ANATOMICAL STUDY OF THE POST-NATAL DEVELOPMENT OF MALE GENITAL SYSTEM OF PATI DUCK (*Anas platyrhynchos*) OF ASSAM

A Thesis
Submitted to the
Assam Agricultural University

In partial fulfillment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

IN

VETERINARY ANATOMY AND HISTOLOGY

By

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Dedicated
to My
Beloved family

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ASSAM AGRICULTURAL UNIVERSITY
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CERTIFICATE I

This is to certify that the thesis entitled **"ANATOMICAL STUDY OF THE POST-NATAL DEVELOPMENT OF MALE GENITAL SYSTEM OF PATI DUCK (*Anas platyrhynchos*) OF ASSAM"** submitted to the Faculty of Veterinary Science, Assam Agricultural University, in partial fulfillment of the requirements for the Degree of **DOCTOR OF PHILOSOPHY** in **VETERINARY ANATOMY AND HISTOLOGY** is a record of research work carried out by **Dr. ELIZABETH VL HMANGAIHZUALI**, under my personal supervision and guidance.

All kinds of help received by her have been duly acknowledged.

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Institutional Animal Ethics Committee
AAU, Khanapara

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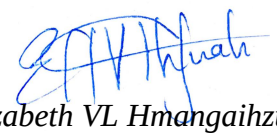

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study was undertaken to elaborate certain gross anatomical, histomorphological, histochemical, ultrastructural, haematological and serum biochemical aspect of male genital organs of Pati duck (*Anas platyrhynchos*) of Assam during the postnatal development. Total 30 (thirty) numbers of apparently healthy Pati ducks (*Anas platyrhynchos*) were utilized for present study.

The testis of Pati duck (*Anas platyrhynchos*) was located within the abdominal cavity. The organ was elongated rice-grain like in 1 month which changed to oval shaped in 20 weeks and bean shaped in 30 and 40 weeks. The epididymis was found on the dorso-medial aspect of testis. The epididymal duct of the testis continued as Vas deferens. The convoluted vas deferens tightly coiled in a zingzag pattern till 20 weeks and the convolutions loosened in 30 and 40 weeks. The vas deferens was translucent in 1 month and 6-8 weeks whereas in 30 and 40 weeks they were opaque white with presence of spermatozoa in the lumen. The phallus coiled in anti-clockwise direction from the base to the apex. The ejaculatory groove and sulcus divide the shaft into two lateral bodies. The length increased with age.

The testis of Pati duck (*Anas platyrhynchos*) had a capsule which had three parts *viz.*, tunica serosa, tunica albugenia and tunica vasculosa. The thickness of the capsule of the testis gradually increased along the advancement of the age i.e. from 1 month to 40 week age group. The collagen, reticular, elastic and nerve fibers were observed within the capsule and as well the peritubular area of the seminiferous tubules. The thickness of the capsule and distribution of all the fibers increased along with the advancement of the age i.e. from 1 month to 40 week age group.

The parenchyma of the testis of the Pati duck (*Anas platyrhynchos*) consisted of complex and convoluted seminiferous tubules separated by interstitial connective tissue. No lobulation and mediastinum testis. The diameter as wells as layers of cell of the ST increased with age. One month and 6-8 weeks birds semiferous tubules were mainly composed of Sertoli cells, spermatogonium cells and vacuolated cells. In 20 weeks the cells were 3 to 5 layers consisting of spermatocyte along with other cells. The ST of 30 and 40 weeks age groups had 8 to 17 layers of cells formed by different stages of spermatogenesis. The interstitial connective tissue decreased with increased in age. The epididymal region consisted of rete testis which was intracapsular and extracapsular, efferent duct with smooth and folded epithelium, collecting duct and epididymal duct having the same epithelial lining. The vas deferens diameter increased with age. Smooth epithelium at the cranial part and folded epithelium at caudal part. The phallus has a narrow lumen which was surrounded by a very large lymphatic space and vascular body.

In the present histochemical study of male genital organ of Pati duck, the reaction of Alkaline Phosphatase enzyme decreased with age in the testis, moderate in the vas deferens and intesnse in the phallus. The reaction of the Acid Phosphatase moderate in the testis and vas deferens of all age group, while phallus had intense and moderate activity area. The Adenosine Tri Phosphatase (ATPase) activity increased with increased in age in the testis, weak activity in the vas deferens and phallus with intense and weak activity area.

Under TEM two types of leydig cells *viz.,* elongated and polygonal shaped was found, they contain numerous lipid droplets along with mitochondria and endoplasmic reticulum. Sertoli cell had large and irregularly shaped nucleus which had intranuclear cleft. The prominent nucleoli of sertoli cell nucleus had a very dense and moderately dense area. In the peritubular space layers of overlapping myoid cells was found. Within seminiferous tubules cellular detailed of spermatogenic cells were observed.

Age related change observed with Testosterone hormone which increased with increased in age. T_3 and T_4 hormones were higher in younger age while Cortisol was higher in older groups. Among the haematological parameters significant changes was found in PCV, WBC, monocyte and neutrophils. ALP was the only serum enzyme which showed significant changes between age groups. Serum metabolites *viz.,* total protein, albumin and creatinine showed significant changes among the differentage group.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

ABBREVIATION		FULL FORM
%	……	Percent
µ	……	Micron
µg	……	Microgram
µm	……	Micrometer
ACPase	……	Acid phosphates
ALP	……	Alkaline Phosphatase
ALT	……	Alanine Transaminase
AST	……	Aspartate Transaminase
ATPase	……	Adenosine Tri Phosphatase
BUN	……	Blood Urea Nitrogen
Etc.	……	Etcetra
Hb	……	Haemoglobin
ICAR-NASF	……	Indian Council of Agricultural Research – National Agricultural Science Fund
i.e.	……	That is
LDH	……	Lactate Dehydrogenase
M	……	Million
mg	……	Milligram
mm	……	Milimeter
nMol	……	Nano Mole
^{o}C	……	Degree Centigrade
PCV	……	Packed Cell Volume
RBC	……	Red Blood Cells
SE	……	Standard Error
T3	……	Triiodothyronine
T4	……	Thyroxine
TEM	……	Transmission Electron Microscopy
viz.	……	Namely
WBC	……	White Blood Cells

CHAPTER - I

Introduction

ANATOMICAL STUDY OF THE POST-NATAL DEVELOPMENT OF MALE GENITAL SYSTEM OF PATI DUCK (*Anas platyrhynchos*) OF ASSAM

CHAPTER-I

INTRODUCTION

Ducks are mostly aquatic birds which have a wide flat beak adapted for dredging. (TNAU Agritech Portal, 2009). In India ducks farming plays an important position next to chicken farming, and form about 10% of the total poultry population contributing about 6-7% of total eggs produced in the country (Bhadauria, 2005) where the Eastern and North-eastern parts of India comprise the major portion of the country's duck population (Mahanta, 2001).

Duck rearing plays an important role in the upliftment of the socio-economic condition of the rural population of Assam as they are very hardy bird and they need less care or management (Sinha *et al.,* 2015). The peculiar agro-climatic condition with marshy and waterlogged areas prevailing throughout the state provides a very congenial environment for rearing ducks. The 'Pati' duck population constitutes a major indigenous non-descript duck variety in the state of Assam as duck husbandry provides a source of income to the rural women folk (Deka *et al.,* 2014). Even under adverse conditions such as high rainfall, temperature, excessive humidity and poor housing, ducks exceed the best of laying strains of chicken in livability (Ola, 2000).

Pati is the non-descript indigenous duck variety of Assam and constitute about 85.6% of the total duck population in Assam (Islam *et al*., 2002). It was approved as a registered breed on 4th August 2017 by ICAR Breed Registration Committee with accession number *INDIA_DUCK_0200*_PATI_*11001* [National Bureau of Animal Genetic Resources, Karnal (Haryana), India. 2010] with the estimated population of about 18.21 lakhs. These are squat in posture. Plumage is dark brown in drakes with greyish black head; tail with black and white feathers. Ducks are solid brown. A white ring may or may not be present at neck in both sexes. The bill, shank and feet are predominantly yellow. Pati ducks are used for meat, egg and ritual sacrifices. The average body weight is 1.58 kg.

Birds have the most variable genital morphology of any amniote group (Herrera *et al*., 2014). This variation ranges from the largest penis for a given body size in any

vertebrates (McCracken *et al*., 2001) to the complete absence of an intromittent penis in 97% of all avian species (Briskie and Montgomerie, 1997); (Brennan *et al*., 2008). The excurrent duct system of avian genital system mainly consist epididymal region adjacent to the testis and a long convoluted ductus deferens (vas deferens) which terminates in the large ejaculatory duct.

Avian do not have a penis such as is found in other animals. The morphological characteristics of the testes in the same species always change according to age and sexual activity cycle (Lin *et al.,* 1992) (Jones *et al.,* 1993). Their size is not constant and they become larger when the birds are actively mating. The reproductive cell continuously proliferate and at the same time it degenerated during the course of differentiation and development (Liu *et al*., 1996)

The testes of adult duck are two large bean-shaped, white and soft (El Jack, 1970) located against the backbone at the front of the kidney. The left testis is usually higher in position and large in size than the right one (King, 1975). In many birds, testes undergo dramatic annual changes in size anatomically and physiologically. Adult testicular function is modulated by a myriad of external factors and orchestrated by numerous hormones that together enable birds to adapt to and breed in diverse habitats worldwide (Deviche *et al.,* 2011). These factors have generated a wide range of avian reproductive strategies, which has further shaped testicular structure and function (Norris and Lopez, 2010).

Testis is a compound tubular gland with an exocrine and an endocrine functions (Deviche *et al.,* 2011). The exocrine function is the production of spermatozoa inside the seminiferous tubules. The endocrine function is the production of the male sex hormone, testosterone, by the specific interstitial (leydig) cell in the intertubular connective tissue.

Testes in birds are located deep in the abdominal cavity and are, therefore, visible only after removal of other organs, in particular the intestine. Testes are surrounded by a fibrous capsule that includes connective tissue and contractile fibers (Aire and Ozegbe, 2007). They contain interstitial tissue and seminiferous tubules, which are the site of spermatogenesis and, in developed testes, make up most of the testicular mass.

Interstitial tissue includes Leydig or interstitial cells, the main source of testicular androgens (Dufty and Wingfield, 1986). Seminiferous tubules of birds are different to those of mammals by forming highly and complexly anastomotic, non-blind-ending network of tubules and the lack connective tissue septa, as well as the absent of lobulation of the avian testis (Bailey, 1953; Marvan, 1969) while leydig cells of birds are generally similar, structurally, to those of mammals (Garnier *et al.*, 1973; Marchand, 1973) which scattered singly or in small groups and are chiefly found in the interlobular spaces (Hodges, 1974). and it seem to form columns of cells in the interstices in the most birds (Russel, 1996; Akingbemi *et al.* 1999) The interstitial tissue of the testis has been described in a number of mammals, but reports on this tissue in birds are rather scanty.

The excurrent duct system of avian genital system mainly consist epididymal region adjacent to the testis and a long convoluted ductus deferens (vas deferens) which terminates in the large ejaculatory duct. Groups of seminiferous tubules fuse together, losing their germ cell lining to form tubuli recti which opens into the rete testis (Hodges, 1974). The rete testis continues into the efferent duct (Budras and Sauer, 1975) where there is an abrupt change in epithelial cell height from the squamous rete cells to the columnar cells of the efferent ducts (Aire *et al.*, 1979). The connecting duct drains several efferent duct and joins the epididymal duct which is a single duct situated on the medial aspect of epididymis (Hodges, 1974). The epididymal duct continued as vas deferens. The paired vas deferens is tubular, convoluted and wavy in appearance, extending from caudal end of epididymis to the cloaca (Saleem *et al.*, 2017).

Although the birds reproduce by internal fertilization, only 3% of them possess a copulatory organ, known as phallus (Montgomerie and Briskie, 2007) and duck are among a few species of avian which have true intromittent phallus. The phallus is intromittent, eversible, with a blind tubular cavity which have two different regions, a fixed base of the phallus and a tubular portion (Previatto *et al.*, 2017).

Testicular secretions are necessary for the normal expression of aggressive behavior. During breeding not only does testosterone increase aggression, but aggressive interactions also increase plasma testosterone levels (Soma, 2006). Major functions of many steroid hormones, including testosterone, are conserved among vertebrates, but

circulating concentrations nonetheless vary widely across species (Hau, 2010). In vertebrates, the primary neural system responsible for regulating reproduction consists of hypothalamic neurons that secrete gonadotropin-releasing hormone (Deviche *et al*., 2011). Of the three GnRH forms identified in birds so far (Berghman *et al*., 2000), GnRH-I is considered the primary secretogogue of the gonadotropins-follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Kuenzel, 2000). Several studies have also demonstrated an important role of thyroid hormone in regulating seasonality of the male reproductive system (Lien and Siopes, 1991; Wilson and Rienert, 1993; Deviche *et al.*, 2011).

Most testicular tissue is devoted to spermatogenesis and testis size is, therefore used to estimate sperm production (Moller, 1988) and the increase and decrease in the diameter of the seminiferous tubules were considered as a parameter for assessing the spermatogenic activity (Simoes *et al*., 2016). Several studies have documented age-related variation in testis size, with older adults generally having larger testis than younger adults (Selander and Hauser, 1965; Morton *et al*., 1990; Hill, 1994; Deviche *et al.*, 2000; Graves, 2004; Laskemoen *et al.*, 2008). Testosterone in vertebrates is essential for spermatogenesis but the negative feedback effect of T on the hypothalamus and pituitary gland can also lead to suppressed testicular functions (Deviche *et al.*, 2011).

Improving the productivity of any animal necessitates the understanding of its physiology including hematological characteristics to establish diagnostic baselines of blood characteristics for routine management practices (Orji *et al.*, 1986). The determination of hematological and plasma metabolite levels may provide valuable information on the physiological state and form cornerstone of the medical diagnosis of diseases (Hauptmanova *et al*., 2006; Harr, 2002). Hematological constituents usually reflect the physiological responsiveness of the animal to its external and internal environments and thus serve as a veritable tool for monitoring animal health (Pascalonpekelniczky *et al.*, 1994).

In the present study, we attempted to find the morphological characteristics of testis and testicular cell by employing light microscopy, electron microscopy, and histochemistry. The morphological features of the Pati duck testes from 4 weeks to adult

will be examined to provide morphological evidence for the developmental biology, the comparative histology and the male reproductive biology, as well as to provide data for prevention of disease in Pati duck. These would help physiologist, pathologist and poultry scientists for effective disease control regime. The present investigation has been undertaken with the following objectives:

1. To study the gross anatomical characteristics of male genital system at different stages of development of Pati duck of Assam.
2. To study the histomorphological and histochemical characteristics of male genital system of Pati ducks.
3. To study the ultrastructure of male genital system of Pati duck.
4. To study the hormonal and haemato-biochemical parameters related to development of male genital system.

CHAPTER - II

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

2.1 GROSS ANATOMY

Hodges (1974) mentioned that the male reproductive system of fowl is rather simple consisting of paired testis, small epididymis, long coiled ducti deferentes opening into the urodeum of the cloaca via the ejaculatory ducts, and certain erectile organs within the cloaca, the lymph folds, vascular bodies and phallus which, together with the ejaculatory ducts form the copulatory organ of the fowl.

King (1975) mentioned that the testis of duck and goose were roughly in cylindrical form which increased in size enormously during sexual activity. He also added that the phallus was essentially a proctodeal structure on the left of ventral midline; the base tightly distended the whole cavity of the proctodeum and its spiral free ends projected ventrally and cranially from the vent.

