gloved hand into the rectum and evacuate faeces if present.

6. Thoroughly clean the vulva.
7. Identify and grab the cervix (landmark structure).
8. Press the forearm gently downwards to compress the vulva thereby dilating it partially. An assistant to spread out the vulva lips is advantageous.
9. Insert the pipette or gun (into the vulva) at an angle of about 45°C with the tip directed cranially and dorsally, into the vulva and along the vaginal wall.
10. Guide the gun into the external cervical os with the aid of the thumb and index finger and there will be a gritty sensation of the cervix.
11. Combine gentle forward pressure on the gun with manipulation of the cervix by rotary movements of the wrist until the desired extent of penetration is achieved i.e. body of the uterus. Sometimes the tip impinges upon a cervical fold, in which case the pipette or gun should be slightly withdrawn and redirected.
12. Carefully withdraw the gun, give the uterus a gentle massage and then carefully withdraw the arm.
13. Dispose all disposable
14. Keep record of the insemination.

Time of insemination: middle to end of oestrus which in practice is refered to as the AM/PM rule. If oestrus is observed in the morning, inseminate in the evening of the same day; if oestrus is observed in the afternoon or evening, inseminate in the morning of the next day.

Conception Rate, CR = no. pregnant/ no inseminated X 100

Pregnancy Rate, PR = no pregnant/ total animal (synchronized) in a group

Insemination procedure for sheep and goats: The hind part of the ewe should be elevated or a rotating platform with the inseminator in a pit for fast inseminations. Insemination with a speculum and light enables the deposition of semen into or through the cervix instead of the vagina. Double inseminations 12hours interval increases fertility.

Insemination procedure for swine: Sow can be inseminated without being restrained since it stands calmly when in oestrus with some rubbing/pressure on the backside. The insemination tube should be guided into the cervix since the vagina tapers directly into the cervix, even though its not possible to pass the catheter into the uterus. With the inflated cuff catheter, most of the 50ml semen suggested for AI will be forced into the uterus.

Factors influencing conception rate in artificial insemination include:

a. Quality of semen (care of semen during collection, processing, storage and insemination).
b. Time of insemination
c. Semen dose for insemination
d. Fertility of female
e. Technician/ inseminators skill

Artificial insemination in Birds

a. **Collection of semen from a cockerel:** The easiest method to collect semen involves two people one person to hold the bird and the other to collect the semen. The collection occurs when the operator places his hand against the tail pushing the feathers out the way. The thumb and index finger should be placed on either side of the cloaca. A rapid, continuous massaging action (milking) is carried out by one hand, and the other is used to hold the collection vessel.

b. **Insemination of a hen:** To inseminate the hen, one person holds the hen close to his body and applies pressure around the cloaca to cause the vagina to evert. A second person inserts a small syringe up the left oviduct about 1 inch where the semen is then deposited.

In chickens, it is generally recommended that hens be inseminated with about 0.05ml of whole semen. To attain maximum fertility, hens should be inseminated at 7 days interval. Turkey hens should be inseminated with 0.025ml of whole semen per insemination. A fertility level in excess of 85% may be attained at an interval of 4 weeks between the first and second insemination, but most producers inseminate more frequently. Two-week intervals should maintain maximum fertility, but the insemination program should be tailored to the particular operation.

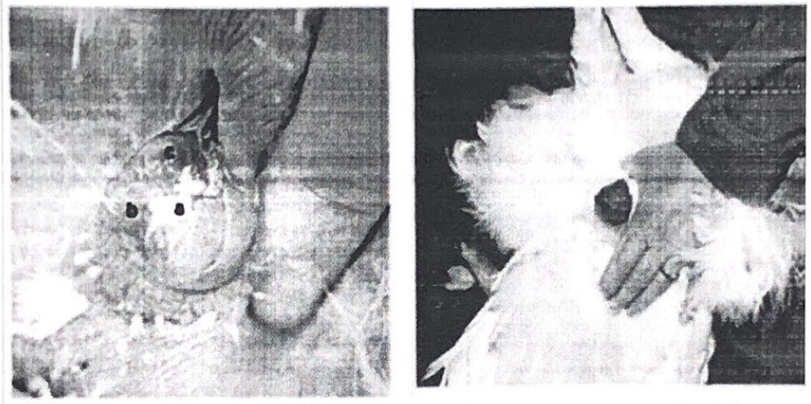


Figure 116.0: Semen collection in adult turkey; Everted oviduct of an adult female turkey for A.I.

2. **OESTRUS SYNCHRONISATION**: Oestrus synchronization is a management technique employed to facilitate the use of AI during which two or more female animals are manipulated to come into oestrus at the same or close period for insemination. The understanding of the hormonal events of the natural oestrous cycle is necessary for synchronization.

a. As long as a high level of progesterone (P_4) or any substance with similar activity (progestagen) is circulating in the blood, the female will not produce a mature follicle, come into heat and ovulate.
b. The effect of P_4 (negative feedback on pituitary with resultant low LH and FSH) is abolished by a uterine luteolysin, prostaglandin F_2 alpha ($PGF_2\alpha$.
c. The events that follows the withdrawal of progesterone, P_4 (withdrawal of negative feedback) from circulation are predictable, well defined and synchronized, which are:
   - Final maturation of a follicle (Graafian follicle)
   - Increased production of oestradiol from the Graafian follicle
   - Onset of heat
   - Release of LH, ovulation and beginning of a new oestrous cycle

From the above set of events, there are two logical ways to control oestrous cycle in female animals

1. By extending the life of the CL or substituting for the CL by the use of progesterone for a specific period of time.
2. By reducing the lifespan of the CL i.e. induction or luteolysis through the use of luteolysins such as prostaglandin or its synthetic analogues.

Progestagen treatments: Progestagens suppress oestrus/ ovulation via a mechanism of negative feedback on LH and FSH secretion. Thus, the use of exogenous P4 simply simulates endocrine events occurring during the luteal phase of the oestrous cycle. Long (18-21days), medium (12-14days) or short (7-10days) term progestagen treatment have been used in controlling oesrous cycle but with the short/ medium term the natural CL may outlive the exogenous progestagen thereby obstructing synchronization. It is necessary to incorporate a luteolytic agent (oestrogens or prostaglandin F_2 alpha) with the progestagen for maximal control. Methods of administration include: oral (melengesterol acetate, MGA; methylacetoxy progesterone, MAP; chlormanacetoxy progesterone, CAP); subcutaneous implants (norgestomet or synchro-mate B); intravaginal sponges or foam (Flugestone acetate, FGA; Medroxyacetate progesterone, MAP) and silastic coils (progesterone releasing intravaginal device, PRID).

Procedure for insertion of PRID

a. Fill a bucket with water/non-irritant disinfectant for cleaning speculum/rod after use in each animal.
b. Lubricate the lip of speculum inside/ outside with savlon cream but not obstetrical lubricants which may increase loss rate.
c. Clean the vulva with suitable wipe.
d. Load the device into beveled end of disinfected speculum with the string clear of the opening. Insert the rod into the speculum.
e. Holding speculum with one hand, spread the lips of the vulva with the other hand and gently insert the loaded speculum into the vagina with the slot in the speculum in the 9 o'clock position for ease of insertion.

f. While still holding the speculum pointed slightly upwards, turn the speculum so that the slot is in the 12 o'clock position, then gently push into the interior vaginal area until it reaches the cervix.
g. Expel the device into the vagina by gently retracting the speculum while holding the rod stationary.
h. Withdraw the speculum/ rod while leaving the string extended from the vulva and gently pull the string to straighten it.
i. Disinfect the speculum/ rod and use on the next animal.

On withdrawal of PRID the blood progesaterone level declines and most of the animals will come into oestrus within 2 -4days.

Prostaglandin F_2 alpha treatments: $PGF_2Ü$ injection results in abortion in pregnant animals, so animal must be tested to be non-pregnant by rectal palpation or other means before its administration. To achieve oestrus synchronization, $PGF_2\alpha$ or its analogue must be injected during dioestrus (luteal phase) for single injection or two injections 11-14days (preferably 13days) apart regardless of the stage of the oestrous cycle. It is ineffective within the first 5days of the oestrous cycle as well as at the end of the cycle when there is no longer a functional CL. A single injection given to a randomly cycling group will synchronize heat in only those with a CL. After injection of PGF2 alpha, observe for heat and inseminate at detected heat over a 7day period. Those that fail to conceive after insemination will return to heat within 17 to 24days later during when they can be re-inseminated. Examples of prostaglandin and its analogues include: Dinoprost (Lutalyse), Cloprostenol (Estrumate), Fenprostalene (Synchrocept B), Luprostiol (Prosolvin), etc.

Advantages of oestrus synchronization

a. Offers opportunities for more rapid genetic improvement through artificial insemination.
b. Help to have a clear breeding season especially during the period when forages are much in quantity during the rainy or close to the end of the rainy season.
c. Makes possible batch rearing/ management of animals of similar age /weight.
d. Better/ more predictable nutritional management of breeding animals.
e. Improved control of entry of replacement stock into breeding herd or flock
f. Offer possibility of induction of heat/ovulation in anoestrus females.
g. Remove pressure on heat detection.
h. Inseminator costs can be materially reduced if effective oestrous cycle control techniques are used.
i. Offers opportunities for the extension of AI to distant places especially where there are no skilled manpower.

Factors influencing the success of synchronization:

1. Nutritional status of the animal. This is the most important single factor governing the success.

2. Clear identification/ records of animal.
3. Animals must be ooestrous cycling/ healthy/ within the correct postpartum interval.
4. Semen/ inseminators must be of adequate quality.
5. Physical animal handling facilities must be good
6. Synchronization of too large number of animals may lead to inseminators fatigue/ mistakes/ impair efficiency.
7. Good unexpired synchronizing agent must be used according to manufacturer's directive.
8. Good working relation between farmer and veterinary surgeon on agreed objective.

3. SUPEROVULATION: This is a process whereby a female animal is stimulated to produce and release two or more ova at ovulation or manipulation of the ovulation rate (Fig. 118.0). In different species and breeds of livestock, litter size is a reflection of ovulation rate and appears to be genetically determined. Although increase in litter size is not desirable in monotocous species like cattle, it is a welcomed idea in polytocous species like sheep, goat and pig but litter size should not be increased beyond the uterine carrying capacity. It could be applied in monotocous species by manipulation of ovulation rate to collect ova that are needed for embryo transfer. The objective of the procedure is to increase the follicular population growing up to the tertiary stage followed by normal ovulation. It affords the opportunity for accelerating genetic progress in the female through embryo-collection/ transfer as well as increased lambing/kidding rate through natural mating. There are two major method of increasing ovulation rate:

a. Genetic method
b. Non-genetic methods
I. Hormonal methods
II. Antisteroid immunization
III. Nutritional methods
IV. Male effect
V. Steroid enzyme inhibitor

a. Genetic methods of superovulation: It may be possible to increase the rate of ovulation since it is genetically determined and breed dependent through selection, cross-breeding of prolific and less prolific breeds. Though heritability is low and it takes a longer time to achieve significant increases

b. Non-genetic methods of superovulation:
I Hormonal methods synthetic hormones that has been used for superovulation include pregnant mare serum gonadotropin (PMSG), follicle stimulating hormone (FSH), human chorionic gonadotropin (HCG) and gonadotropin releasing hormone(GnRH). However, animals vary in responses depending on the breed, type of drug/ dosage, season,

nutritional status of animals and even the batch of the drug used. E.g. in ewe, as PMSG increases, ovulation rate increases (750 i.u. PMSG gives 5.5±0.5 ovulation rate i.e. mean number of corpora lutea; 1000 i.u. PMSG gives 7.0±1.2 ovulation rate). Superovulation in pigs is by a single dose of 1,000 15,000 i.u. PMSG given early in the follicular phase or a mixture of 600 i.u. PMSG + 200 i.u. HCG given as single dose.

Procedure for superovulation

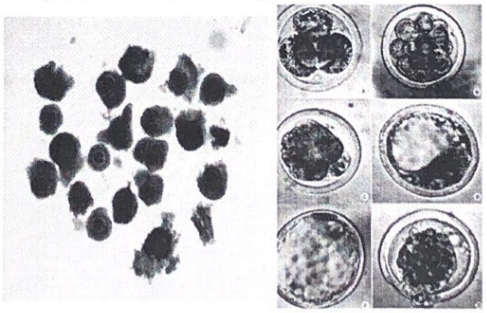
1. Insert progestagen sponges intravaginally
2. Leave progestagen sponges for 12 - 14days
3. Inject 0.4ml PGF_2 alpha intramuscularly (i.m.) 2days before sponge withdrawal to increase tightness of synchrony (though it may be skipped)
4. Withdraw pessaries and inject appropriate dose of PMSG depending on desired rate of ovulation.

II. Antisteroid immunization The synthesis and release of gonadotropins are under the influences of the steroid hormones via the feedback mechanism. Therefore, the removal of the negative feedback will lead to increased secretion of gonadotropins and high ovulation rates. However, immunization with antisera to androstenedione, testosterone, oestrone and oestradiol-17 Beta would neutralize these hormones, remove the feedback mechanism, cause an elevation in gonadotropins and hence ovulation.

III. Nutritional methods Nutrition is an important factor influencing ovulation and reproductive rates in livestock. High plane of nutrition (energy) have resulted in increased ovulation rates and fertility in polytocous animals, a phenomenon refered to as "**flushing**". The duration of the increased nutritional level is however important and must be at least 3weeks before breeding.

IV. Male effect The presence of the male, introduced at the beginning of breeding resulted in multiple ovulations and higher reproductive rates.

V. Steroid enzyme inhibitor It been reported that treatment of ewes with 3-hydroxysteroid dehydrogenase (3HB HSD) inhibitor cause decreased peripheral progesterone and an elevation in both ovulation rate (2.00 vs 1.27) and mean number of lambs per ewe (1.55 vs 1.19) lambing.



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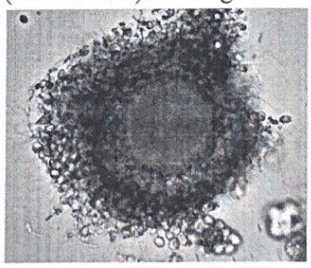
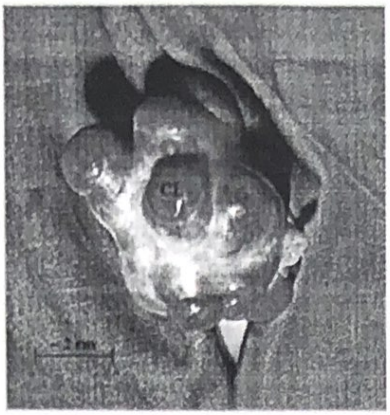
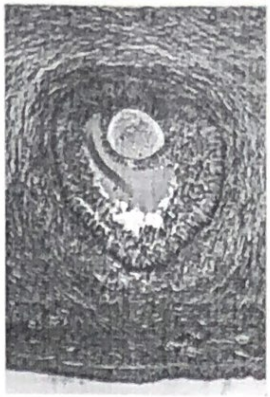


Figure 117.0: Oocyte collected from slaughtered cow ovary, ova stages (morula) and *In-vitro* Oocyte surrounded by spermatozoa