King and McLelland (1975) mentioned that avian testis were bean shaped and were symmetrically arranged on either side of the midline, in the dorsal celom, near the caudal end of the lung and cranial end of the kidney. The left testis tended to be larger than the right till 6 months of age, after which the right testis became heavier during the reproductive period. The ductus deferens formed a tight zigzag and ran parallel with the ureter and connected with the dorsal wall of urodeum.

Hess *et al.* (1976) reported that the testis of turkey were large, soft and similar to the chicken testis. The white epididymal region was approximately 3 cm in length. It contained the rete testis, ductuli efferentes, connecting ductules and ductus epididymidis.

Nickel *et al.* (1977) mentioned that topographically the cranial end of the avian testis lay close to the ventral border of the lungs, while their caudal border lay cranio-ventral to the cranial divisions of the kidney

Moller (1988) reported that with the increase of body weight, the weight of the testis increased, as well as increase of ejaculatory volume with increase of sperm number per ejaculation in avian.

Mulder and Cockburn (1993) compared the development pattern and size of cloacal protuberances of male Superb fairywren differing in age and social status. They observed that protuberance size increased with body mass. Age, intragroup dominance, and pairing status did not influence the overall size of the protuberance, but old males had a larger tip on their protuberance. This prominent tip has not been reported in other species, and they speculate that it serves as an intromittent organ. Other birds with large testes and cloacal protuberances have high copulation rates, but copulation in Superb Fairy-Wrens is only very rarely observed. They propose that the cloacal protuberance and large testes of Superb Fairy-Wrens provide large sperm reserves primarily for extrapair copulations.

Kundu and Panda (1990) mentioned that the amount and quality of avian semen varies with seasons. The exposure to extreme temperatures could limit the reproduction of birds, and severe temperature fluctuations could often result in reduced production of spermatozoa.

McCreken (2000) observed that Argentine lake duck testis dimensions were 40.8 ± 3.6 × 17.8 ± 1.8 mm for the left and 41.0 ± 2.3 × 17.2 ± 3.1 mm for right testis. Mass of the two testis combined averaged 10.8 ± 3.1g or 1.7 ± 0.5% of total body mass.

Coker *et al.* (2002) reported that the size of the testes, length of intromittent organ, the height of the intromittent organ ridges and knobs increased significantly with the frequency of forced extra pair copulations.

Ghosh (2006) mentioned that the testicles of fowl were placed symmetrically on either side of the midline at the sub lumbar region in the abdomen. They were oval or bean shaped yellowish structures with the left generally larger. Epididymis were elongated and placed at the dorsomedial aspect of testis. Vas deferens was highly torturous and placed medial to the corresponding ureter which opened at the dorsal wall of cloaca.

Wei *et al.* (2006) reported that the position of male reproductive organs, their morphology and structure of the testes were club-shaped in African ostrich. They were asymmetrically situated on either side of the vertebrae. The penis had a penis gap

posteriorly for ejection of semen, which differ from the reproductive tract of mammals and the spiral penis gap of other fowls.

Aire (2007) reported that the testis were intra-abdominal, closely related to kidneys and were located on either side of the midline in birds. They were attached from their dorso-medial borders to the dorsal abdominal wall by a short mesorchium.

Brennan *et al.* (2007) reported that the length of phallus was 1.5 – 40 cm in male water fowl. It varies morphologically among species and were positively correlated with the frequency of forced extra-pair copulations.

Bull *et al.* (2007) mentioned that the testes were even organs, internally located, parallel to each other, displaced at the sides of the median line of the body, presenting rounded surface, with varied shape which were oval, elongated, curve, tortuous and, sometimes, with fine caudal extremity till 20^{th} week, and from the 21st week of age there was great increased in size. The extra testicular seminiferous path was made up bilaterally of the epididymis and ductus deferens. The epididymis was firmly attached to the corresponding testis on the dorsomedial face and continued with the ductus deferentia which were lateral to the ureters to open themselves through the ductus deferens papilla in the urodeum. The phallus was on ventral floor of the proctodeum.

Akers and Denbow (2008) mentioned that the paired abdominal testes were located anterior to the cranial lobe of each kidney in male birds. The vas deferens emerged medially and passes caudally alongside the ureters to the cloaca where it has common opening with the ureter in the urodeum.

Fails and Magee (2008) mentioned that testes were normally paired and located cranioventral to the kidneys in domestic birds. The ductus deferens conveyed sperm from the testis to the raised papilla on the lateral aspect of the urodeum. The phallus, a copulatory organ, lay on the ventral floor of the proctodeum in rooster and male turkey.

Dyce *et al.* (2009) mentioned that avian reproductive system consisted of paired testis, epididymides, vas deferens, and a single phallus, which was the copulatory organ. They also mentioned that the testis remained at the site of origin; spermatic cord, tunica vaginalis, scrotum, accessory reproductive glands and urethra were absent. The bean

shaped testes were relatively large and white during breeding season while they are yellowish and shrink to half the size during quiescent period. Attach by short mesorchia, they were placed symmetrically against the cranial end of kidneys, and related ventrally to the abdominal sacs, proventriculus, liver and intestines.

Brennan *et al.* (2010) reported that the average length of penis (19.23+0.70 cm) which had two distinct regions which differed in their curvature of the anti-clockwise coil in domestic Muscovy ducks. The basal region was tight coils approximate length 7-8 cm while the apical region with was more open coil in nature with approximate length 11-12 cm.

Razi *et al.* (2010) reported that the length of testes in turkey was 4-5 cm. their comparative observation revealed that the left testis was longer in most cases with significant decrease in the width than the right one in 24 weeks of age.

Zhang and Ren (2011) studied the male reproductive organs of ostrich and observed that the testes were similar, elliptical, and thicker towards the heart. They appeared off-white, and the vessels could be seen clearly on the surface. Testes of sexual mature male ostriches were mainly made up of the seminiferous tubules, which were formed like a web. Immature testes were as big as an adult's thumb and appear ivory. The weight and volume of testis increased during the breeding season. The color turned grey-white when the ostrich became sexually mature. The penis was divided into two parts, the base and the free hanging portion. The penis was a small club shaped and light pink in clolour at the age of 1 year.

Jacob and Pescatore (2013) noticed that the testis of male chicken were elliptical in shape and light yellow in colour. The vas deferens transported sperm from the testis to papilla of cloaca which served as mating organ

Herrera *et al.* (2014) observed that development of the genital tubercle is similar throughout the pre-hatching period in chicken and duck. They suggested that differences in penis morphology arose post-hatching and were controlled by steroids. In females, the genital tubercle underwent severe reduction due to a mass of apoptotic cells at the tip of the female genital tubercle and suggested that programmed cell death might play a role in

regression of the female phallus. In males, the tubercle continued to grow and elaborate, became thicker, pigmented and coiled.

Kadhem (2014) reported that the testes of adult duck were found suspended from its abdominal surface to the roof of the abdominal cavity by mesorchium which bore the blood and nerve vessels on its way. Each testis appeared elongated bean shape and was pinkish in colour. The mean average length of the duck testis was 1.48cm, and 0.47cm in width. Its weight was 1,54gm.

Kannan *et al*. (2015) studied the age related change in gross anatomy of Japanese quail and reported that the testis were yellowish in immature (6-8 weeks) birds and white in mature (7-40 weeks) birds which was retained in the abdominal cavity. The length and width of the testis increased from day old to 22 weeks of age.

Gerzilov *et al.* (2016) reported that the morphometric studied reflected the age and seasonal related differences which were logically associated in Muscovy duck. The weight and size of testes were the greatest during the reproduction period from the 8th to 12th month and at 24 months of age. At 18 months of age, their weight and size was similar to those in 4-month-old birds in Muscovy drakes.

Saleem *et al*. (2017) reported that the testicles were elongated bean shaped and creamy in appearance in adult local fowl of Uttarakhand. The left testis was larger and heavier, while the right one was smaller and lighter. The epididymis was located on the dorsomedial aspect of the testis. The anterior part was closely associated with the adrenal gland and was particularly extensive for left epididymis. The paired ductus deferens was convoluted and wavy in adult birds. It began at the caudal end of the epididymis and extended to the cloaca parallel to the respective ureter.

Abdul-Rahman *et al.* (2018) studied the age-related changes in the gross anatomy of the reproductive organs in male guinea fowls. Authors observed that, the testes and vas deferens were not discernible until about 4 weeks of age which was bean shaped and creamy in colour. Testicular weight increased significantly from 8 to 20, and between 24 and 28 weeks of age. Testicular height showed inconsistent growth pattern. Testicular

length showed similar growth pattern as width. Unlike other traits of the male reproductive organs, initial suspension of growth occurred much earlier in the vas deferens which increased significantly in length between 4 and 8 weeks, remained unchanged thereafter until puberty and increased again between 12 and 16 weeks of age. No change was recorded in the length of the duct thereafter.

Khatun *et al.* (2019) reported that the weight of the paired testis was 0.004 ± 0.009g at day one in Khaki Campbell duck old and gradually increased with the age and attained maximum weight (23.767± 0.804g) at 6 months of age, thereafter it decreased after 1 year of age. The mean length and width of the paired testis also increased gradually with age.

Kareem *et al.* (2020) reported that the indigenous Iraqi adult duck testis were elongated bean shaped which appear pink in colour. The two testes situated asymmetrically cranioventral to the kidney on each side of the midline of the body

2.2 HISTOLOGY

Davis *et al.* (1970) stated that the testis of most vertebrates was enclosed in a testicular tissue capsule through which blood vessels and nerves entered and left the substance of the organ. Histologically, the avian testicular capsule displayed an outer tunica serosa, a thick middle tunica albuginea and a poorly differentiated innermost layer of tunica vasculosa. The avian capsule was generally very thin.

Hodges (1974) mentioned that the avian testis was contained within a thin connective tissue capsule, the tunica albuginea which did not give off septa to divide the testis into lobules. The tunica consisted of fine collagen and elastic fibres with fibroblast. The seminiferous tubules formed a complex anastomosing network, whose epithelium lies on the basement membrane and surrounded by a thin layer of elastic fibres. The interstitial tissues were reduced to minimal amounts except in large interstices between the seminiferous tubules. Three types of tubules were found in the epididymis, the efferent duct, epididymal duct or connecting duct and epididymal canal. The vas deferens possessed a well-developed muscular wall enveloped by a dense layer of fibrous connective tissue.

King (1975) mentioned that the bulk in testis was formed by seminiferous tubules which were highly anastomosed with few interstitial cells occupying the space in between in fowl. The tubules were connected to the straight tubule which opened into the rete testis. The efferent ductules of epididymis were lined by ciliated pseudostratified columnar which opened to the epididymal duct.

Hess *et al*. (1976) reported that the rete testis consisted of squamous or low cuboidal epithelium channels which opened from the seminiferous tubules and abruptly change into columnar cell at the beginning of efferent ducts in Turkey. The efferent ducts were large and highly convoluted with ciliated or non-ciliated pseudo stratified columnar epithelium. Narrow and wide connecting duct of epididymal region was lined by pseudo stratified epithelium. Highly tortuous epididymal duct were found both in the boundary of epididymis and near the epididymis. The vas deferens was morphologically similar to the epididymal duct which was lined by pseudostratified columnar epithelium with more basal cells distally.

Osman (1980) reported that in domestic fowl the seminiferous tubules were connected to the rete testis in three different ways which include the link either by terminal segment and a tubuli rectus, by a terminal segment only, or opened directly into the rete cavities.

Aire (1982) observed rete thesis histologically and found simple squamous to high cuboidal epithelium in domestic fowl, Japanese quail, guinea-fowl and drakes. A cilium like structure projected from the luminal portion of most cells into the rete lumen, and the outline of the cells varied from polygonal to elongated. Sparse, stubby microvilli were concentrated on the cell borders.

Rohss and Silverlin (1983) reported that the only difference in the interstitial tissue of adult and juvenile testis of Great tits parus major bird is that in juvenile bird the interstitial tissue has considerably higher amount of Leydig cells.

Van Nassauw *et al.* (1993) observed the presence of smooth muscle cells of peritubular tissue and of tunica albuginea in testis of quail. The peritubular cells formed a

pseudo-stratified tissue which suggest the requirement of smooth muscle cells for testicular contractions.

Aire and Ozegbe (2007) reported that the avian testicular capsule displayed an outer tunica serosa, a thick, middle tunica albuginea and a poorly differentiated innermost layer of tunica vasculosa. The testicular capsule, other than the tunica serosa and tunica vasculosa, comprised of smooth muscle-like or myoid cells running mainly in one direction, and disposed in one main mass. Peritubular tissue was similarly composed of smooth muscle-like cells disposed in several layers. Only one, broad, distinct layer of smooth muscle cells interspersed with sparse collagen tissue was reported. A few profiles of cell groups appeared longitudinally sectioned, while others appeared transversely or obliquely sectioned, histologically, in the orchido-epididymal zone of the testicular capsule, especially in the duck.

Bakst *et al.* (2007) mentioned that regardless of age of the male turkey birds, the wall of the seminiferous tubule consisted of a basement membrane and connective tissue cells and fibers in male turkey birds. The connective tissue cells had an attenuated fibroblast-like appearance with long oval nuclei, which in the pre-pubertal males circumscribed a more readily apparent basement membrane. Leydig cells were characterized by their round nuclei and the numerous lipid droplets typical of steroid secreting cells. Spermatogonia were predominantly cuboidal to low cuboidal and restricted to contact with or close proximity to the inner wall of the seminiferous tubule.

Wei *et al.* (2008) observed that many primordial germ cells and a few spermatogonia were found while seminiferous tubule integrity was not evident in testis of 1-day-old ostrich chick. Spermatogonia were completely differentiated in 30 days old, but very few primary spermatocytes were observed in testes at 45 days of age. The quantity of mitochondria in spermatogonia and lipid droplets in Leydig cells increased gradually with increase in age. Testicular cell apoptosis was observed and the number of apoptotic testicular cells showed a peak in the testis of 45-days-old ostrich.

González-Morán and Soria-Castro (2010) studied the sequence of histological and quantitative changes which occurred in the interstitial tissue of domestic fowl from 8 days old embryo to 28 weeks chicken. They also observed increase in total number of

components of all interstitial tissue: Leydig cells, blood vessels and interstitium with the advancement of age differed markedly according to age. There was increased in total volume and number of Leydig cells per testis, and individual cell volume along the age of the animal. They also observed that total volume of Leydig cells was the dominant component of the interstitial tissue in the testes of 6-week-old chickens,

Al-Tememy (2010) reported that two types of parenchymal tissue were found in quail testis: the interstitial tissue and the seminiferous epithelium. The interstitial tissue contains Leydig cells blood and lymphatic vessels. There were no well-developed interstitial tissues (septa) to divide the testes into lobules because there were very little connective tissue between adjacent seminiferous tubules and the Leydig cells were sparse. The leydig cells were found singly or in small clusters, primarily in the larger interstitial spaces, they are recognized by their small, round and an acidophilic nucleus, with foamy cytoplasm. Seminiferous tubules of birds are highly and complexly anastomotic, non-blind-ending network of tubules and lack connective tissue septa, as well as the non lobulation of the avian testis.

Brennan *et al.* (2010) reported that the cross-sectional study of Muscovy duck showed a large area of fluid filled lumen and collagen fibres. The large lymphatic lumen of the waterfowl penis was surrounded by a thin layer of collagen fibres only 200–300 nm thick which was composed of two distinct layers: an inner layer (40–50 nm thick) next to the lumen which is made of circumferential fibres that encircled the lumen, and an outer layer (150-250 nm thick) that lacks any recognizable organization in either cross or longitudinal sections.