Oocyte on a hillock of the granulosa cell

Figure 118.0: Superovulated ovary showing graafian follicle

4. **FERTILITY CONTROL**: Human chorionic gonadotropin (HCG) and Gonadotropin releasing hormone (GnRH) has been used in improving conception rates based on their ability to induce ovulation and enhance corpus luteum (CL) development. Extension of CL function and delaying subsequent oestrus will give the fertilized egg greater time to become established in the uterus and inhibit the luteolytic factor. Therefore, HCG or GnRH injection after service early in metoestrus phase could enhance luteal activity and increase fertility rates. Significant increase in fertility and litter size of sheep/ goats can be achieved with gonadotropins treatments.
   The standard techniques for oestrus/ litter size control in sheep/goat involves intravaginal synchronization with suitable progestagen pessary for a period of 12days, followed by a single intramuscular injection of PMSG at sponge withdrawal and introduction of sexually mature ram/buck (1 ram or buck to 4 ewe or doe) at 48 hours after sponge withdrawal. Varying doses of progestagen and PMSG have also been used with FGA 30mg and 759 i.u. PMSG having highest conception rate.The timing of ram introduction affect conception rate of PMSG treated ewes with highest conception obtained by introducing ram at 48hours after pessary withdrawal in FGA-PMSG treated ewes in late anoestrus. With AI, cervical method should be used in goats and intra-uterine in sheep with two inseminations of 200 x 10^6 sperm in 0.2ml volume each at 48 and 56 hours (or a single 400 x 10^6 sperm at 56hours) after progestagen withdrawal, though may be breed dependent in relation to ovulation timing.

5. **EMBRYO TRANSFER:** Embryo transfer (ET) is the process whereby fertilized ova (embryo) or ova are collected from a female animal

and transferred to a primed female (surrogate mother) for natural gestation and parturition. It is done to increase the progeny numbers from females of outstanding merit by

a. Using gonadotropic hormones to stimulate ovulation of large numbers of ova and then

b. Transplanting fertilized ova or early embryo to recipient females for pregnancy and parturition.

In combination with AI genetic qualities of a superior male can be additive to that of the superior female with fast propagation in the offspring. The resulting offspring would genetically be progeny of the outstanding female (the recipient of the embryo i.e. surrogate-mother just serving as an incubator). Splitting or cloning of embryos into two or more segments with each developing into an offspring can also be done. Such individuals are monozygotic since they are derived from a single fertilized egg and they are the same genetically as the infrequent identical or monozygotic twins that occur naturally. Embryo transfer technique involves the following stages:

a. Selection of donors
b. Superovulation with PMSG or FSH
c. Synchronization of donors and recipients
d. Recovery of embryo (flushing) by surgical or non-surgical means
e. Embryo morphology and evaluation
f. Transfer of embryo or storage.

a. Selection of donors the criteria for selection include genetic superiority, reproductive ability, heat/disease resistance and market value. Regularly cycling females should be selected, maiden (young) or too old females have low response to superovulation.

b. Superovulation with PMSG or FSH superovulation is the key in embryo transfer to increase embryo number.

c. Synchronization of donors and recipients synchronization of donors and recipients can be achieved by injecting the animals with PGF_2 alpha on day 12 of the oestrous cycle.

d. Recovery of embryo (flushing): There are two methods of recovery of embryos - surgical and non-surgical means. Surgical recovery was an earlier method done by either midline (in young cows/heifers) or flank incision which results in adhesions of reproductive tract. The non-surgical method involves the use of the two-way or three-way Foley catheter (18-guage size in cattle). Laparoscopic method is use in small ruminants and swine. The procedure is as follows:

- Collect the embryos 6 to 8 days after the onset of oestrus.
- Stiffen the catheter with stainless steel to enable passage into the reproductive tract.

- Ensure superovulation by rectal palpation in cows and laparoscopy in sheep and goats.
- Clean the vulva, locate the cervix rectally and pass the catheter through the cervix into the uterine horn, inflate balloon and withdraw stiffner.
- Flushing medium is phosphate-buffered-saline (PBS) with 1% heat-inactivated calf serum or foetal calf serum.
- Flush using the continuous flow, closed-circuit method or the interrupted-syringe method.
- Keep "flush" at room temperature for 30minutes to allow embryos to settle, siphon the top fluid.
- Examine the embryos for normal morphology in a Petri dish.
- Place embryos in smaller Petri dish containing PBS with 2% foetal calf serum or 0.4% Bovine Serum Albumin.

e. Embryo morphology and evaluation embryos should be evaluated for normal morphology since it ET success depends on it. Embryo should be spherical, symmetrical with cells of uniform size.

Cell stage	Estimated age (days)
Morula	5
Compact morula	6
Blastocyst	7
Expanded blastocyst	8
Hatched blastocyst	9

F. Transfer of embryo or storage Embryos can be transfered surgically or non-surgically into the uterine horn ipsilateral to the corpus luteum. Place the embryo in a 0.25cc French straw and load into a cason inseminating gun. Cover inseminating gun with a straw. Clean the vulva and locate the cervix (by rectal palpation in cow). Insert inseminating gun into vagina, force with the protective straw through the cervix into the uterine horn and discharge embryo.

Excess embryo can be stored by deep freezing method. Addition of cryoprotectant (dimethylsulphoxide, DMSO or glycerol) is done in several steps or one step; cooled from room temperature to ?7°C at 1°C/min; seed with cooled forceps; cool from -7°C to 28°C at 0.3°C/min; cool to -37°C at 0.1°C/min.; thaw rapidly in water bath at 37°C and remove cryoprotectant using sucrose solution before transfer.



Figure 119.0: A Cow, the ET offspring and the surrogate dam



Figure 120.0: Two sets of identical twins produced by embryo- splitting

6. **CLONING**: Cloning is the science of producing offspring from a single cell, usually asexually and the offspring are highly identical like identical twins, to the parent. A clone is a group of genetically identical progeny produced by the natural or artificial asexual reproduction of a single organism cell. Thus any pair of monozygotic twins is a clone of two. Cloning involves the use of genetic material to artificially create a genetically identical copy of a living creature. It enables the production of multiples of identical animals greater than that of embryo splitting. Cloning can be carried out in three basic ways by blasomere separation, blastocyst division or body (somatic) cell nuclear transfer.

   Blastomere separation in this technique the embryo is allowed to develop to the blastula stage or the ova is collected and fertilized with a spermatozoon, allowed to divide to a four cell mass (blastula). After which the outer membrane is removed and placed in a special media which cause the individual cell mass (blastomere) to develop separately.

Each can now be transferred to a recipient (surrogate mother) to develop into separate identical individuals.

Blastocyst division this technique involved allowing the embryo to develop to the blastocyst stage which is then split into two equal halves, implanted in the uterus of primed mother to develop into separate identical individual, just like the natural identical twins.

Body or somatic cell nuclear transfer this technique involve the transfer of the nucleus of a differentiated adult cell (donor) to an enucleated oocyte (oocyte that have had its nucleus removed) and the resulting embryo implanted in a primed recipient to develop into an individual that is identical to the nuclear donor. This technique unlike the other two results in the production of offspring (clone) from only one parent. The procedure involves the following

1. The activation of the recipient oocyte, this initiates development in the absence of fertilization.
2. Enucleation of the recipient ovum i.e. removal of the recipient nucleus intact.
3. Transfer of a nucleus intact, from either a blastomere (cell from early or presumably undifferentiated cleavage stage embryos) or a somatic cell (fibroblast, skin, heart, nerve, or other body cell) to the enucleated oocyte (unfertilized female egg cell with the nucleus removed).

The basic technique can be traced to the work of McGrath and Solter (1983) who developed a non-invasive method for ovum enucleation and a Sendai virus for blastomere fusion. It involves pretreatment of the recipient ova with cytochalasm which makes the recipient plasma membrane more elastic and the ovum more condusive to manipulation without lysis. The zona pellucida is circumvented by either a cut made with a glass knife to allow the insertion of a blunt enucleation pipette or a sharpened beveled pipette to puncture it and subsequently enucleate the ovum.

When inside the zona pellucida, the pipette is placed against the oolemma (near the egg chromatin in case of mice) and a gentle suction applied, pinching off the chromatin plus varying amount cytoplasm in self-contained karyoplast. The karyoplast or cytoplast is then placed in a recipient zona pellucid and both halves can be stained with DNA binding fluorescent dye (Hoechst 33342) to determine which domi-oocyte contains the metaphase chromosomes (Westhusin *et al.*, 1990). Staining of enucleated half does not result in reduced viability. The recipient ovum is now ready to receive a donor blastomere after enucleation.

A donor blastomere can be derived from the different embryonic cell stages (from the 2-cell stages to the blastocyst stage). Blastomere separation can be improved by placing in calcium ion free medium. After separation, a single blastomere is placed inside the zona pellucida and positioned tightly against the plasma membrane. The blastomere and enucleated ovum are now ready for fusion. Fusion may be induced by the use of electrical pulse (electroporation) to generate small pores on the plasma membranes in a non-ionic solution such as 0.3M mannitol in distilled water

and a fusion chamber consisting of two electrodes within a range of 1 - 2mm apart (Robl *et al.*, 1986). The ovum plasma membranes and the blastomere must be in close contact when the electric current passes perpendicularly through the ovum or blastomere interface for fusion to occur. Embryo cloning procedure requires 2 - 3hours from oocyte enucleation to blastomere fusion if a 30 - 40 cell donor embryo is used.

The cloned embryos are embedded into the ligated oviduct of a ewe for incubation for 5 to 6days, when surgically recovered about 13 - 15 % will be at the late morula or blastocyst stage during which they can now be transferred to recipient (surrogate) dam by normal ET technique.

7. **IN-VITRO FERTILIZATION**: This is a technique whereby immature oocytes (female eggs) are obtained from ovaries of infertile or aged females, or from regular embryo donors and fertilized with spermatozoa in the laboratory outside the animal body (*in-vitro)*. The procedure of ovum collection is non-surgical whereby ultrasound and a guided needle is used to aspirate immature oocytes from the ovaries. After collection, they are matured, fertilized and cultured *in vitro* for up to seven days until they develop to a suitable stage for transfer or freezing.

8. EMBRYO OR SPERM SEXING: This is a technique in which the sex of the foetus is determined through the manipulation of the sperm chromosome that is involved in the process of fertilization. This involves methods made to determine the sex of sperm or embryos so that farmers can control the sex of the offspring of their livestock. A beef producer will prefer male calves which tend to have higher body weights and higher feed efficiency (compared to female or heifer calves) when placed in feedlots for growing/ finishing stages of meat production. In contrast, a dairy farmer will prefer heifer calves that will ultimately produce offspring/ milk for the market. Hence the need to determine the sex of sperm or embryos in order to control the sex of the offspring. Through the use of a specific dye that binds to DNA (Hoechst 33342) and a flow cytometer/ cell sorter, the DNA content of individual sperm is measured, (X-bearing sperm contain 3.8% more DNA than the Y-bearing sperm in cattle). In mammal the presence of a Y-chromosome (and one X chromosome) determines that the individual will be a male, while females contain 2 X- chromosomes. Although the process to sort the X and Y bearing sperm is slow (approximately 10 million live sperm of each sex can be obtained per hour i.e. about the number of live sperm required for one conventional dose of frozen semen for AI)it determines sex with more than 95% accuracy.

FISH HATCHING TECHNOLOGY

Identification of male and female fish

i. The female catfish particularly the one with eggs is easily identified because it has a bigger stomach compartment(protruding) while the male has a smaller compartment.

ii. The genital (sex organ) of the female is round while that of the male fish is long.

Identification of suitable broodstock (spawners)

In identifying the female catfish suitable for hatchery purposes, you may discover those of 100g body weight are having enough eggs but it is preferable using those with eggs of 400g body weight and above.

Make sure that the female catfish is up to 8 months old (their eggs must be between dark green and golden in colour) in order to ensure better results.

Note that the older and bigger the broodstocks, the higher the proportion of shooters you will get from the fingerlings population.

As for the male fish, the genital which is located immediately below the anus must be longer than the upper end of the anal fin.

Furthermore, you can also test for maturity of fish suitable for breeding using the colour of their genitals. Check if their genitals (male and female) are reddish in colour. If they are, then they are suitable.

DIAGRAM DIFFERENTIATING MALE AND FEMALE GENITALS

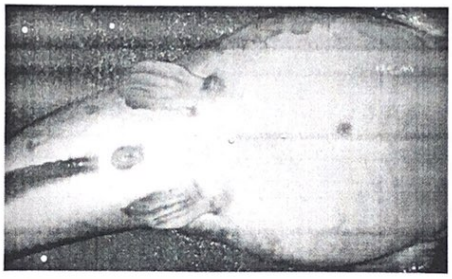


FIGURE 121: showing *the female genital and the protruding belly.*

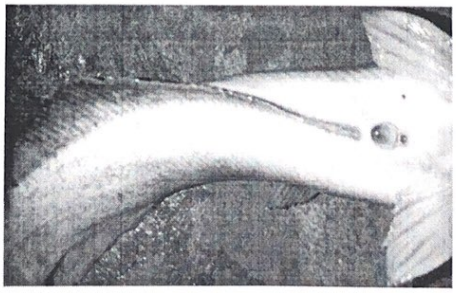


FIGURE 122: *showing the male genital papillae.*

Care for the broodstock fish

Keep the catfishes meant for the hatchery purposes separately in different plastic containers filled with good water and cover with aerated lid and supported with a heavy object like stone. If you do not do this, they will injure themselves and injury can affect the hatchery results.

Types of hormones

Any of the following can be used to inject the female fish:

i. Pituitary gland
ii. Ovaprim
iii. Ovatide
iv. Human choronic gonadotropin (HCG) etc.

Hormone administration

The pituitary gland can be extracted from either a male or female catfish whether up to six months or not. But the body weight of the fish (donor) whose pituitary gland you are using must be slightly more than that of the female fish (recipient) you are injecting. As such, you can use one or two fishes whose weight is slightly more than that of the female fish as donor. The injection dose is 2ml of syringe of the grinded pituitary gland and saline solution.

Requirements:

1. Sharp knife
2. Matured male and female catfish
3. Handkerchief / hand-towel
4. Tissue paper
5. Syringe (2ml / 5ml) and needle
6. Cutter
7. Small mortar and pestle (or use water bottle cover and syringe head)
8. Saline solution
9. Weighing scale
10. Clean water
11. Plastic buckets

Procedures:

- Cut off the head of the donor catfish and remove the lower soft part of the head gently so as not to break the skull of the fish. Thereafter, dress the skull gradually removing all the succulent parts and then use the cutter to prize open the brain compartment where the pituitary gland is located and extract the gland carefully using pincers or the tip of the knife.
- Put the gland into the mortal (glassware) or water bottle cover.
- Take your syringe and draw 2-3ml of the saline solution. Thereafter, use the syringe head to grind the pituitary gland or with mortar and pestle.

- Put the saline solution in the syringe into the grinded pituitary gland and mix together with the syringe head or pestle before finally drawing with syringe all available liquid from the gland mixture and adjust it to 2ml before injecting the fish.

In case you are using ovaprim or HCG, use a syringe dosage of 0.2ml for fish weighing 500g and less. As for fish weighing 600g and above, use a dosage of 0.5ml of syringe.

How to inject the female fish

With the syringe, hold the dorsal fin of the female fish and the injection is given either between the base of dorsal fin and lateral line or between the anal fin and lateral line (intra-muscular) at angle of 45°.After this, place the fish in any plastic water container like plastic bucket. Half-fill it with good water and cover it carefully with an aerated lid. Secure the lid with a heavy stone so as to prevent the fish from jumping out of the container.

Thereafter, wait for 10-12 hours before stripping. This period of waiting is known as **latency periodor time** i.e. it is the period (time) between when the fish is injected and when it is ready for stripping or stripped.