Elbajory *et al.* (2013) reported that the connective tissue capsule of duck testis consist mainly of collagenous fibres and reticular fibres. No mediastinum testis was observed as the rete testis was found outside the testicular parenchyma. The latter was constituted of convoluted seminiferous tubules and interstitial tissue. A thin fibrous basement membrane, collagenous fibres and reticular fibres surrounded and supported the tubules.

Islam *et al.* (2013) reported that histology of mule hybrid testis showed the accumulation of primary spermatocytes with irregularly highly condensed chromosomes

in the seminiferous epithelium, while secondary spermatocytes and post meiotic cells were absent and many testicular cells undergo apoptosis.

Hassanzadeh *et al.* (2013) reported that in ostrich the spermatogonial stem cell and sertoli cells were located within the seminiferous tubules adjacent to the basement membrane. Within the tubules meiotic cells up to spermatozoa were located in centripetal manner. Outside the tubules one to three layers of peritubular myoid cells were present surrounded by loose interstitial connective tissue. A thick tunica albuginea which forms major part of capsule contained many myoid cells and some rete ducts. Straight seminiferous tubules were distributed in the lateral surfaces and hilus portions of the capsule but were rare in the free surface.

Bowles (2014) mentioned that avian testis lack septa that divided the testicle and there was no mediastinal testis. Unlike mammals, the seminiferous tubules anastomose with each other. Each seminiferous tubules was composed of a lining of spermatogonia and sustentacular or Sertoli cells. In most avian species the tubules converged to smaller number of short, straight tubules that continued as rete testis. The rete testis was a meshwork of tubules embedded in in connective tissue, located dorsomedially to each testicle and adjacent to the epididymis. Both the rete testis and straight tubules were line by sustentacular cells.

Kadhem (2014) reported that, histologically each testis of duck was covered by a thin capsule, the stroma of the testis was homogenous due to lack of mediastinum which resulted into absent testicular lobules because there was no septa. Connective tissue occupy the space between the seminiferous tubules and contain Leydig cells which commonly found in groups, fibroblast, macrophages, mast cells, and numerous blood and lymphatic vessels. The seminiferous tubules were surrounded by outer layer of connective tissue contains fibroblast and myoepithelial cells. Germ cells form more than three lines associated with Sertoli cells. The germ cells and Sertoli cells of the seminiferous tubules were supported by the basement membrane.

Shil *et al.* (2015) reported that the seminiferous tubules diameter, circumference and germinal epithelium height increased during summer and rainy season in Japanese

quail. Seminiferous tubules reveal two phases reproductive active phase and regressive phase.

Kannan *et al*. (2015) reported that there was no difference in the histoarchitecture of either of the testis of Japanese quail studied at different age group. The testis were covered by a fibrous capsule mainly composed of collagen, elastic and reticular fibres. There was not lobulation due to the lack of septuli testis and the parenchyma was made up of seminiferous tubules and interstitial tissue. Canalisation of the seminiferous tubules was complete at 4 weeks of age. The tubules were lined by a double layer of polyhedral cells in immature birds which later transform into typical seminiferous epithelium in mature birds. The interstital tissue consisted of Leydig cells, blood vessels, nerves, lymphocytes, macrophages, plasma cell and formed elements of blood. Melanin pigments and mast cells were absent in the interstitium.

Gerzilov *et al.* (2016) studied the testicular development in Muscovy duck and observed that there were two cell types in seminiferous tubular wall immediately after hatching – Sertoli cells and spermatogonia. At 2 months of age, spermatogonia formed one row in ST with lumen and the spermatogenesis had started. At 5 months of age, all generations of germ cells were present in ST and histological picture was the typical one for the testis of a sexually mature bird. After the 2nd month of age, the increase in testes weight and ST diameter became more pronounced at each subsequent age period after 2 months. During the breeding period (at 8–12 months of age and at 2 years of age), the weight and size of testes were the greatest. From hatch to 1 month of age, the interstitial tissue (IT) prevailed over ST, whereas at age periods that followed, the opposite relationship was observed.

Olea *et al.* (2018) reported that organization of the tubules could be visualized at the time of hatching and testicle was constituted of closed seminiferous tubules in Columba livia. The Leydig cells were evident outside the tubules. In juvenile stages, the differentiation of germline cells and the organization of small vessels that irrigated the developing testicle begin to be visible.

Khatun *et al.* (2019) observed that the testis of adult Khaki Campbell duck was covered by connective tissue capsule which formed three main layers: outer thin tunica

serosa, thick tunica albuginea and innermost tunica vasculosa. There was no connective tissue dividing the testis into lobules and stroma of the testis lacking mediastinum. Leydig cells were found in groups within the connective tissue between the spaces of seminiferous tubules. Germ cells and Sertoli cells constituted the main components of seminiferous tubules.

Kareem *et al.* (2020) reported that, histologically the indigenous adult duck of Iraq testis was observed to be covered by a thin capsule. The testis consisted of seminiferous tubules which consisted of two cell types, spermatogenic and sustentacular (sertoli cells) which was surrounded by outer layer of connective tissue. The interstitium was consisted of wide connective tissue, that include the Leydig cell.

2.3 HISTOCHEMISTRY

Lake (1962). Alkaline phosphatase in the testis was present in the cytoplasm and in the nuclei of cells of the intertubular tissue. A much weaker reaction of the enzyme was observed in the nuclei of germ cells which formed the basal layer in tubules. Very feeble reactions were given by the cytoplasm and nuclei of Sertoli cells, secondary spermatocytes and spermatids. The acid phosphatase reaction was feeble in the basal cell nuclei. These findings would indicate that the fowl testis was similar to most mammalian testes with regard to the distribution of the alkaline and acid phosphatases.

Lake (1962) and Angulo and Bosch (1964) observed that there was high degree of activity of acid phosphatase in efferent duct and vas deferens of male fowl.

Varute (1971); Gutitrrez *et al. (*1972); Urry *et al. (*1975); Silverin (1978) reported that enzymes in testicular tissues have been shown to undergo yearly cycles in some vertebrate species and the increase in enzyme activity were correlated with spermatogenesis.

Kugler (1975) reported that testicular interstitial cells of rooster showed an extraordinarily high activity of acid phosphatase in adult. In the seminiferous tubules there was strong reaction of alkaline phosphatase in the peritubular cells might play a part in energy disposition for contractions.

Silverin (1978) reported that during development of testis the Leydig cells were increased in Pied flycatcher with high metabolism which result in no enzymatic changes during this period.

Gunawardana *et al.* (1982) reported the ACPase activity in germinal epithelium of testis in domestic fowl and observed that ACPase activity in spermatogonia and spermatocyte was confined to golgi complex. In spermatids ACPase activity was seen in the endoplasmic reticulum and nuclear envelope. In Sertoli cells ACPase activity was predominant in the lysosomes

Gunawardana (1985) observation revealed the localization of alkaline phosphatase in the testis of domestic fowl. After 30 minutes of incubation, sections examined under light microscope showed prominent enzyme activity in the boundary tissue of the seminiferous tubules and in the interstitial areas. More diffuse and apparently weaker staining reaction was recorded in the germinal epithelium and impossible to locate the site of activity.

Gunawardana (1990) reported that the intertubular tissue, the interstitial tissue, the Leydig cells, transitional cells and the fibroblasts displayed alkaline phosphatase enzyme activity on their cell membranes of testes of the domestic fowl:. Vacuoles located in the transitional cells were lined by reaction products of enzyme activity, whereas the vacuoles present mainly in the Leydig cells were free of enzyme activity. In the peritubular tissue and in pinocytotic vesicles the cell processes of fibroblasts showed enzyme activity on the cell membranes.

2.4 ELECTRON MICROSCOPY

2.4.1. Transmission Electron Microscopy

Connel (1972) reported a small sized lipid droplets, numerous rough endoplasmic reticulum, appearance of bizzare mitochondrial shapes and a stiking development of polysomes in Leydig cell of a 2 day old chicks.

Nicholls and Graham (1972) reported that birds which were subjected to short photoperiod (6 hr of light per day) had undeveloped testis and the interstitial region

contained only fibroblast-like cells. When birds were subjected to 20 hr of light per day, fibroblast-like elements were in the more central regions of the interstitial zone which differentiated into Leydig cells similar to those of mammals in ultrastructure. Mature Leydig cells were present after 10 days of subjection to long photoperiods. These contain a spherical nucleus, numerous mitochondria, large quantities of tubular smooth membrane, little granular endoplasmic reticulum, and variable numbers of lysosomes and lipid droplets. The Golgi complex did not appear to be extensive.

Garnier *et al*. (1973) reported that in Pekin duck the changes in the ultrastructure of the Leydig cells had been shown to be parallel to changes in plasma levels of testosterone and a close correlation between the development and regression of the smooth endoplasmic reticulum and tubular cristae of the mitochondria, on one hand, and the levels of testosterone in the testes and plasma on the other which conclude that the testis sequesters testosterone early in the period of development of spermatogenesis.

Rothwell (1973) reported that the ultrastructural characteristics of Leydig cell in domestic fowl had an elongated transitional cell with well-developed rough endoplasmic reticulum and lipid droplets, and a polygonal series of cells possessing mitochondria with tubular cristae, smooth endoplasmic reticulum and lipid droplets were found.

Rothwell and Tingari (1973) reported that the ultrastructure of the boundary tissue of the seminiferous tubules of the domestic fowl consisted of inner fibrous lamella of homogeneous dense material, collagen fibers and non-striated fibrils, and peripheral to this a multilayered peritubular cellular component. Two distinct cellular forms were observed - an inner fibroblast cell and an outer myoid cell containing many cytoplasmic filaments and interfilamentous dense bands indicative of a contractile function.

Cooksey and Rothwell (1973) reported that in 11 week old domestic fowl testis the Sertoli cell lay between the germ cells of the seminiferous tubule; its basal plasma membrane is in association with the basal lamina, while its apical portion reaches the lumen. It was easily distinguished from the germ cells by its larger size, irregular shape and indented nucleus. During the development of the fowl from hatching to 11 weeks the Sertoli cell changes from a simple columnar type epithelial cell to the type described. For the first 6 weeks the seminiferous epithelium consisted of a ring of Sertoli cells and

spermatogonia. The Sertoli cells increased in number until the seventh week, when spermatogenesis begans. Thereafter their number remained stable throughout the succeeding spermatogenic cycles.

Humphreys (1975) reported that Sertoli cell cytoplasm showed great increase in size and number of residual body, while smooth endoplasmic reticulum was reduced in Budgerigar bird when photoperiod was 8 hours or less. At photo period of 10½ hours the prominent interstitial tissue had more than 3 cell thickness, most of which were fusiform in character with elongated nuclei which was difficult to be differentiated from endothelial cells. The cells near the intertubular junction were more pyramidal with nucleus located in one side most of them having a dark cytoplasm. Cytoplasmic contents included rounded mitochondria, rough and smooth granular endoplasmic reticulum and aggregations of osmiophilic material without defined borders around groups of mitochondria. A few cells contained accumulations of defined inclusion bodies which were not electron dense as well as smaller osmiophilic particles

Lam and Farner (1976) reported that in male White-crowned Sparrows subjected to 20 h daily photoperiods there was an approximately 3-fold increase in the plasma concentration of luteinizing hormone (LH) on the first long day after which a quasi-stable level was maintained for at least 42 days. This increase was followed by an increase in numbers of cells of Leydig and an enhancement of their steroidogenic features, a decrease in transitional interstitial cells, and an increase in plasma level of testosterone. With the decline in plasma LH, as photo refractoriness developed the steroidogenic features of the cells of Leydig undergwent disorganization. For as yet unexplainable reasons the plasma levels of testosterone declined before the decrease in plasma LH and before the degeneration of the steroidogenic features of the cells of Leydig.

Osman (1980) reported that the ultrastructure of domestic fowl testis showed that the seminiferous tubules terminal segments were lined by columnar cells which were a modified sertoli cell. These cells were characterized by presence of an indented nucleus with a prominent nucleolus, many mitochondria, a sizeable Golgi apparatus, electron dense bodies and many cytoplasmic protrusions into the lumen.

Barker and Kendall (1983) reported that in the ultrastructural study of rete testis in three different species of wild birds showed two types of cells in the epithelium which were numerous non-ciliated cells intermixed with a smaller number of ciliated cells. These two cell types contained a large number of vesicles which suggested the influence of epithelium on the luminal contents of the excurrent duct system.

Rohss and Silverlin (1983) reported that during autumn when the testis regressed in adult male Great Tits Parus major birds the electron microscopic view showed the prominent interstitial tissue consisted mainly of fibroblasts, transitional cells and very few scattered Leydig cells. The transitional cells were usually more or less oval-shaped, with a thin layer of cytoplasm. Contrary to the fibroblasts, they occasionally contained a few lipid droplets and few endoplasmic reticulum of both the smooth (SER) and the granular (RER) type. Leydig cells showed large nucleus and a thick layer of cytoplasm with more and larger mitochondria, and they always contained few and small lipid droplets. The testis enlarged during spring and breeding season where the enlarges seminiferous tubules compress the interstitial tissue between tubuli which resulted in the decrease in size and number of Leydig cells along with decreased in number and size of lipid droplets and mitochondria. A Golgi complex is difficult to distinguish.

Rikihisa and Lin (1987) reported that the ultrastructure of testis of Japanese quail showed that the basal lamina were relatively thin and followed the contours of the cell lining the periphery of the seminiferous tubules. Leydig cell had mitochondria, with tubular cristae, abundant smooth endoplasmic reticulum and lipids.

Aire (1997) reported that the ultrastructures of the interstitial tissue of testis were similar in domestic fowl, guinea fowl, duck and Japanese quail. Ultrastructural study showed that thin lymphatic vessels run between the peritubular tissue, blood vessels, leydig cell and macrophages. The basal lamina of the seminiferous tubules was dense homogenously and occasionally invaginated into the seminiferous epithelium especially sertoli cells as spike-like folds or plicae. The basal lamina of inactive testis was highly irregular, relatively electron-dense, contained electron-lucent globules.

Similarly author reported that under electron microscope the Leydig cells of the gonadally active birds possessed oval or polygonal nuclei which were generally

euchromatic. In gonadally inactive birds they accumulated lipid droplets, dense heterogeneous bodies, probably lysosomes, and appeared to degenerate.

Aire and Josling (2000) reported that the testis of gallinaceous birds under transmission and scanning electron microscope showed that the epithelial surface of the rete testis was regular and each cell bore a single cilium, as well as numerous, or in some parts, very few, short, regular microvilli. Each of the Types I and II non-ciliated cells of the proximal and efferent ducts displayed abundant, moderately long and regular microvilli, and a solitary cilium. The ciliated cells exhibited tufts of cilia. The Type III non-ciliated cell of the connecting and epididymal ducts exhibited a solitary cilium, and numerous microvilli which were intermediate in length between those of the rete testis and those of the efferent ducts.

Aire and Ozegbe (2007) reported that the ultrastructure of spindle-shaped cells of the tunica albuginea were typical of smooth muscle cells. The nuclei were regularly elongated, euchromatic and displayed an eccentric nucleolus. The organelles such as mitochondria and short strands of rough endoplasmic reticulum (RER) were few and located mainly at both poles of the nucleus, from which they extend towards the central portion of the cytoplasm. The cytoplasm contained abundant filaments which radiated obliquely, from the nucleus or central zone occupied by organelles toward the sarcolemma. Some cells that were uncommonly scattered between the smooth muscle cells contained elongated heterochromatic nuclei, which were surrounded by a small rim of cytoplasm and displayed very poorly developed cytoplasmic filaments. These were probably fibroblasts

Similarly author reported that ultrastructure of the peritubular tissue was composed of several overlapping layers of cells which was consisted of moderate abundance of filaments attaching to cytoplasmic densities that lay within the cytoplasm or were adherent to the plasmalemma, which bore numerous micropinocytotic vesicles. Organelle content included an elongated, generally euchromatic nucleus, a number of mitochondria, free and rosettes of ribosomes, and a few, short profiles of RER, all contained in a central, filament-free zone of the cytoplasm, extending for variable lengths from either pole of the nucleus into the rest of the cytoplasm.