Milt extraction from the male fish

- Get the male fish already selected for the hatchery purpose.
- Cut off its head (optional) and slit the belly vertically with a sharp knife in order to expose the stomach compartment.
- Remove the fatty substance / tissue and the intestines. After these, you will see the milt (sperm) attached to the flesh.
- Remove and clean it carefully using tissue paper to remove blood and other stains on it.
- Wrap it in a clean tissue paper.

Stripping of the injected female fish

Catfishes are ready for stripping 10-12 hours after injection.

- Get a dry semi-deep small sized plastic plate, remove the female fish from the latency container and begin to strip the eggs into the plastic plate, gently massaging the belly of the female fish.
- Stop stripping as soon as you noticed blood coming out of the female fish.

Egg fertilization (artificial insemination)

Take the milt, slit it vertically with a sharp new razor blade and allow the milky liquid to drop on the eggs gently. Then rinse it by dropping some drops of saline solution over slit milt and allow it to drop over the eggs by spreading it gently over the eggs in the plastic plate. Shake the eggs while dropping water over it gently and gradually for about 80seconds.

Incubation of fertilized eggs on the egg mat

Gently spread the eggs over the incubator's spawning mat evenly, and then begin to run the water inlet and outlet and wait for 24-30 hours for the eggs to hatch on their own. This period of waiting is known as the ***incubation period or time i.e. it is the period (time) between when the fish eggs are spread on the spawning mat and when they hatch into larvae.***

CHAPTER FOURTEEN
OSTEOLOGY

Osteology is the study of bones. Skeleton, which includes bones, cartilage, ligaments and connective tissues, provides the basic scaffolding for the body (Constantinescus and Constantinescus, 2004).

Functions of the skeletal system

1. Support it provides the structure to which muscles attach, as well as the structural support for the entire body.
2. Protection it protects vital organs of the body. The ribs surround the delicate lungs and heart while the brain is encased in the skull and the spinal cord in the vertebra column.
3. Blood production (haemopoiesis) bone marrow is the site of blood cells formation.
4. Storage of minerals and lipids it provides a major storage for various minerals particularly calcium as well as contain a substantial amount of lipid.
5. Leverage joints act as levers to assists with movements.

Bones can be classified into five (5) categories i.e. long bones, short bones, flat bones, irregular bones and sesamoid bones.

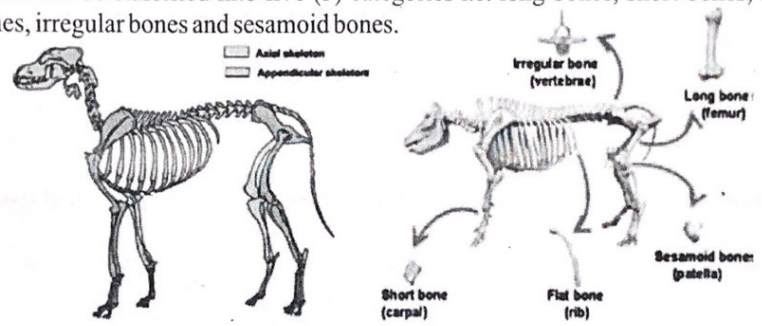


Figure 123.0: Structure of an animal showing type of skeleton and the five categories of bones

- **Flat bones** are longer than they are wide and characterized by an elongated shaft and enlarged ends that bear articular surfaces e.g. humerus, radius, femur, tibia, metacarpals, and metatarsals.
- **Short bones** are thin and flattened, and are made up of two plates of compact bone separated by cancellous or spongy bone e.g. sternum, ribs, scapula and certain skull bones.
- **Irregular bones** are complex irregularly shaped bones e.g. vertebrae and certain facial bones.
- **Sesamoid bones** are small bones embedded in a tendon and shaped like sesame seed e.g. patella, proximal and distal sesamoid bones of the digits.

Structure of the bone (Gross anatomy): A bone can be divided into two components i.e. compact bone and cancellous bone (Fig. 122.0). **Compact,**or **dense,** or **cortical bone**is a solid looking structure found on the surfaces of bones where they form a protective outer coating. **Cancellous** or **Spongy bone** is found on the interior and is made up of an irregular lattice network of bone (trabeculae or spicules) interspersed with spaces that are filled with red or yellow bone marrow. Cancellous bone is most common in the short, flat and irregular bones as well as the epiphyses of long bones and narrow lining of medullary cavity of the diaphysis of long bones. The epiphyses consist mostly of cancellous bone with a thin outer coat of compact bone. The medullary cavity (medulla "innermost part") is the space in the diaphysis containing bone marrow; unlike compact bone it does not contain osteons. The joint surface of bone has an articular surface made up of a smooth layer of hyaline cartilage covering the epiphysis where one bone forms a joint with another. The fibrous covering (contains nerve fibres, lymphatic vessels and blood vessels) surrounding the part of the bone that is not covered with the articular cartilage is called **periosteum** (made up of dense irregular connective tissue). Its innermost layer consists of an osteogenic layer containing **osteoblasts** (bone germinators) that make new bone and **osteoclasts** that breakdown bone. The periosteum is attached to the underlying bone by Sharpey's fibres extending from the fibrous layer into the bone matrix. There is a high density of Sharpey's fibres where tendons and ligaments attach to the periosteum. The endosteum lines the medullary cavity in long bones and covers the trabeculae of spongy bone. Osteocytes are found in lacunae within the trabeculae, and canaliculi radiate from the lacunae. There is variation in the proportion of compact and cancellous bones present in the short, irregular and flat bones. There is also the absence of shaft or epiphyses, although they contain bone marrow between their trabeculae, they have no bone marrow cavity. The internal spongy layer in flat bone is called diploë (folded).

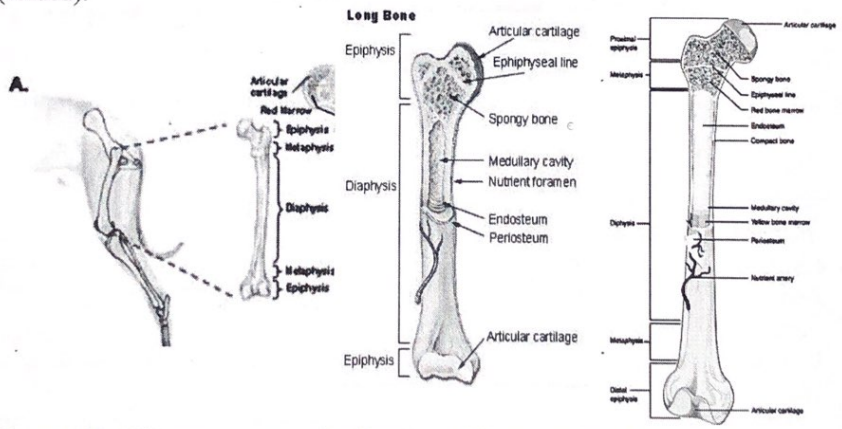


Figure 124.0:(A)Femur as an example of long bone; (B) cross-section of an equine humerus showing exterior and interior anatomy

Microscopic anatomy of bone: Bones have four (4) major cell types Osteocytes, Osteoblasts, Osteoclasts and Osteoprogenitor or stem cells.