2.5 HAEMATOLOGY

Mulley (1979) reported that in male black duck the haematological parameters were Hb (g/100 ml) 12.88 ±1.25, PCV (%) 40.00 ±3.82, Erythrocytes (× 106/mm^3) 2.79 ± 0.19, M.C.V. (Mm^3) 143.15±7.73, M.C.H. (pg) 46.06±2.39, M.C.H.C. (%) 32.18± 0.65, Leucocytes (× 103/mm^3) 19.93±5.67, Heterophils (×103/mm^3) 4.54±1.31, Lymphocytes (× 103/mm^3) 13.71±4.00, Monocytee (×103/mm^3) 1.46±1.06, Eosinophils (× 103/mm^3) 0.15±0.14 and Basophils (× 103/mm^3) 0.07±0.12.

Driver (1981) reported the hematological changes in captive and wild drake mallards, *Anas p. platyrhynchos*, observed from the nesting and post-nesting period to the start of' fall migration and observed that in mallard drakes the hemoglobin, packed cell volume, erythrocyte and total leukocyte counts, as well as the number of heterophils and lymphocytes, declined significantly during and after remige moult compared to values recorded prior to remige moult.

Fourie and Hattingh (1983) reported that in male Yellow-bill duck the haemoglobin, RBC, MCV, MCH AND MCHC were 13.38 g%, 3.17 million/mm^3, 128.53μ^3m, 42.29ng and 32.88% respectively.

Olayemi *et al.* (2003) studied the comparative assessment of the white blood cell values, plasma volume and blood volume in the young and adult Nigerian duck (Anas platyrhynchos) and observed that mean total WBC, heterophil, lymphocyte, eosinophil and monocyte counts in the adult duck were 10.42, 4.22, 5.93, 0.09 and 0.17 ×109/L, respectively. These counts in adult birds were similar to the values in young ducks. Mean plasma volume and blood volume in adult ducks were 36.30 and 57.43 ml/kg body mass, respectively. He concluded that the values of adult did not differ significantly from those of young ducks.

Okeudo *et al.* (2003) reported the haematological characteristics of ducks of Southern Nigeria were Packed cell volume (PCV) 46.00 ± 1.73%, hemoglobin concentration (HBC) 15.67 ± 0.29 g%, Erythrocytes (RBC) × 106/mm^3 3.31 ± 0.10, Leucocytes (WBC) × 103/mm^3 23.81 ± 0.88, Heterophils (Neutrophils) % 15.33 ± 4.16

Eosinophils % 5.67 ± 2.08 and Lymphocytes % 79.00 ± 3.61 for adult drakes of about 4 months of age.

Mostaghni *et al*. (2005) reported that the packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) number, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), MCH concentration (MCHC), heterophils, lymphocytes and thrombocytes were found to be 35.21±1.6 (%), 117.8±59 (g/l), 2.27±0.29 (×1012/l), 5.93± 1.25(×109/l), 201.84±86(fl), 62.54±5.73(pg), 329±1.6(g/l), 64.71±4.47(%), 35.14±2.1(%) and 76.4±9.2(109/l), respectively. In black-headed gull PCV, Hb, RBC, WBC, MCV, MCH, MCHC, heterophils, lymphocytes and thrombocytes were found to be 39± 2.52 (%), 123±13.3 (g/l), 2.89±0.45 (×1012/l), 2.25±0.42 (×109/l), 184±17.32 (fl), 60.33±6.74 (pg), 327.6±3.8 (g/l), 57.33±12.2 (%), 42.66±4.7 (%) and 61.44±8.25 (109/l), respectively

Olayemi *et al.* (2006) compared the hematological and plasma parameters of the Nigerian laughing dove and Nigerian duck and observed that the Nigerian duck had significantly higher mean corpuscular volume, total white blood counts, plasma urea, total protein and globulin, but significantly lower red blood cell counts, haemoglobin concentration, mean corpuscular haemoglobin concentration, plasma potassium and albumin/globulin ratio than the Nigerian laughing dove. However, the packed cell volume, mean corpuscular haemoglobin, plasma sodium, creatinine and albumin were similar in the two species of bird.

Odalede *et al.* (2007) reported that the mean values for packed cell volume (PCV), haemoglobin (Hb), total protein (TP) and whole blood coagulation time (WBCT) in male mallard duck were 41.46%, 0.78%, 13.82%, 13.02 0.26g% and 3.70 0.20 min respectively

Kececi and Col (2011) conducted a study to determine selected haematological and biochemical parameters of pheasants bred in Konya city in Turkey at 1, 5, and 12 months of age and observed that some haematological and biochemical values were influenced by age differences. The red blood cell counts, haemoglobin amount, haematocrit values, lymphocyte percentage, and total protein and albumin levels increased with the advancement of age. However, glucose, length and width of

erythrocyte, length and width of erythrocyte nucleus, and percentages of monocyte and basophile decreased with the advancement of age. The white blood cell counts of the chick and the adults were lower than those of the young pheasants. While thrombocyte count of the chicks was higher than that of the young pheasants

Ismoyowati *et al.* (2012) studied the different haematological condition, immune system and comfort of Muscovy duck and local Indonesian duck reared in dry and wet seasons and observed that Muscovy duck and local duck reared in wet season had a higher haematological status than those of dry season. Leukocyte amount was higher in Muscovy duck than local duck, while Muscovy duck had a lower heterophile-lymphocyte ratio than that of local duck.

Avni-Magen *et al.* (2016) reported that in the ferruginous ducks haematological study the blood parameters showed that there was lower white blood cell counts, which were dominated by heterophils rather than by lymphocytes.

2.6. HORMONE

2.6.1. Testosterone

Connel *et al.* (1966) reported that White Leghorn and White Rock chicks possess a considerable interstitial tissue 2 days after hatching and produce testosterone at this early age *in vivo*. Increased amount of androgen were produced after stimulation by gonadotropin.

Kerlan and Jaffe (1974) studied the plasma testosterone levels during the testicular cycle of the red winged blackbird and observed the fluctuations in plasma testosterone concentration during the testicular cycle by radioimmunoassay. A peak in testosterone was associated with small testes that weighed less than 100 mg in the photosensitive stage. In the regressive stage, testosterone concentration was uniformly low. Testosterone was detected in birds in the refractory stage maintained on a long photoperiod for 35 days and in two birds kept for about 27 weeks.

Rohss and Silverlin (1983) reported that in male Great Tits Parus major birds there was a significant age related difference in plasma levels of testosterone only in the

month of September. Juvenile males had significantly higher plasma levels as compared adult males.

Silverlin (1984) reported that in male cape cormorant bird there is increase in luteinizing hormone concentration in corresponds to the time of peak testicular growth. The functional significance of this LH peak is that it is always associated with the rise in testosterone secretions.

Jallageas *et al.* (1974) reported that a Pekin duck exposed to long day lengths result in increase in testicular size along with plasma testosterone and LH level. During natural photoperiod the testicular concentration reached peak level few months before the maximum testicular size and LH concentration was obtained.

Tanabe *et al.* (1983) reported that the plasma testosterone concentration on a day old duck was 141.5 + 7.5 ng/dl and on 14 days the hormone concentration was 111.8 ± 16.0 ng/dl estimated using radioimmune assay.

Yang *et al.* (2005) reported that in male Shao ducks plasma testosterone level increase significantly from 95 days of age onwards

Washburn *et al.* (2007) reported the plasma testosterone level in Mourning Doves, White-eyed Vireos, Red-eyed Vireos, and Indigo Buntings were 93.9 ± 4.9, 100.6 ± 2.3 , 102.6 ± 4.9 and 92.8 ± 3.2 respectively.

Gryzinska *et al*. (2011) determine the level of testosterone in roosters of the Polbar breed at 8, 12 and 18 weeks of age and observed that testosterone in roosters of the Polbar breed showed upward trends of the hormone in blood serum with the maturation of sexual organs responsible for its production.

Simões *et al*. (2016) reported that the plasma testosterone concentration of the domestic duck showed seasonal variations. The highest mean concentration was observed during peak reproduction (76.91 ± 0.20 ng/dL), as well as at the beginning of testicular quiescence (70.84 ± 5.71 ng/dL), with no significant difference between these two phases

Abdul-Rahmana *et al.* (2018) observed that in male guinea fowl the peripheral testosterone concentrations increased from 4 weeks of age and reached the peak concentrations at 20 weeks of age. After 20 weeks of age the hormone concentration decreased and remained at the value which was observed at 12 weeks of age. The also mentioned that the correlations between all testicular anatomical characteristics, testicular weight and peripheral testosterone concentration were positive and highly significant.

2.6.2. Triiodo thyronine (T_3) and thyroxine (T_4)

Singh *et al.* (1967) reported that the thyroxine secretion rate observed were Chicken 6.5 weeks 2.03 ± 0.150, 7 weeks 2.03 ± 0.178, 56 weeks 1.02 ± 0.242, Bobwhite quail 56 – 68 weeks 2.49 ± 0.493 and Japanese quail 2.78 ± 0.349 µg/100gm body weight/ day.

Sadovsky and Bensadoun (1971) reported that Thyroxine in rooster plasma ranges from at 4.9 ± 0.30 to 11.1 ± 1.0 µg per 100ml of plasma. Triiodothyronine ranges from 3.6 ± 0.1 to 5.6 ± 0.9, µg per 100 ml of plasma.

Astier and Newcomer (1978) observed that in Peking drakes the thyroxine concentration was 7.30 ± 0.95 ng/ml, the mean triiodothyronine was 0.55 ± 0.04 ng/ml. The mean T3/T4 ratio was 7.74 ± 1.04%.

Yang *et al.* (2005) reported that in male Shao ducks there was a significant increase in plasma thyroxine (T_4) at 95 days of age which decrease afterward and plasma triiodothyronine (T3) was at high level during early stages of development which significantly decrease at 55 days of age.

Biswas *et al.* (2010) studied the age- dependent variation in hormonal concentration in blood plasma of Indian native fowl and observed that the hormonal profile of Triiodothyronine (T3) and Thyroxine (T4) in blood plasma was found highest around 6 to 12 weeks of age, after which a linear and significant decrease was recorded till the end of experiment that is 30wks of age.

2.6.3. Cortisol

Kalliecharan and Hall (1974) reported that chicks 2 days after hatching have cortisol 8.75 ± 0.62 ng/ml which was determined using the competitive protein-binding globulin method from plasma.

Tanabe *et al*. (1983) stated that the plasma cortisol concentration in male post embryonic duck had peak value at 1 day post hatching i.e. 8.02 ± 1.82ng/ml which declines thereafter and the value at 14 days old was 0.56 ± 0.17ng/ml.

Schmidt and Soma (2008) reported that the plasma level of cortisol in song bird was highest at 0-day old which decrease with age.

Flament *et al.* (2012) reported that cortisol concentration was 4.25 ± 1.36 ng/dl at 8 week of age in male mule duck estimated using radioimmunoassay.

2.7. BIOCHEMISTRY

Driver (1981) reported that the blood biochemical values of mallard drakes before, during and after remige moult and observed that no significant changes were observed in total protein, alkaline phosphatase, glutamic oxaloacetic transaminase or creatine phosphokinase in pre- or post-moult periods.

Mulley (1979) reported that in the male black duck (*Anas superciliosa*) the concentration of different serum biochemicals *viz*. Lactate Dehydrogenase 351.80 ± 80.50 µ/l, Alkaline Phosphatase 17.80 ± 5.60 µ/l, Serum Glutamate Oxaloacetate Transaminase 61.00 ± 24.00 µ/l, Serum Glutamate Pyruvate Transaminase 12.90 ± 7.70v/v, Blood Urea Nitrogen 1.50 ± 0.28 mg/100ml, Total Protein 4.57 ± 0.22 gms/100ml and Albumin 3.2 ± 0.22gms/100ml respectively.

Bowes *et al*. (1988) reported the biochemical profile of male broiler at different age group and stated that the total protein levels was significantly lower at 9 and 20 days of age, creatinine at 9 days of age and albumin at 20 days of age. Lactate dehydrogenase level was significantly higher at 20, 30 and 42 days of age.

Mostaghni *et al.* (2005) reported that in flamingo the concentrations of glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatine phosphokinase (CPK), were 8.45±1.65 (mmol/l), 55±4.7 (g/l), 17.1±2.7 (g/l), 70.83±19.77 (IU/l), 4.2±0.2 (IU/l), 19.78±5.38 (IU/l), and 197.16±57.45 (IU/l), respectively. Whereas in black-headed gull, the serum concentrations of glucose, total protein, albumin, AST, ALT, ALP, CPK, were 10.78±1.39 (mmol/l), 51±8.1 (g/l), 18.3±2 (g/l), 92.66±17.14 (IU/l), 9.21±1.2 (IU/l), 27.73±5.37 (IU/l), and 164.33±48.81 (IU/l) respectively.

Biswas *et al.* (2010) reported that in Indian native fowls blood plasma the activities of acid phosphatase (ACP) increased with the reduction of alkaline phosphatase (ALP) activities at different time intervals. Transaminases (glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)) activities of blood plasma increased linearly with the advancement of the age.

Flament *et al.* (2012) reported that the Aspartate aminotransferase (AST) concentration in male mule duck at 8 weeks of age was 32.33 ± 15.55a (U/l).

Sinha *et al.* (2017) observed that Alkaline phosphatase decreased significantly with age i.e. for 2 to 40 weeks while AST, ALT, CPK, glucose, protein, cholesterol, creatinine and triglycerides increased significantly ($P<0.0001$) with age in Pati duck.

Deka *et al.* (2017) reported that the serum ALT (175.77 ± 3.85 Vs 56.32 ± 2.45) and ALP (51.03 ± 1.52 Vs 28.10 ± 1.87) level were found to be significantly higher in Chara-Chemballi duck than in Pati duck.

Rezende *et al*. (2017) reported the serum metabolites in male broiler breeder observed were *viz*. total protein was 24.60 ± 4.20 (g/L), Albumin 10.60 ± 1.90 (g/L) and Urea (mmol/L) 7.34 ± 3.75.

Jerabek *et al.* (2018) reported the biochemical indicator of 40 day old mallard duck i.e. Albumin (%) 49.1, Total protein (g/l) 32.6, ALP (µkat/l) 14.4, ALT (µkat/l) 0.70 and AST (µkat/l) 0.92 respectively.

Rath *et al.* (2019) compared the serum biochemistry profile between three breeds of adult duck i.e. Khaki Campbell, White Pekins and a native duck breed of Odhisa and reported that the indigenous ducks (of Odisha region) possessed the most-desirable estimates. Khaki and Pekins had higher total protein and albumin compared to native duck, SGOT (Serum glutamate oxaloacetate transaminase) was lower in Pekins than in Khakis and Kuzhis (native duck) while SGPT (Serum glutamate pyruvate transaminase) value was significantly higher in Khakis than Kuzhi ducks.

CHAPTER - III

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

The present research was conducted on 30 (thirty) apparently healthy male Pati duck of Assam at different stages of development. They are put into five (5) groups according to their age as group 1 (1 month), group 2 (6-8 weeks), group 3 (20 weeks), group 4 (30 weeks) and group 5 (40 weeks) maintained under ICAR-NASF Project, Department of Veterinary Physiology, College of Veterinary Sciences, Assam Agricultural University, Khanapara Campus.

LOCATION AND DURATION OF THE EXPERIMENT

The experiment was conducted in the Department of Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Assam Agricultural University, Khanapara, Guwahati. Haematological parameter was carried out in the Teaching Veterinary Clinical Complex (TVCC) laboratory, biochemical parameter was conducted on the Department of Biochemistry and Department of Animal Biotechnology, and hormonal parameter was conducted on the Department of Veterinary Physiology, respectively at College of Veterinary Sciences and Animal Husbandry, Assam Agricultural University, Khanapara, Guwahati, while the electron microscope was carried out in the Sophisticated Analytical Instrument Facility (SAIF), NEHU, Shillong.

The animal used for the experiment was ethically approved by the institutional Animal Ethics Committee, Faculty of Veterinary Science, AAU, Khanapara, Guwahati-22 vide Approval No. *770/GO/Re/S/03/CPSEA/FVSc/AAU/IAEC/18-19/630 dated 28.12.2018.*

The experiment was carried out from February, 2018 to October, 2020.

TABLE 3.1: EXPERIMENTAL DESIGN SHOWING NUMBER OF PATI DUCKS UTILIZED IN THE EXPERIMENTAL GROUP

Group	Age group	Number of birds
Group 1	1 month	6
Group 2	6-8 weeks	6
Group 3	20 weeks	6
Group 4	30 weeks	6
Group 5	40 weeks	6
Total		**30**

3.1. GROSS ANATOMY AND BIOMETRY

Upon reaching the respective age the experimental birds were brought to the Department of Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22. The blood of each individual bird were collected from the jugular vein and then sacrificed according to the technique of Gracy (1986). After exsanguination the skin and fascia were reflected, and then a ventro median incision was given in the abdominal region to remove the abdominal viscera without disturbing the topographic position of genital organs.

Thereafter, length, breadth and thickness of the testis, vas deferens and phalluses of all age group were recorded with the help of the Vernier caliper (Mc Cance, 1974). While weight of the testis of all age group was recorded with the help of electronic balance.

3.2. HISTOLOGY

For histological study the tissue samples from the male genital organs of all the age group were fixed in 10% neutral buffered formalin solution. The fixed tissue samples were processed to prepare paraffin blocks as per the procedure described by Luna (1968). The paraffin blocks were sectioned in Shandon Finesse microtome in 5µm thickness and the sections were stained by following methods as described by Luna (1968).

a. Mayer's Haematoxylin and Eosin stain.
b. Van Gieson's method for collagen fibres.
c. Gomori's method for reticular fibres.
d. Hart's method for elastic fibres.
e. Bielschowsky's method for nerve fibers.

Different micrometrical parameters were recorded on Hematoxylin and eosin stained sections by means of standard method of micrometry using Nikon E 200 camera mounted microscope and Image Pro Express Ver-2.0 Software.

3.3. HISTOCHEMISTRY

For histochemical parameters, tissue samples were preserved in deep freeze maintained at -80^{o}C immediately after collection. The preserved tissue samples were shifted directly to cryostat microtome (Leitz) which was maintained at -20^{o}C at Central Instrument Facility (CIF), C.V.Sc, Khanapara. The frozen sections were cut at 10 µm thickness and were collected on clean slides. They were temporarily stored at -22^{o}C and were then treated as per the method of Singh and Sulochana (1978) for demonstration of following histochemical parameters:

a) Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1978)
b) Gomori's method for acid phosphatase (Singh and Sulochana, 1978)
c) Lead method for Adenosine Tri-Phosphatase (Bancroft, 2008)

3.4. ULTRASTRUCTURE (ELECTRON MICROSCOPY)

For ultrastructral study (Electron Microscopic) the tissue samples were processed as per the techniques of Parsons *et al.*, (1991) which was slightly modified by SAIF, NEHU, Shillong. The samples were cut into small pieces of 2 mm size and fixed in 2% glutaraldehyde solution for 4 hours at 4ºC. After that the fixed tissue samples were kept in Na-cacodylate buffer. The processing of sample was done in SAIF, NEHU, Shillong.

For TEM Study:

- Fixed tissue in Gluteraldehyde were then post fixed using 2% osmium tetra oxide in sodium phosphate buffer (0.5M, pH – 7.2) for 2 hours.
- Dehydrate in graded alcohol.
- Infiltrate and embedded in Araldite resin (Araldite 6005)
- Ultrathin sections were cut (60-70nm) with glass knife using Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome and mounted on grid.
- Stain with uranyl acetate and counter stained with 4% lead citrate and observe at various magnifications.

3.5. HAEMATOLOGY

For haematological parameters whole blood were collected in EDTA vial and stored at 4°C. haematological study was immediately conducted in the laboratory of TVCC, College of Veterinary Science. For this study RBC, WBC, lymphocyte, monocyte, neutrophil, eosinophil, basophil and haemoglobin value of different age group were recorded.

3.6. HORMONE ANALYSIS

For hormonal analysis serum samples were collected from whole blood using clot activator and kept in room temperature for 4 hours after which serum was separated using micropipette in a cryovial and stored at -20°C. The samples were processed in the Radio Immuno Assay (RIA) Laboratory, Department of Physiology, College of Veterinary Science, AAU, Khanapara, Guwahati-22. Estimation of hormone level in serum was conducted using commercially available hormone kit. Total T3 C.T. RIA Kit for T3 hormone, Total T4 C.T. RIA Kit for T4 hormone, Cortisol C.T. RIA Kit for Cortisol and Testosterone (Direct) C.T. RIA Kit for testosterone hormone.

3.6.1. T_3 (Tri-iodothyronine)

Serum T_3 concentrations were estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using ^{125}I – labeled T_3 tracer and anti T_3 monoclonal anitibody coated tubes.

Step 1 Additions	To antibody coated tubes, add successively: 20µl of calibrator, control or sample and 500µl of tracer mix
Step 2 Incubation	Incubate 1 hour at 18-25ºC with shaking (>280 rpm)
Step 3 Counting	Aspirate carefully the contents of the tubes (except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min.

*Add 500µl of tracer to additional tubes to obtain total cpm

3.6.2. T_4 (Thyroxin)

Serum T_4 concentrations were estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using ^{125}I – labeled T_4 tracer and anti T_4 monoclonal antibody coated tubes.

Step 1 Additions	To antibody coated tubes, add successively: 20µl of calibrator, control or sample and 500µl of tracer mix
Step 2 Incubation	Incubate 1 hour at 18-25ºC with shaking (>280 rpm)
Step 3 Counting	Aspirate carefully the contents of the tubes (except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min.

*Add 500µl of tracer to additional tubes to obtain total cpm.

3.6.3. Testosterone

Serum testosterone concentrations were estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using ^{125}I – labeled testosterone tracer and anti-testosterone antibody coated tubes.

Step 1 Additions	To antibody coated tubes, add successively: 50µl of calibrator, control or sample and 300µl of tracer mix
Step 2 Incubation	Incubate 1 hour at 18-25ºC with shaking (≥400 rpm)
Step 3 Counting	Aspirate carefully the contents of the tubes (except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min.

*Add 500µl of tracer to additional tubes to obtain total cpm.

3.6.4. Cortisol

Serum testosterone concentrations were estimated using a RIA Kit (IMMUNOTECH, Czech Republic) using ^{125}I – labeled testosterone tracer and anti-testosterone antibody coated tubes.

Step 1 Additions	To antibody coated tubes, add successively: 50µl of calibrator, control or sample and 300µl of tracer mix
Step 2 Incubation	Incubate 1 hour at 18-25ºC with shaking (≥400 rpm)
Step 3 Counting	Aspirate carefully the contents of the tubes (except the 2 tubes <<totalcpm>>) Count blood cpm (B) and total cpm (T) for 1 min.

3.7. BIOCHEMISTRY

For biochemical parameter the serum samples of different age group were collected from jugular vein using clot activator and kept in room temperature for 4 hours after which serum is separated using micropipette in a cryovial and stored at -20^{o}C. The serum sample were processed in biochemistry laboratory and biotechnology laboratory. The serum biochemistry value were estimated using UV spectrophotometry for ALP, AST, ALT, BUN, LDH, creatinine and albumin.

3.7.1. Alkaline phosphatase

Sample	**20µl**
Working reagent	1000µl
Mix well, and incubate for 1 min at 37ºC. Read Absorbance and at the same time start the stop watch. Read the absorbance again exactly after 1, 2 and 3 min.	

ALP activity = ΔA/min × Factor

Factor 2750

3.7.2. Aspartate aminotransferase (AST/SGOT)

Sample	**100µl**
Working reagent	1000µl
Mix well, read absorbance after 1 min and start stopwatch. Read absorbance again exactly after 1, 2 and 3 minutes at 37ºC.	

AST activity (U/L) = ΔA/min × Factor
Factor 1746

3.7.3. Alanine aminotransferase (ALT/SGPT)

Sample	**100µl**
Working reagent	1000µl
Mix well, read absorbance after 1 min and start stopwatch. Read absorbance again exactly after 1, 2 and 3 minutes at 37ºC.	

AST activity (U/L) = ΔA/min X Factor
Factor 1746

3.7.4. Urea

Pipette into test tubes	**Standard**	**Test**
Standard	10µl	-
Sample	-	10µl
Working reagent	1000µl	1000µl
Mix well, incubate for approx. 30 sec. at 37ºC then read absorbance A1. After exactly 60sec, further read absorbance A2 at 340nm.		

ΔA = {(A1-A2) sample or standard

$$\text{Urea [mg/dl]} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \text{Conc. of std. mg/dl}$$

3.7.5. Lactate dehydrogenase (LDH)

R1: N-Methyl-D-Glucamin 325 mmol/, L-Lactate 50mmol/l

R2: NAD + 10 mmol/l

R1 Prefill + 100µl of R2 + 10µL of sample.

Read change of absorbance (ΔA) with delay time 60 sec and Read time 180 sec at 37ºC.

LDH Activity in U/L = ΔAbs/min of test × 10665

3.7.6. Creatinine

Pipette into test tubes	**Standard**	**Test**
Standard	50µl	-
Sample	-	50µl
Working reagent	1000µl	1000µl
Mix well, incubate for approx. 30 sec. at 37ºC then read absorbance A1. After exactly 60sec, further read absorbance A2 at 505nm.		

ΔA = {(A1-A2) sample or standard}

$$\text{Creatinine [mg/dl]} = \frac{\Delta A \text{ of sample}}{\Delta A \text{ of standard}} \times \text{Conc. of std. mg/dl}$$

3.7.7. Albumin

Pipette into test tubes	**Blank**	**Standard**	**Test**
Standard	-	10µl	-
Sample	-	-	10µl
Reagent	1000µl	1000µl	1000µl
Mix well, incubate for approx. 5 min. at room temperature and read the absorbance against reagent blank at 630nm			

$$\text{Albumin} = \frac{\text{Abs of test}}{\text{Abs of standard}} \times \text{Conc. of std. g/dl}$$

3.7. STATISTICAL ANALYSIS

The data were analysed as per the methods described by Snedecor and Cochran (1994) and were presented accordingly.

Fig. 3.1. PHOTOGRAPH OF THE EXPERIMENTAL PATI DUCKS OF 1 MONTH OF AGE

Fig. 3.2. PHOTOGRAPH OF THE EXPERIMENTAL PATI DUCKS OF 6-8 WEEKS OF AGE

Fig. 3.3. PHOTOGRAPH OF THE EXPERIMENTAL PATI DUCKS OF 20 WEEKS OF AGE

Fig. 3.4. PHOTOGRAPH OF THE EXPERIMENTAL PATI DUCKS OF 30 WEEKS OF AGE

Fig. 3.5. PHOTOGRAPH OF THE EXPERIMENTAL PATI DUCKS OF 40 WEEKS OF AGE

CHAPTER - IV

Results and Discussions

CHAPTER-IV

RESULTS AND DISCUSSIONS

In the present investigation, the testis, vas deferens and phallus of Pati duck of Assam were studied at different stage of development. The changes that occur during post-natal development were observed by changes in the gross shape, size, structure and location of testis, vas deferens and phallus. The findings were discussed under the following subheads.

4.1. GROSS MORPHOLOGY

The gross morphometry of testis, vas deferens and phallus of Pati duck of Assam at different stages of post-natal development were presented in Table 4.1 and 4.2.

4.1.1. Testis

Location

The paired testis of Pati duck (*Anas platyrhynchos*) of Assam was located within the abdominal cavity. They were located ventral to the kidney till the Pati duck reached 20 weeks of age (Fig. 4.1 to Fig. 4.3). With the advancement of age, the testis increased in size and were located between the cranioventral aspect of the kidney and caudoventral aspect of lung in adult birds (Fig. 4.4 and Fig. 4.5). The findings were in agreement with the findings of Bull *et al.* (2007) in domestic fowl, Fails and Magee (2008) in domestic birds, Razi *et al*. (2010) in rooster and Saleem *et al.* (2017) in local fowl of Uttarakhand.

The paired testis of Pati duck were attached to the dorsal body wall by connective tissue called mesorchium and located asymmetrically on either side of the midline. The finding was in consonance with the findings Nickel *et al.* (1977), Johnson (1986), Banerjee (1991), Ghosh (2006), Dyce *et al.* (2009) and Saleem *et al.* (2017) in domestic fowl Kadhem (2014) and Kareem *et al*. (2020) in adult duck. However the findings were in conflict with King (1975) who mentioned that the two testes were symmetrically arranged on either side of the midline in avian. The difference in the findings might be attributable to the difference in species or breed of birds studied.

The left testis of Pati duck was located slightly more cranial than the right testis (Fig. 4.1 to Fig. 4.5). This finding was similar to the findings of Elbajory *et al.* (2013) in Sudanese duck. The cranial end of the two testis lay in between the level of 5^{th} and 6^{th} rib in 1 month of age, which extended up to 5^{th} rib in 6-8 weeks of age, while the caudal end at the level of last rib. At 20 weeks of age the cranial end lay between the 4^{th} and 5^{th} rib while the caudal end of the testis extended beyond the last rib. At 30 and 40 weeks of age the cranial end of the testis extended up to 4^{th} to 3^{rd} rib and covered up to $1/3^{rd}$ of the lung in some cases. At 30 weeks the caudal end of testis extended up to the junction of cranial and middle lobe of kidney while at 40 weeks it reached beyond half of the kidney. The present findings were similar to the findings of Kadhem (2014) in indigenous duck of Iraq.

Shape, size and appearance

The testis of 1 month of age Pati duck was opaque white in colour with elongated rice grain like appearance (Fig. 4.1) while the colour turn slightly yellowish at 6-8 weeks of age (Fig. 4.2). At this age the epididymis area of the testis was not distinguishable. At 20 weeks it was creamy colour and oval shaped (Fig. 4.3). The finding was similar to the observation of Bull (2007) in domestic fowl by who stated that they were oval till the birds were 20 weeks. The testis of 30 and 40 weeks were bean shaped with creamy to pinkish colour (Fig. 4.4 and Fig. 4.5). The finding was similar to the findings of Elbajory *et al.* (2013) in Sudanese duck and Saleem *et al.* (2017) in local fowl. The finding in Pati duck was in conflict with Jacob and Pescatore (2013) observation in male chicken where it was elliptical in shape. However the testes were clubbed shaped in Ostrich as reported by Wei *et al.* (2008). The difference in the findings might be due to difference in species. The testis of 30 and 40 weeks of age had prominent vascularization which (Fig. 4.4 and 4.5) while it was not noticeable at 1 month, 6-8 weeks and 20 weeks age group (Fig. 4.1 to 4.3).