- **Osteocytes** are the mature cells within a lacuna of bone that makes up most of the bone cells.
- **Osteoblasts** are cells that secrete the extracellular matrix on bone as well as collagen and ground substance that makes up unmineralized bone (osteoid) which later become embedded within the matrix to form osteocytes (Fig. 124.0).
- **Osteoclasts** are giant multinucleated cells involved in resorption of bone and are therefore present in areas where bone is being removed. The breakdown of bone by osteoclasts is called bone **resorption**. Osteoclasts bind tightly to either the endosteum or periosteum to form a leak-proof seal. It releases lysosomal enzymes and acids into this sealed region which digest collagen fibres and organic matrix while the acid digests the minerals. The digested components are engulfed by osteoclasts (endocytosis), packaged into vesicles, translocated across osteoclast (transcytosis) and released into the interstitial space (exocytosis) where the material is absorbed into the capillaries. The two hormones involved in bone formation (calcium homeostasis) are parathyroid hormone (from parathyroid gland) and calcitonin (from thyroid gland). Parathyroid is released in response to low plasma calcium level while calcitonin is released when plasma calcium levels rise. Increased resorption cause the bones to get weak as in osteoporosis while increased deposition of calcium lead to development of Spurs. Oestrogens help to reduce bone resorption while parathyroid hormone promotes bone resorption. The decrease in oestrogen level associated with menopause is linked to the weakening of bones. Bone deposition requires vitamin C for collagen synthesis, vitamin D for calcium absorption in the gut, and vitamin A for bone deposition and removal, in addition to calcium, phosphorus, magnesium, and manganese.
- **Osteoprogenitor or Stem-cell** are mesenchymal cells that can produce osteoblasts located in the inner cellular layer of the periosteum that lines the marrow cavity(endosteum), and the lining of vascular passage way in the matrix, they are important in fracture repa

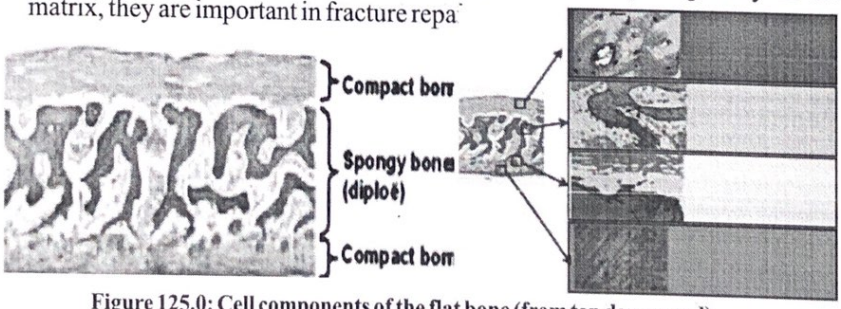


Figure 125.0: Cell components of the flat bone (from top downward) showing Osteocyte, Osteoblasts, Osteoclast and Osteoprogenitor cells

Composition of bones: Bones are made up of both organic and inorganic components. The major inorganic component is Calcium-phosphate, $Ca_3(PO_4)_2$ which makes two-third of bone weight. $Ca_3(PO_4)_2$interacts with Calcium hydroxide, $Ca(OH)_2$ to form hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. The formation of crystals of hydroxyapatite incorporates other inorganic materials such as calcium carbonate, sodium, magnesium and fluoride. The organic portion is made up of cells (osteoblasts, osteocytes and osteoclasts) and osteoid (includes collagen fibres and ground substance i.e. proteoglycans/ glycoproteins). The osteoid is secreted by osteoblasts.

The haemopoietic (blood forming) portion of bone is the **red bone marrow** (consists of mature/immature RBC, WBC and stem cells) found in spongy component of the long bones and the diploe of flat bones. The medullary cavities of spongy-bones contain red bone marrow in newborns but in adults, the medullary cavities of spongy bone of the long bones are fat-filled with **yellow-bone marrow** which extends into the epiphysis. Yellow marrow functions in fat-storage, and contains mostly fat cells. Therefore blood cell production in adult long bones is restricted to the head of the femur and humerus. The yellow bone marrow can transform into a red bone marrow if an animal is anaemic inorder to supplement RBC production. In contrast, the spongy components of flat-bones found in the hips remain haematopoietic and a good source of bone marrow for samples. Blood cell formed in the marrow enters circulation through the osteonal/ lateral canals. Venous vessels running through these channels are connected to the sinuses of the bone marrow making it easy for newly formed blood cells to be released into them, from where they enter the general circulation. While bone may appear dormant after adulthood, but in actual sense it is very active with constant breakdown (resorption) and replacement in response to various physical or hormonal changes (remodeling).

The process of bone formation is called **osteogenesis** or **ossification**. **Calcification** (process of calcium salt deposition) occurs during ossification, though calcification is associated with bone formation, it can occur in other tissues. Bone formation occurs generally in two ways, they are **intramembraneous ossification** and **endochondral ossification**.

- **Intramembraneous ossification** occurs when bone develops from a fibrous membrane. Early in embryonic development, elongated mesenchymal cells (messenchyme is tissue from which connective tissue develops) migrate and aggregate in specific regions of the body, condense and form the membrane from which the bones develop. They become more vascularized, enlarged and rounder with time. It differentiate into osteoblasts as the mesenchymal cells change from eosinophilic (stained with eosin dyes) to basophilic (affinity for basic dyes) cells.These cells secrete the collagen/proteoglycans (osteoid) of the bone matrix. Increased deposition of the osteoid makes the osteoblasts become increasingly separated from one another,

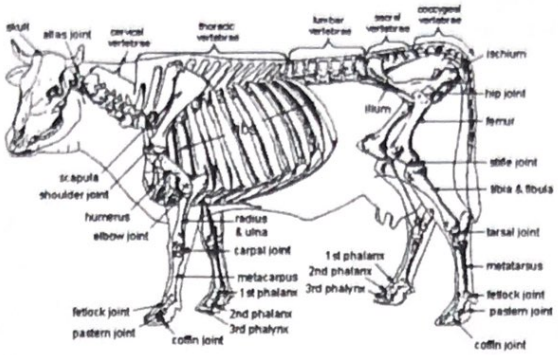
although they remain connected by thin cytoplasmic processes. The flat bones of the skull/ face, mandible, clavicle (if present) and bones in abnormal locations such as testes or whites of the eyes (heterotopic bones, *hetero* different; *topos* - place) are formed by this method. If cartilage serves as the precursor for the bone, the formation is called endochondrial ossification, though the initial bone laid down by either method is eventually replaced as a result of later remodeling that occurs later. **Remodeling** is the constant breakdown of bone by osteoclasts and its reformation by osteoblasts which occur at both the periosteal and endosteal surfaces. The area where calcification starts in the matrix is called the ossification centre. As the matrix becomes calcified, osteoblasts changes to osteocytes (contained in canaliculi). The surrounding primitive cells in the membrane proliferate to give rise to osteoprogenitor cells which also changes to osteoblasts when it comes in opposition to the spicules, thereby increasing the matrix. This results in appositional growth in which the spicules (areas of calcification extending from the ossification centre) enlarge and become joined into a trabecular network having the shape of bone

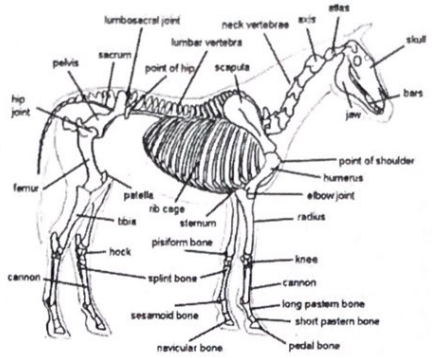


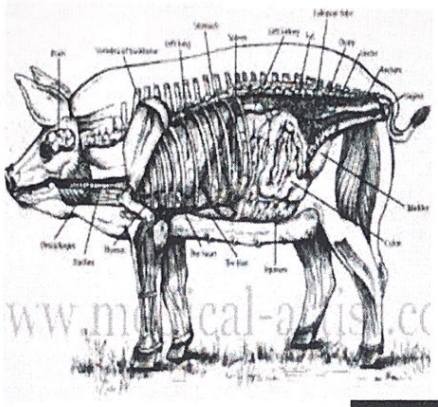
Figure 126.0: (A) Mesenchymal cells (Osteoblast (top), Osteocyte (left) and condenced mesenchyme (right) indicated by black line; (B) Ossification centre (top) and marrow (down) indicated by yellow arrow.