In the present investigation, the epididymis of the testis of Pati duck in 1 month, 6-8 weeks and 20 weeks of age was not distinct (Fig. 4.8). While in 30 weeks and 40 weeks of age at the dorso-medial aspect of testis was occupied by group of tubules strongly attached to the testis which was the epididymis. The epididymis extended upto

the middle 2/3rd of the dorsomedial aspect of testis (Fig 4.6). The finding was in contradicted with the findings of Saleem *et al.* (2017) in domestic fowl where it extended from cranial pole to caudal pole. The difference in the findings might be attributable to the difference in the species of avian studied. The epididymal region was mainly comprised of efferent duct and connecting duct that joined the main epididymal duct which was in agreement with Tingari (1971) and Hodges (1974) which continued as a single highly coiled tube ventrally towards the cloaca as vas deferens. The epididymal duct was a single convoluted large duct found on the medial end of the epididymal region which extended from cranial end to caudal end of the epididymis (Fig. 4.7).

Length, width, breadth and weight

The average length of the left and right testis of Pati duck of Assam at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 7.10±0.60 mm and 6.67±0.55 mm, 9.40±1.48 mm and 8.08±0.60 mm, 15.23 ±0.53 mm and 14.18±0.69 mm, 42.34±1.63 mm and 41.66±1.47 mm, and 51.26±2.12 mm and 45.99±1.31 mm respectively (Table 4.1). There was significant difference among the age group. It was observed that the length of left testis was longer than that of the right testis in all the age groups (Fig. 4.19). Similar opinion was put forth by Bull (2007) in domestic fowl up to 24 weeks of age, Khatun (2019) in Khaki Campbell and Razi *et al.* (2010) in turkey upto 24 weeks. However the finding was in conflict with the findings of Kareem *et al.* (2020) in adult indigenous duck of Iraq and Artoni (1993) in quail where right testis was longer than the left. The difference in the observations between the two studies might be attributable to the difference in species of avian, difference in breed of the duck and difference in the environment.

The average breadth of testis of Pati duck of Assam was recorded as 1.42 ± 0.08 mm and 1.41±0.08 mm, 2.13±0.31 mm and 2.22±0.32 mm, 5.85±0.32 mm and 5.94±0.36 mm, 15.12±0.87 mm and 16.18±0.94 mm, and 21.47±0.99 mm and 23.70±0.74 mm respectively for the left and right testis at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks (Table 4.1). It was observed that except in 1 month age group, the testis of all the other age group has the right testis with more breadth than the left

testis (Fig. 4.20). The breadth of 40 weeks testis was significantly higher than the other age group.

The average thickness of the testis of Pati duck of Assam was recorded as 1.29±0.09 and 1.31±0.07 mm, 1.89±0.29 and 1.95±0.22 mm, 3.95±0.3 and 4.69±5.09 mm, 13.07±0.59 and 14.11±0.53 mm, and 20.97±0.99 and 22.60±0.43 mm respectively for the left and right testis of Pati duck at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks (Table 4.1). It was observed that the average thickness of the right testis was more than the left testis with a few exceptions where it was thicker in the left testis in all the age groups (Fig. 4.21). The findings were in accordance with the findings by Artoni (1993) in quail.

The average weight of the left and right testis at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was recorded as 0.01±0.00 g and 0.01±0.00 g, 0.04±0.01 g and 0.03±0.01 g, 2.23±0.01 g and 2.24±0.01 g, 11.14±0.12 g and 12.74±0.11 g, and 16.00±1.21 g and 17.38±1.15 g respectively (Table 4.1). The weight of 30 and 40 weeks testis was significantly higher than the other age group. The weight of both the testis increased with the increased in age of the birds which supported the reported of Moller (1988) in avian and Khatun *et al.* (2019) in Khaki Campbell duck. From the study it was observed that the left testis was larger and heavier at young age from 1 month till 20 weeks of age. From 30 weeks till the end of study i.e. 40 weeks the right testis was found to be heavier than the left (Fig. 4.22). This observation was found to be similar to the findings of King (1975) in fowl. The significant increased in weight observed at 40 weeks might be due to active breeding season as Gerziilov *et al.* (2016) observed that testis were largest during reproduction period from 8^{th} to 12^{th} month.

4.1.2. Vas deferens

The paired vas deferens of Pati duck of Assam was observed to emerge from the epididymis and run parallel to the ureter (Fig. 4.9 to 4.13) at the ventral aspect of kidney as to reach the cloaca (Fig 4.13). The observations was similar to the findings of Tingari (1971) in domestic fowl, Aire *et al.* (1971) in Japanese quail, Mercadante *et al.* (1983) in male pigeon and Saleem *et al.* (2017) in local fowl. The course of the vas deferens was not discernable at 1 month of age group at the ventral aspect of kidney, while it was

traceable from the cloacal aspect. Identical finding was observed by Abdul-Rahman *et al.* (2018) in guinea fowl. In the present study the vas deferens at 6-8 weeks were observed to run ventral to the kidney and their course was clearly observed at 20 weeks, 30 weeks and 40 weeks of age.

The vas deferens of Pati duck of Assam was highly convoluted coiled tube. It appeared tightly coiled like zigzag pattern at younger age (Fig 4.9 to 4.11). With the increase in age of the duck the diameter of vas deferens increased and the tightly coiled tube loosened which could be observed in (Fig. 4.12 and 4.13). The vas deferens appeared translucent at 1 month and 6-8 weeks, while they appeared opaque white in 20 weeks, 30 weeks and 40 weeks of age.

The average length of the vas deferens at 1 month, 6-8 weeks, 20 weeks 30 weeks and 40 weeks was 59.92 ± 1.45mm, 68.55 ± 3.39mm, 85.69 ± 1.9mm, 121.98 ± 4.09mm, 120.95 ± 8.1mm respectively (Table 4.2). The vas deferens of 20 weeks ducks was significantly longer than 1 month and 6-8 weeks. Significantly longer vas deferens in 30 weeks than all the other age group, however 40 weeks vas deferens was significantly longer than 1 month, 6-8 weeks and 20 weeks. The longest vas deferens was observed in 30 weeks of age group (Fig 4.23).

4.1.3. Phallus

In the present study the body of the phallus was observed to be attached to the ventral floor of the cloaca (Fig. 4.18). The finding was similar to the findings of Bull *et al.* (2007) in domestic fowl. From the base, the phallus coiled in anti-clockwise direction towards the apex (Fig. 4.14 to 4.18). Similar result was reported by Brennan *et al.* (2010) in Muscovy drakes. The ejaculatory groove and sulcus of the phallus ran along the length of phallus and divide the shaft into two lateral bodies which ends at the tip of apex (Fig. 4.13). The present finding in Pati duck was in consonance to the findings of Brennan *et al.* (2008) in Crypturellus, in which the sulcus originated from the ejaculatory fossa where sperm were ejected from the vas deferens and run along the outside of the coiled body of the phallus.

The phallus of Pati duck was long, thick at the base with tapering coiled end (Fig 4.14 to 4.17). The finding was similar with the findings of Herrera *et al*. (2014) in Pekin and Laysan duck, however, the finding differ with Mandarin duck phallus which was thinner and short. The difference in phallus shape might be attributable to the difference in the breed of duck or difference in environment and management.

The average length of the phallus at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 14.93±1.27 mm, 15.40±1.26 mm, 20.80±2.58 mm, 19.60±1.40 mm and 21.10±2.26 mm, respectively (Table 4.2). It was observed that the length of the phallus increased with age but the rate of increase was not significant. The length of the phallus observed in the present study support the finding of Brennan *et al.* (2010) in Muscovy drakes. The length of the phallus might range from 1.5 to 40 cm in any waterfowl (Brennan *et al.* 2007). The changes in phallus length and size could be due to season as the size increased during mating season and regressed when breeding season was over (Hohn, 1960; Herrera *et al.*, 2014).

The length of the phallus of Pati duck of Assam increased with age where maximum length was observed in 40 weeks of age group (Fig. 4.24). The increased in length might be attributable to age or forced extra pair copulation (FEPC) as reported by Cocker *et al.* (2002). In waterfowl, phallus length and elaboration were correlated with the frequency of FEPCs, so species with high levels of FEPCs had longer and more elaborate genitalia than species where FEPCs were uncommon (Coker *et al.*, 2002; Brennan *et al.*, 2007). Whereas Mulder and Cockburn (1993) reported that in Superb fairywren bird age, intragroup dominance, and pairing status did not influence the overall size of the protuberance, but old males had a larger tip on their protuberance which might serves as primary sperm reserves.

The phallus of Pati duck was an intromittent copulatory organ, unlike a non-intromittent organ found in domestic fowl and turkey as reported by Brennan (2008). The difference might be due to species difference as 97 per cent of avian species lack intromittent phallus (Briskie and Montgomerie, 1997; Brennan *et al.*, 2008).

TABLE 4.1. AVERAGE LENGTH, BREADTH, THICKNESS AND WEIGHT OF THE LEFT AND RIGHT TESTIS OF PATI DUCK DURING POSTNATAL DEVELOPMENT

Measurements		AGE				
		1 month	**6-8 weeks**	**20 weeks**	**30 weeks**	**40 weeks**
Length (mm)	**Left**	7.10 ± 0.6^{D}	9.40 ± 1.48^{D}	15.23 ± 0.53^{C}	42.34 ± 1.63^{B}	51.26 ± 2.12^{A}
	Right	6.67 ± 0.55^{C}	8.08 ± 0.6^{C}	14.18 ± 0.69^{c}	41.66 ± 1.47^{B}	45.99 ± 1.31^{A}
Breadth (mm)	**Left**	1.42 ± 0.08^{C}	2.13 ± 0.31^{C}	5.85 ± 0.32^{C}	15.12 ± 0.87^{B}	21.47 ± 0.99^{A}
	Right	1.41 ± 0.08^{C}	2.22 ± 0.32^{C}	5.94 ± 0.36^{C}	16.18 ± 0.94^{B}	23.70 ± 0.74^{A}
Thickness (mm)	**Left**	1.29 ± 0.09^{D}	1.89 ± 0.29^{CD}	3.95 ± 0.3^{BC}	13.07 ± 0.59^{B}	20.97 ± 0.99^{A}
	Right	1.31 ± 0.07^{B}	1.95 ± 0.22^{B}	4.69 ± 5.09^{B}	14.11 ± 0.53^{B}	22.60 ± 0.43^{A}
Weight (g)	**Left**	0.01 ± 0.00^{c}	0.04 ± 0.01^{c}	2.23 ± 0.01^{c}	11.14 ± 0.12^{B}	16.00 ± 1.21^{A}
	Right	0.01 ± 0.00^{c}	0.03 ± 0.01^{c}	2.24 ± 0.01^{c}	12.74 ± 0.11^{B}	17.38 ± 1.15^{A}

* Means with the different superscript are significantly different.

TABLE 4.2. AVERAGE LENGTH OF VAS DEFEREN AND PHALLUS OF PATI DUCK DURING POSTNATAL DEVELOPMENT

	AGE				
	1 Month	**6--8 Weeks**	**20 Weeks**	**30 Weeks**	**40 Weeks**
Vas Deferens (mm)	59.92 ± 1.45^{D}	68.55 ± 3.39^{D}	85.69 ± 1.9^{C}	121.98 ± 4.09^{A}	120.95 ± 8.1^{B}
Phallus	13.13±1.27	14.40±1.26	17.10±2.58	19.60±1.4	21.10±2.26

* Means with different superscript are significantly different.

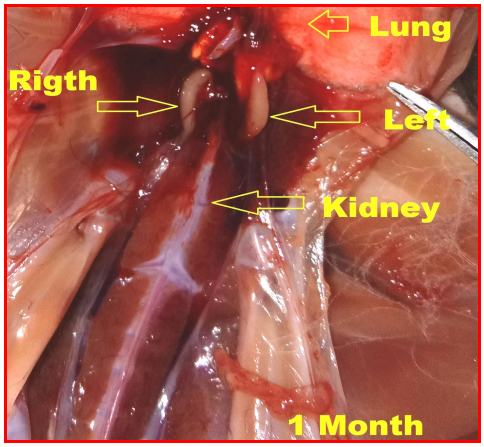

Fig. 4.1. PHOTOGRAPH SHOWING THE IN-SITU POSITION OF THE TESTIS OF PATI DUCK (1 MONTH)

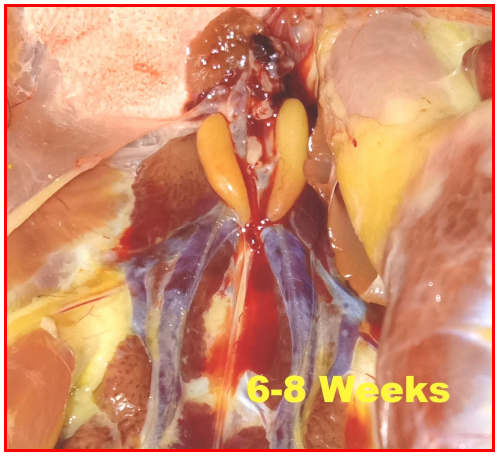

Fig. 4.2. PHOTOGRAPH SHOWING THE IN-SITU POSITION OF THE TESTIS OF PATI DUCK (6-8 WEEKS)

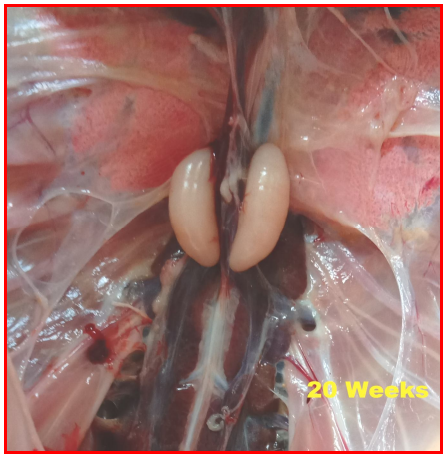

Fig. 4.3. PHOTOGRAPH SHOWING THE IN-SITU POSITION OF THE TESTIS OF PATI DUCK (20 WEEKS)

Fig. 4.4. PHOTOGRAPH SHOWING THE IN-SITU POSITION OF THE TESTIS OF PATI DUCK (30 WEEKS)

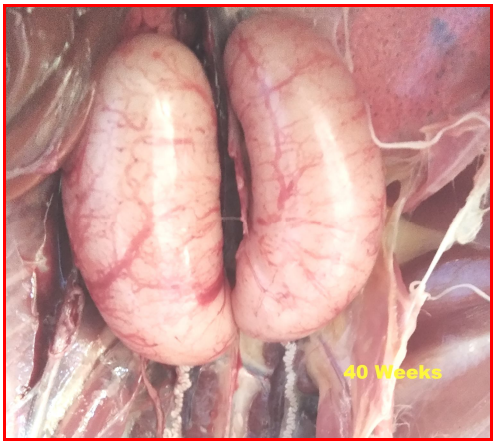

Fig. 4.5. PHOTOGRAPH SHOWING THE IN-SITU POSITION OF THE TESTIS OF PATI DUCK (40 WEEKS)