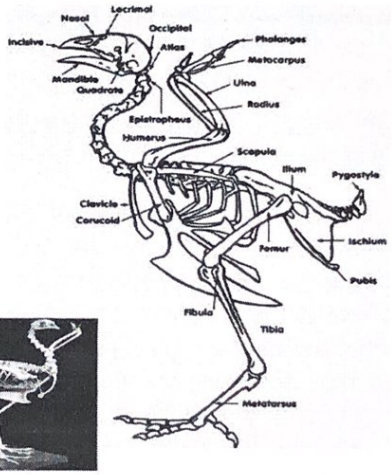
- **Endochondral ossification** begins in a similar way to intramembraneous ossification with the mesenchymal cells migrating and aggregating to become chondroblasts (instead of osteoblasts as in intramembraneous ossification) which begin to make a cartilage matrix. The cartilage matrix grows by both interstitial and appositional growth. The interstitial growth is responsible for the increase in length while the increase in width is due to the production of new chondrocytes that differentiate from the chondrogenic layer of the perichondrium surrounding the cartilage mass.

  Cervical vertebrae thoracic vertebrae lumbar vertebrae sacral vertebrae alanges.









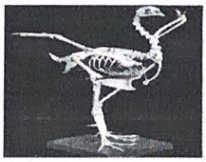


Figure 127.0: Skeletons of Cow, Horse, Pig and Chicken

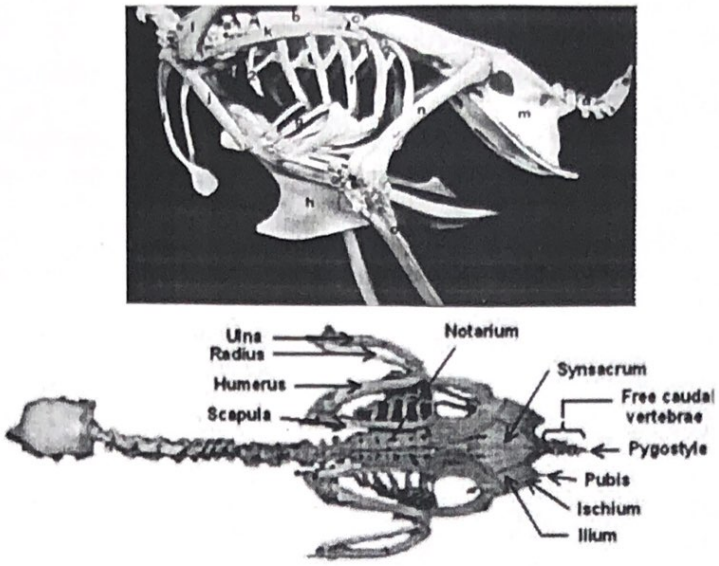


Figure 128.0: Skeleton of chicken (Lateral view) showing cervical vertebrae (a), notarium (b), 4th thoracic vertebrae (c), free caudal vertebrae (d), pygostyle (e), vertebral rib (f), sterna rib (g), sternum (h), clavicle (i), coracoids bone (j), scapula (k), humerus (l), pelvis (m), femur (n), tibia/fibula (o), floating rib (2), and uncinate processes (1) of the vertebral ribs which provides attachment for muscles/ligaments and strengthen thoracic wall.

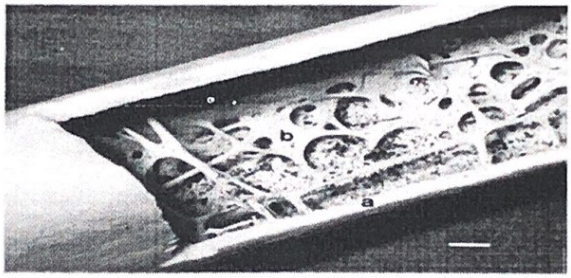


Figure 129.0: Chicken humerus showing cortex (a) which is exceptionally thin and the medullary cavity (b) contains a network of trabeculae which increases the strength of the bone. The thin and brittle nature of the outer shell makes the bone unsuitable for plating in the treatment of fractures, while intramedullary pinning would have the effect of destroying the internal strutting. Many of the bones of the post-cranial skeleton are pneumatized by diverticula of the air sacs which invade the medullary spaces of the spongy bones where they replace the bone marrow. In female birds, prior to the laying season, a cancellous type of bone, medullary bone is laid down in the medullary cavity of the long bones under the influence of oestrogens and androgens. During the egg laying cycle this bone is alternately undergoing formation and destruction and, along with the diet, acts as a source of calcium for the manufacture of the egg shell. The presence of medullary bone increases the weight of the chicken skeleton before laying by approximately 20 percent (scale, 2mm)

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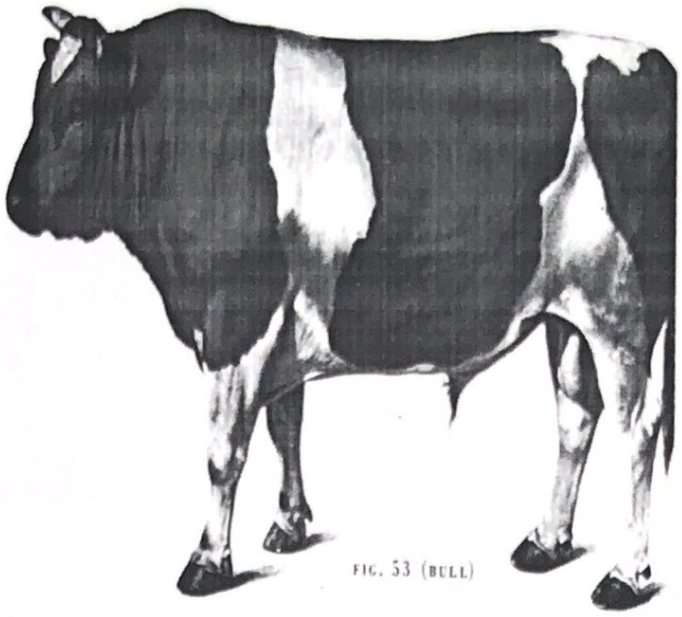


FIG. 53 (BULL)

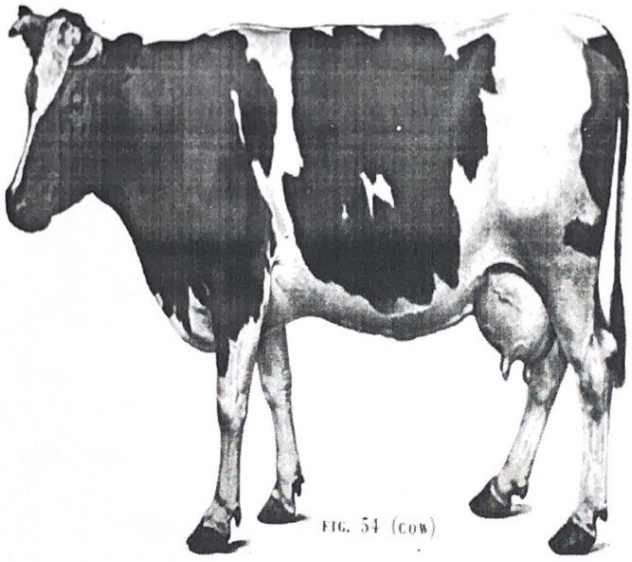


FIG. 54 (COW)

Appendix

NORMAL LABORATORY VALUES

It should be noted that normal values of laboratory data are offered as a guide or reference and that the values may vary depending on the sex, age and geographical location or habitat of the animal and on the laboratory.

Appendix 1: Haematology and biochemical values of Small ruminants' species in Nigeria.

Parameters	WAD goats (Range)	WAD goats (Mean±SE)	Red Sokoto goat (Mean±SE)	WAD Sheep (Mean±SE)	WAD bucks (Mean±SE)	WAD doe (Mean±SE)	WAD buck-kid (Mean±SE)	WAD doe-kid (Mean±SE)
PCV,%	21-35	29.4±0.9	25.7±3.1		22.1±0.5	31.3±2.1	25.3±1.2	30.0±2.1
Hb, g/dl	7-15	9.8±0.3	11.4±1.6		7.8±0.1	10.3±1.7	7.6±0.3	7.9±0.1
RBC,x10^6/µL	9.2-13.5	11.5±0.4	10.9±2.1		9.8±0.3	10.8±1.0	10.2±1.1	10.1±0.4
MCHC,%	32-34.6	33.1±0.1	44.7±8.2		37.8±1.4	33.9±0.2	32.1±1.4	32.8±0.6
Total WBC,x10^3/ µL	6.8-20.1	13.5±0.8	10.6±2.8		10.1±0.2	14.0±3.9	13.1±1.4	13.5±0.3
Lymphocytes, %	47-82	65.8±1.1	51.6±3.0		68.8±1.2	56.6±2.1	73.0±2.0	67.9±1.0
Neutrophils,%	17-52	33.5±1.7	36.4±2.5		31.2±1.2	42.9±1.3	26.7±2.2	30.7±0.1
Eosinophils,%	1-7	0.8±0.2	3.9±1.5		-	0.5±0.3	0.3±0.1	1.4±0.5
Monocytes,%	0-1	0.1±0.0	7.4±1.7		-	0.4±0.2	0.2±0.1	0.2±0.1
Calcium,mmol/L	1.15-2.4	1.6±0.1	-	9.6±1.6	2.2±1.1	1.5±0.3	1.8±0.2	1.8±0.4
Phosphorus,mmol/L	0.58-4.5	2.4±0.2	-	5.7±3.2	-	2.6±0.2	2.3±1.4	2.7±1.4
Potassium,mmol/L	3.0-6.0	4.8±0.1	5.3±1.8	5.3±1.6	6.5±0.3	4.3±0.1	5.3±0.1	5.2±0.1
Sodium,mmol/L	124-146	135.1±1.7	138.0±0.6	138.8±5.2	140.3±1.3	131.0±1.0	138.0±0.1	138.1±1.0
Chloride,mmol/L	-	-	-	-	103.3±0.1	-	-	-
Magnessium,mmol/L	-	-	-	-	1.1±0.4	-	-	-
Carbonate,mmol/L	-	-	-	-	18.9±0.6	-	-	-
Glucose,mmol/L	-	-	-	-	3.1±0.2	-	-	-
Urea,mmol/L	0.8-9.7	2.7±0.3	4.7±2.1	4.2±1.4	1.8±0.6	2.6±1.2	1.4±0.4	3.7±0.6
Total Protein,g/100ml	6.3-8.5	7.1±0.1	4.4±1.5	6.3±0.7	6.2±0.3	70.6±1.2	70.0±1.3	71.3±0.3
Albumin,g/100ml	2.8-4.3	3.4±0.7	2.5±1.8	2.5±0.3	2.4±0.1	33.1±1.1	29.7±0.5	35.7±0.4
Cholesterol,mmol/L					3.2±0.1	-	-	-
Triglyceride,mmol/L	0.16-1.6	0.4±0.1	-	-	-	0.5±0.2	0.3±0.4	0.4±0.1
SGPT,IU/L	2–22	8.9±0.9	-	10.0±1.1	5.0±3.2	9.1±1.4	10.6±0.1	8.3±0.5
SGOT,IU/L	12–38	20.9±1.2	-	67.9±4.9	27.7±9.4	24.3±1.4	17.3±0.3	19.7±0.2
ALP,IU/L	1.4-25.7	10.7±1.2	3.1±2.8	10.7±7.3	24.0±26.9	11.9±0.1	10.2±0.1	9.9±0.8

Sources: Adapted from Oduye and Adadevoh, 1976; Tambuwal *et al.*, 2002; Daramola*et al.*, 2005; Fatoba, 2013 and Okukpe, 2014

Appendix 2: Some physiological parameters of some animals

Parameters	WAD buck	Cattle	Sheep/ Goat	Pig	Poultry
Body weight, Kg	6.91				
Pulse rate, beat/min	94.49	60 – 80 or 100-120 for young calves	70-90		
Respiratory rate,beat/min	71.05				
Heart rate, beat/min	111.00				
Rectal temperature, °C	39.32	38.5	39.0	39.0	40.6-41.7

Appendix 3: Haematology and biochemical parameters of some animals in University of Saskatchewan

Parameters	Ox	Horse	Swine	Sheep
PCV, %	24.0-46.0	32.0-53.0	32.0-50.0	27.0-45.0
Haemoglobin, g/dl	8.0-15.0	11.0-19.0	10.0-16.0	9.0-15.0
RBC, $x10^6/\mu l$	5.0-10.0	6.8-12.9	5.0-8.0	9.0-15.0
MCV, fl	40.0-60.0	37.0-58.5	50.0-68.0	28.0-40.0
MCH, pg	11.0-17.0	12.3-19.7	17.0-21.0	8.0-12.0
MCHC, g/dl	30.0-36.0	31.0-38.6	30.0-34.0	31.0-34.0
WBC, / µl	4000-12000	5400-14300	11000-22000	4000-12000
Thrombocytes, $x10^5/\mu l$	1.0-8.0	1.0-3.5	3.2-5.2	2.5-7.5
Neutrophils (mature)	600-4000	2260-8580	3080-10450	700-6000
Neurophils (bands)	0-120	0-1000	0-880	Rare
Lymphocytes	2500-7500	1500-7700	4290-13640	2000-9000
Monocytes	25-840	0-1000	200-2200	0-750
Eosinophils	0-2400	0-1000	55-2420	0-400
Basophils	0-200	0-290	0-440	0-300
Plasma proteins, g/dl	6.0-8.5	5.8-8.7	6.0-8.0	6.0-7.5
Fibrinogen, mg/dl	300-700	100-400	100-500	100-500

Appendix

Biochemical				
Sodium, mmol/L	132-152	132-146	135-150	139-152
Potassium, mmol/L	3.9-5.8	2.4-4.7	4.4-6.7	3.9-5.4
Chloride, mmol/L	95-110	99-109	94-106	95-103
HCO_3^-, mmol/L	24-33	23-32	18-27	20-27
Calcium, mg/dL	8.0-11.0	11.2-13.8	8.6-11.0	8.2-11.2
Phosphorus, mg/dL	4.0-7.0	3.1-5.6	6.7-9.3	4.0-6.0
Magnessium, mg/dL	2.0-3.5	2.1-3.5	2.7-3.7	2.2-3.0
Urea, mg/dL	6.0-27	10-20	8.0-24	8.0-20
Creatinine, mg/dL	1.0-2.7	1.2-1.9	1.0-2.7	1.2-1.9
Glucose, mg/dL	35-55	60-100	65-95	35-60
Cholesterol, mg/dL	50-230	60-230	152-154	-
Bilirubin, mg/dL	0-1.4	0.2-4.0	0-0.2	0-0.4
SGOT, iu/L	50-150	120-300	-	-
CPK,iu/dL	10-30	10-30		
SDH, iu/L	0-15	0-5		
Protein, g/dL	6.0-8.5	5.8-8.7	6.0-8.0	6.0-7.5
Albumin, g/dL	2.1-3.6	2.9-3.8	3.4	2.96
□-Globulin, g/dL	0.7-1.2	0.7-1.3	1.5	0.28
ß-Globulin,g/dL	0.6-1.2	0.4-1.2	1.1	0.45
y-Globulin, g/dL	1.6-3.2	0.9-1.5	1.4	1.30

*Source:*Veterinary haematology, Schalm, Jain and Carroll (1975) and Clinical biochemistry of domestic animals, Kaneko and Cornelius (1970).

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