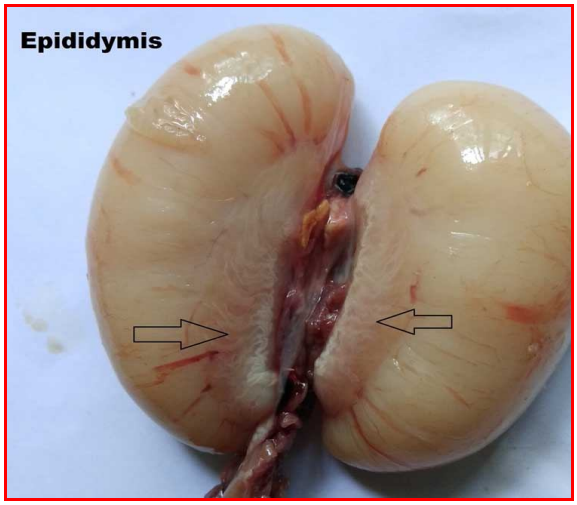

Fig. 4.6. PHOTOGRAPH SHOWING THE EPIDIDYMIS OF PATI DUCK

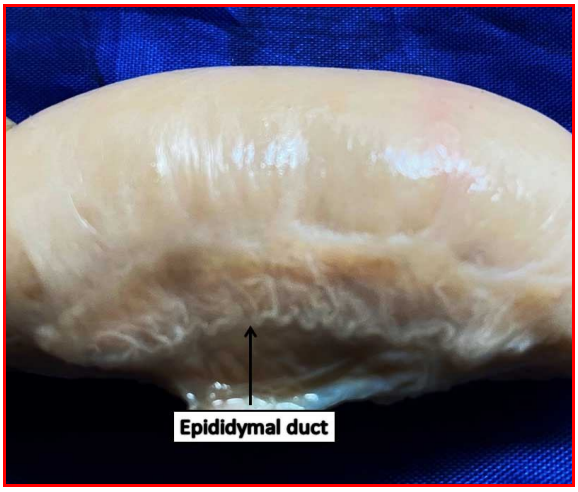

Fig. 4.7. PHOTOGRAPH SHOWING THE EPIDIDYMAL DUCT OF PATI DUCK

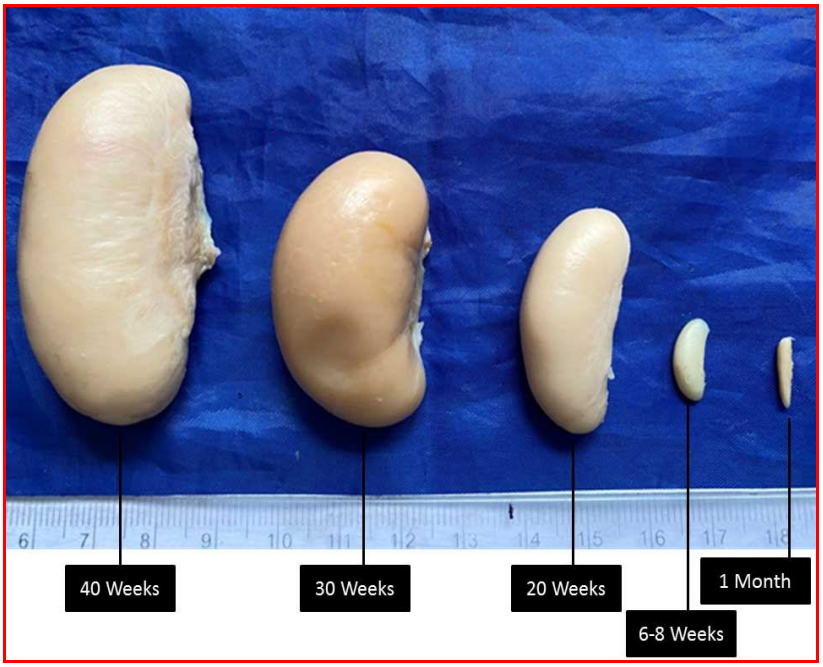

Fig. 4.8. PHOTOGRAPH SHOWING THE TESTIS OF PATI DUCK AT 1 MONTH, 6-8 WEEKS, 20 WEEKS, 30 WEEKS AND 40 WEEKS

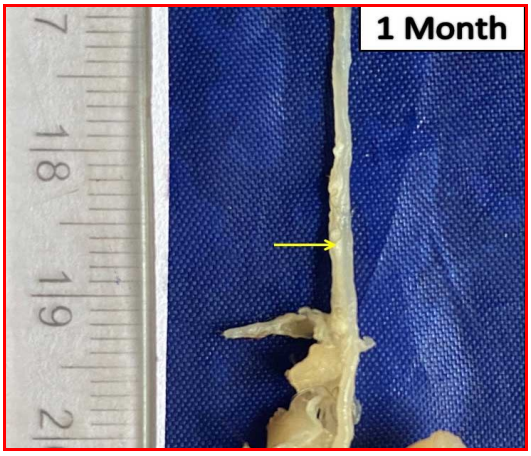

Fig. 4.9. PHOTOGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK (1 MONTH)

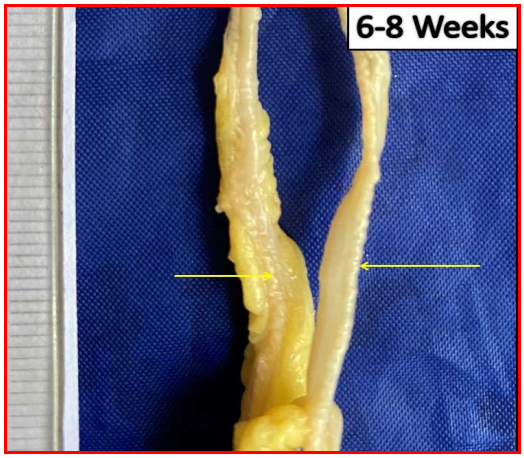

Fig. 4.10. PHOTOGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK (6-8 WEEKS)

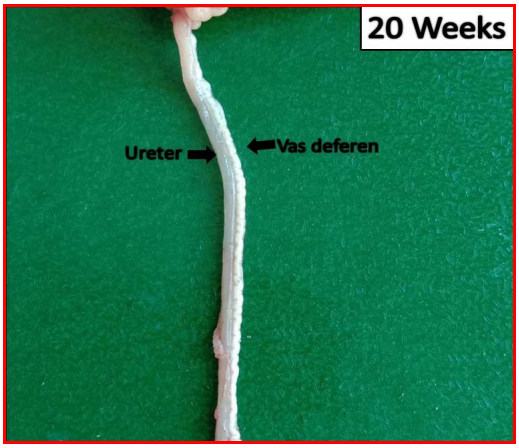

Fig. 4.11. PHOTOGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK (20 WEEKS)

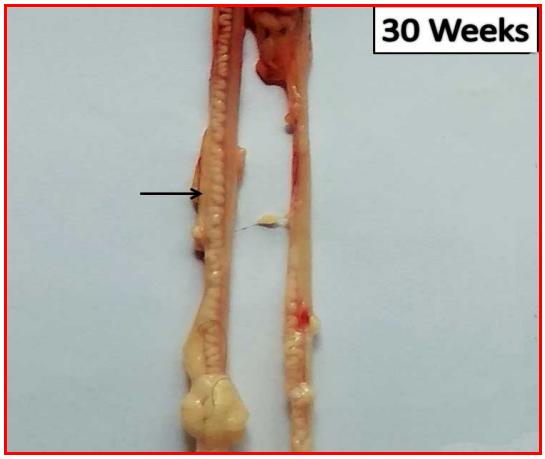

Fig. 4.12. PHOTOGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK (30 WEEKS)

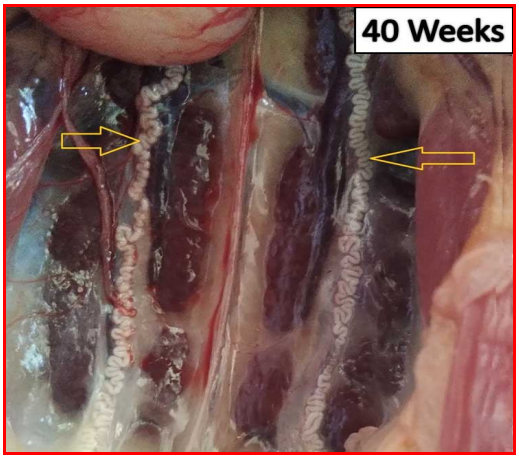

Fig. 4.13. PHOTOGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK (40 WEEKS)

Fig. 4.14. PHOTOGRAPH SHOWING THE PHALLUS OF PATI DUCK AT 1 MONTH OF AGE

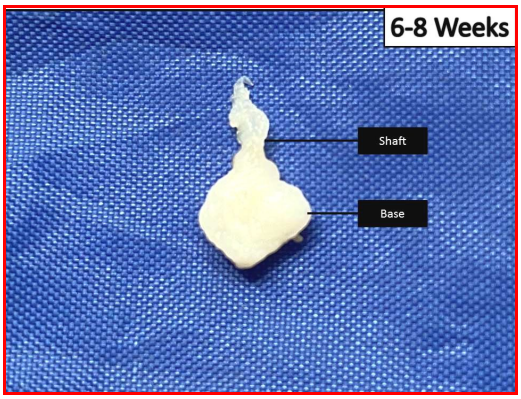

Fig. 4.15. PHOTOGRAPH SHOWING THE PHALLUS OF PATI DUCK AT 6-8 WEEKS OF AGE

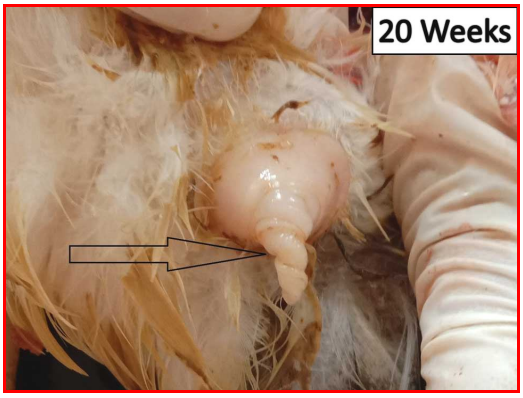

Fig. 4.16. PHOTOGRAPH SHOWING THE PHALLUS OF PATI DUCK AT 20 WEEKS OF AGE

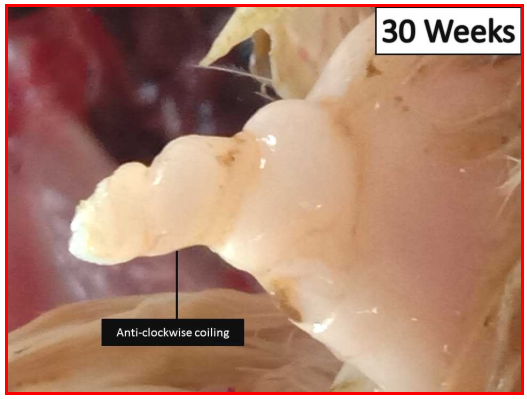

Fig. 4.17. PHOTOGRAPH SHOWING THE PHALLUS OF PATI DUCK AT 30 WEEKS OF AGE

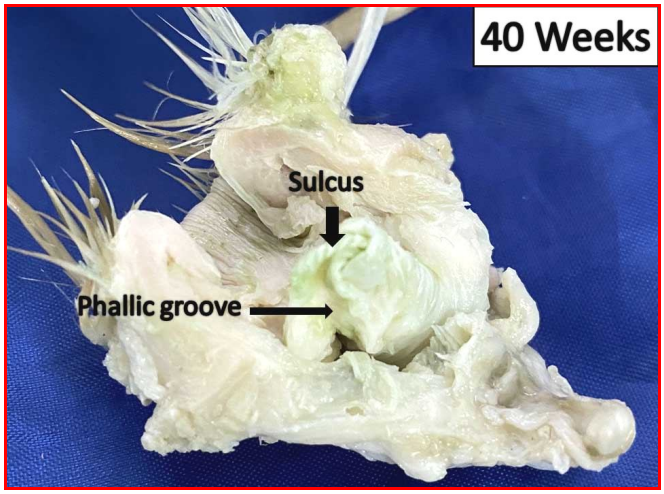

Fig. 4.18. PHOTOGRAPH SHOWING THE PHALLUS OF PATI DUCK AT 40 WEEKS OF AGE

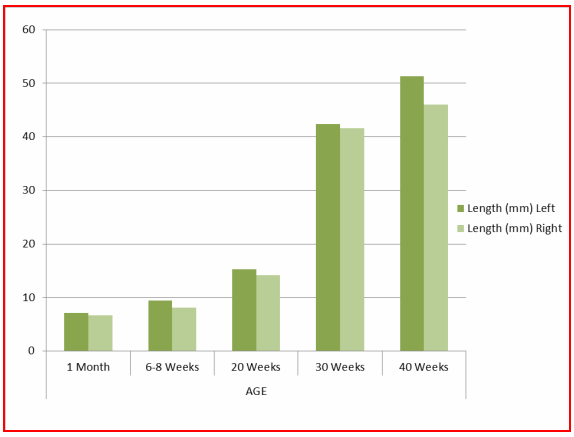

Fig. 4.19. GRAPHICAL REPRESENTATION OF THE AVERAGE LENGTH OF THE TESTIS OF PATI DUCK

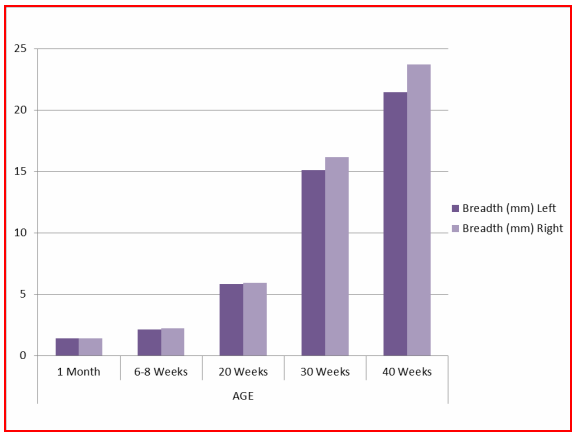

Fig. 4.20. GRAPHICAL REPRESENTATION OF THE AVERAGE BREADTH OF THE TESTIS OF PATI DUCK

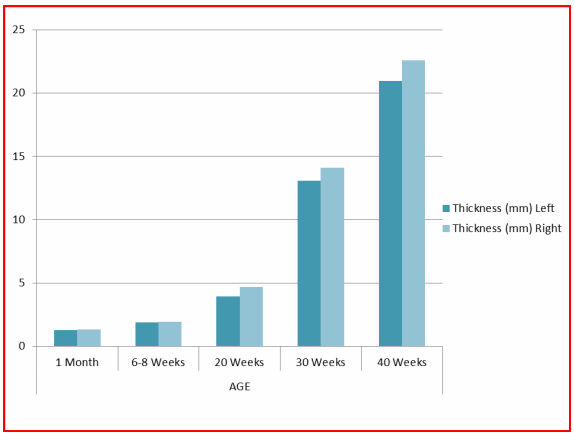

Fig. 4.21. GRAPHICAL REPRESENTATION OF THE AVERAGE THICKNESS OF THE TESTIS OF PATI DUCK

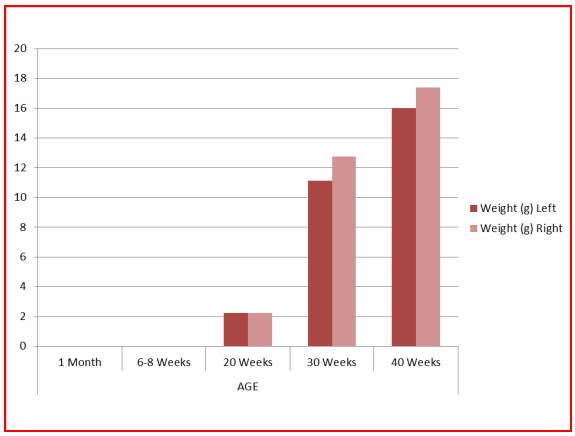

Fig. 4.22. GRAPHICAL REPRESENTATION OF THE AVERAGE WEIGHT OF THE TESTIS OF PATI DUCK

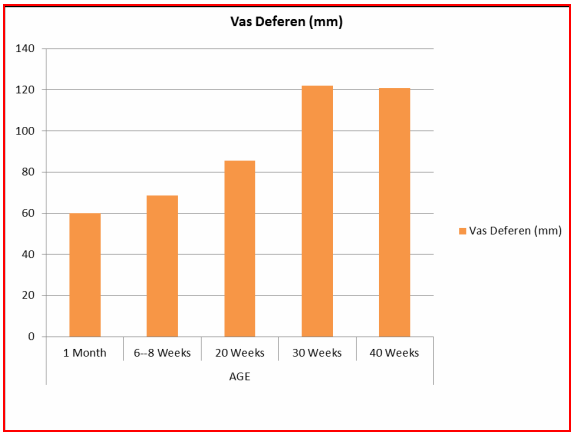

Fig. 4.23. GRAPHICAL REPRESENTATION OF THE AVERAGE LENGTH OF VAS DEFERENS OF PATI DUCK

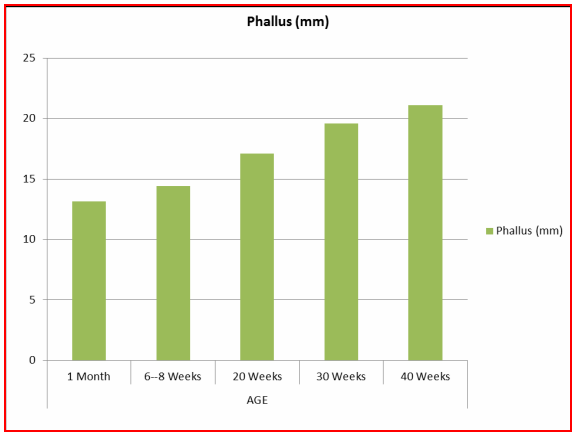

Fig. 4.24. GRAPHICAL REPRESENTATION OF THE AVERAGE LENGTH OF PHALLUS OF PATI DUCK

4.2. HISTOLOGY

The architecture of testis, epididymis, vas deferens and phallus were studied by histomorphological observation on paraffin section. The sections were stained with routine Mayer's hematoxylin and eosin method, Van Gieson's method, Gomori's method, Hart's method and Bielschowsky's method (Luna, 1968) subjected to light microscope. The histological observations were discussed in the following subheads.

4.2.1. Testis

4.2.1.1. Capsule of Testis

In the present study, the testis of Pati duck of Assam were covered by a thin capsule which had 3 layers – tunica serosa, tunica albuginea and tunica vasculosa (Fig. 4.25). The finding was in accordance with the observations by Aire and Ozegbe (2007) in Japanese quail, turkey, domestic fowl, duck and Khatun *et al.* (2019) in Khaki Campbell duck. However the finding was in contrast with the findings of Al-Tememy (2010) in Quail where the testis was covered by dense irregular connective tissue, the tunica albuginea covered by a peritoneum.

The thickness of capsule of the testis in Pati duck at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 9.17±0.98 µm, 12.67±1.26 µm, 11.67±1.43 µm, 12.17±0.6 µm and 14.00±3.39 µm, respectively (Table 4.3). There was no significant difference in capsular thickness among the different age groups while the capsule was thickest in 40 weeks age group (Fig. 4.137). According to Budras and Sauer (1975) there was steady thickening of the capsule till sexual maturity in cock. The capsular thickness observed in the present study was much thinner than the reports forwarded by Hodges (1974) in domestic fowl, Aire and Ozegbe (2007) in quail, Al-Tememy (2010) in quail and Khatun *et al.* (2019) in Khaki Campbell duck. The difference in the capsular thickness of the testis might be attributable to difference in breed and species of the bird studied. It was also observed that the thickness of the capsule of testis of Pati duck testis was thicker in the epididymal region as compared to the free capsule. The finding was similar to the findings of Al- Tememy (2010) in quail testicular capsule.

The tunica serosa was the outermost layer and consisted of simple squamous epithelium (Fig. 4.25). The finding was in accordance with Khatun *et al.* (2019) in Khaki Campbell duck. Whereas Aire and Ozegbe (2007) reported occasional cuboidal and presence of few stubby microvilli on the surface.

The tunica albuginea formed the bulk of the capsule, and is composed of dense connective tissue (Fig. 4.25). The connective tissues were formed by thick collagen fibres (Fig. 4.31 to 4.35), reticular (Fig. 4.36 to 4.40), few elastic fibers (Fig. 4.41 to 4.45), and nerve fibers (Fig. 4.46 to 4.50). Blood vessels were also observed within this layer. The collagen fibres were dispersed in the capsule of 1 month age group (Fig. 4.31) which increased with age at 6-8 weeks, 30 weeks and it appeared like a thick bundle in the capsule of 40 weeks age group (Fig. 4.35). The finding was similar to the findings by Kannan *et al.* (2015) in quail and Razi *et al.* (2010) in white Rooster. According to Fitzgerald (1969) and Breucker (1982) increased in collagen fibre bundles lead to increase thickness of the capsule. However Aire and Ozegbe (2007) report bundles of collagen were infrequently seen between the smooth muscle cell in Japanese quail, turkey, domestic fowl and duck. The difference in the findings could be attributed to the difference in species and breed of the birds.

n the testis of Pati duck, beneath the dense tunica albuginea was a thin layer of loose connective tissue, which consisted of fibroblasts and blood vessels. This layer formed the tunica vasculosa. The finding was similar to the reports forwarded by Aire and Ozegbe (2007) in Japanese quail, turkey, domestic fowl and duck; by Razi *et al.* (2010) in White Rooster.; by Aire (1997); Jamieson (2007) and Salwa *et al.* (2013) in Sudanese duck and Khatun (2019) in Khaki Campbell duck.

4.2.1.2. Seminiferous Tubules of Testis

The parenchyma of the testis of Pati duck of Assam consisted mainly of complex tubules which were highly convoluted and separated by interstitial connective tissue (Fig 4.26 to 4.30). The testes were not divided into lobules and had no mediastinum testis. The finding was supported with the findings of Razi *et al.* (2010) in White Rooster, Kadhem (2014) in indigenous duck of Iraq, Gerjilov *et al.* (2016) in Muscovy duck,

Khatun *et al.* (2019) in Khaki Campbell duck. The diameter of the seminiferous tubules of testis in Pati duck of 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 16.50±1.20 µm, 29.50±2.81 µm, 36.33±1.87 µm, 104.17±7.00 µm and 124.33±13.37 µm, respectively (Table 4.3). The diameter of the seminiferous tubules increased with age and there was significant difference between the different age groups (Fig. 4.138). The diameter of the seminiferous tubules observed in adult was approximately similar with the findings of Tingari *et al.* (1984) in adult chicken (126 and 124 µm) and duck (135 and 134µm); and Razi *et al.* (2010) in white Rooster (162.07±4.70 µm). Whereas the mean width of the seminiferous tubules was 231.46±41.68 µm in Khaki Campbell duck (Khatun *et al.*, 2019). The diameter of the seminiferous tubules increased with age due to the increase in number of layers of germ cells. The diameter of seminiferous tubules also increased during active breeding period which was decreased in non-breeding period [Jamieson (2007); Simoes *et al.* (2017) in domestic duck; Baradi-artoni *et al.* (1999) in gander and Gumulka and Rozenboim (2015) in domestic ganders].

The thickness of the cellular layers of the seminiferous tubules of testis in Pati duck of 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 6.00±0.37, 7.67 ± 0.49, 8.50±0.76, 46.33±4.35 and 48.83±2.98 µm, respectively (Table 4.3). The thickness of cellular layers of the seminiferous tubules increased with the age as the number of layers of cells increase in the testis along with age increased (Fig. 4.139). There was significant different among the different age groups.

Microscopic structure of 1 month old testis showed that there was no distinct layer of cells in the seminiferous tubules which was mainly composed of Sertoli cells along with few spermatogonium and vacuolated cells which had prominent nucleus (Fig. 4.26). This finding was tallied to the findings of Kannan *et al.* (2015), Al-tememy (2010) in quail and Gerjilov *et al.* (2016) in Muscovy duck. The seminiferous tubules of Pati duck did not have a clear cut lumen and the tubules were separated by interstitial cells which were thicker comparatively at 1 month old. But a few tubules were lined by columnar cells and appeared like ducts (Fig. 4.26). The numbers of sertoli, spermatogonium and vacuolated cells were gradually increased and were more distinct at 6-8 weeks of age. The cells within the seminiferous tubules were not arranged in layers as sertoli cells were dispersed in between tightly packed spermatogonium cells (Fig.

4.27). The finding was in contrast to the finding of Gerjilov *et al.* (2016) in Muscovy duck where germ cells formed a layer above the Sertoli cell in 2 months testis. In the present study the lumen of the seminiferous tubules could be identified which was devoid of spermatozoa. The interstitial tissue was lesser in the testis of 6-8 weeks than 1 month age group.

The testis of 20 weeks of age Pati duck of Assam showed that the seminiferous tubules had an increased number of cells which were arranged in 3 or 5 layers (Fig 4.28). There was a large number mitotic dividing cell near the basement membrane as well as towards the lumen while the lumen was devoid of spermatozoa (Fig. 4.28). Numerous sertoli cells and dividing spermatocytes were observed. Sertoli cells and spermatogonium were present near the basement membrane while the primary spermatocytes were found near the lumen. Vacuolated cells were found in the seminiferous tubules up to 20 weeks of age. Interstitial spaces were lesser and seminiferous tubules were closer to adjacent tubules as compared to 1 months and 6-8 weeks testis. The ST were mostly 3-5 layered consisting of sertoli cells, spermatogonium and dividing spermatocyte cells. Some ST didn't have dividing spermatocyte, while some tubules had early and late spermatid in the tubules where the number of layers increased. The present findings contraindicated with the finding of Gerjilov *et al.* (2016) in Muscovy ducks where testis of 5 months old had various stages of spermatogenesis. The difference in findings might be due difference in the age of maturity and different breeds studied which could be achieved due to environmental difference or difference in management.

In the present study 30 weeks and 40 weeks testis showed more or less a similar architecture of the seminiferous tubules which was characterized by a multiple layered cell consisting of 8 to 17 layers with distinct layers formed by different stages of spermatogenesis (Fig. 4.29 and 4.30). However an average of 12 layers in the seminiferous tubules of white Rooster was reported by Razi *et al.* (2010). The basement membrane was formed by connective tissue fibres, cells and smooth muscle fibres. This observation was similar to the findings of Al-Tememy (2010) in quail. Adjacent to the basement were spermatogonium and sertoli cells which formed the first layer of ST, second layer was formed by a mixture of sertoli cells, primary and secondary spermatocytes which resumed till 5 to 6 layers. At around 6^{th} to7^{th} layer the

spermatocytes were replaced by early spermatid which developed to late spermatid from 7th or 8th layers. At around the 11th layer spermatozoa were observed invaded with few late spermatids. The lumens of the seminiferous tubules were occupied by mature spermatozoa (Fig. 4.29 and 4.30).

4.2.1.3. Interstitial Connective tissue

The testis of Pati duck of Assam was not divided into lobes. The spaces between the adjacent seminiferous tubules were formed by the interstitial connective tissue and leydig cells. The interstitial connective tissue occupied large portion of testis in 1 month and 6-8 weeks of age and were located in the space between the small seminiferous tubules. At 20 weeks of age, as seminiferous tubules enlarged and the interstitium part of the testis decreased. The interstitial connective tissues were greatly reduced to a small space between the adjacent seminiferous tubules in 30 and 40 weeks. (Fig. 4.26 to 4.30). The observation of present study was similar to the findings of Al-Tememy (2010) in quail.

The interstitial space consisted of leydig cells, fibroblast-like cells, collagen fibres (Fig. 4.31 to 4.35) and reticular fibres (Fig. 4.39 and 4.40), few elastic fibres (Fig. 4.41 to 4.43), nerve fibres (Fig. 4.46 to 4.50) lymphatic vessels and blood vessels. The finding was in accordance with the findings of Kannan *et al.* (2015) in quail, Al-Tememy (2010) in quail and Khatun *et al.* (2019) in Khaki Campbell duck. In the present study, the connective tissue cells were observed to have some fibroblast-like cells with oval nuclei. The finding was in accordance with Bakst *et al.* (2007) in turkey.

Leydig cells were noticeable in the testis of all age group which might appear in single or small cluster. The finding was in contrast to the report of Kannan *et al.* (2015) in quail where they were noticed only from 6 weeks of age. In the present study the leydig cells were of different shapes *viz.,* oval, spherical and elongated like fibroblast in shape and had a spherical or elongated nucleus and prominent nucleolus. The observation was in accordance with the findings of Khatun *et al.* (2019) in Khaki Campbell duck. However Rosenstrauch *et al.* (1998) in aging rooster and Kareem *et al.* (2020) in adult duck observed polyhehral, uninucleated and large leydig cells.

4.2.2. Epididymis

The epididymal region of testis of Pati duck consisted of rete testis, efferent duct, collecting duct and epididymal duct which were separated by connective tissue. The seminiferous tubules passed directly into the rete testis. The finding was in accordance with the observation of Tingari (1971) in domestic fowl, Abd-Elmaksoud *et al.* (2009) in Sudani duck and Razi *et al.* (2010) in white Rooster. However, Aire *et al.* (1979) in Guinea fowl, Barker (1983) in wild bird and Al-Tememy (2010) in quail reported the presence of tubuli recti which was dilated tubules between the seminiferous tubules and rete testis. The difference in findings with the present study might be due to the difference in species of the avian studied.

Rete testis were found within the capsule (intracapsular) and outside the capsule (extracapsular) of the testis some of which were in direct contact with efferent ducts (Fig. 4.51 to 4.55). It was a narrow channel lined by simple low cuboidal or squamous epithelium and surrounded by connective tissue with smooth muscle fibre. The observation was similar to the findings of Barker and Kendall (1984) in starling wild bird, Clulow and Jones (1988) in Japanese quail, Abd-Elmaksoud *et al. (*2009) in Sudani duck, Al-Tememy (2010) in Quail and Razi *et al.* (2010) in White Rooster. However, Aire (1982) reported the presence of intratesticular rete testis in addition to intracapsular and extra testicular part in domestic fowl, Japanese quail and duck but was absent in Pati duck of Assam. Whereas Tingari (1971) reported that rete testis were located entirely outside the testis in domestic fowl. The difference in findings might be due to difference in species and breed of avian studied.

In the present study rete testes were small and mainly present within the capsule (intra capsular) as extra capsular rete testis were less developed in 1 month and 6-8 weeks. In 20 weeks a few and small extra capsular rete testis which was surrounded by abundant connective tissue was observed. The lining epitheliums were mostly cuboidal in 1 month, 6-8 weeks and 20 weeks which changed to simple squamous epithelium in 30 and 40 weeks of age. In younger ducks connective tissue was more abundant than the rete testis which drastically changed to a few connective tissues surrounding the large dilated rete testis in 30 and 40 weeks. At the junction between the rete testis and efferent

duct the epithelium changed from simple squamous to columnar epithelium. Similar finding was reported by Aire *et al.* (1979) in Guinea fowl and Hess *et al.* (1976) in Turkey.

In Pati duck the different ducts of the epididymis *viz.,* efferent duct, connecting duct and epididymal duct were all extra capsular. The volume of connective tissue in the epididymis surrounding the ducts was comparatively higher in 1 months, 6-8 weeks and 20 weeks age group. Similar finding was reported by Tingari (1971) in immature and inactive testis of domestic fowl. In the present study the connective tissue decreased in 30 and 40 weeks age group. The difference in volume of connective tissue might be due to enlargement of the efferent duct, connecting duct and epididymal duct at 30 and 40 weeks as spermatozoa filled the ducts when the bird matured. The connective tissue consisted of numerous mononuclear cells and lymphoid nodules which was scattered in the periductal region. The observation was similar to the findings of Aire (1979) in domestic fowl, Japanese quail and Guinea fowl. These lymphoid cells were indicative of the day-to-day immunological responses of the bird to its environment Aire (1979) while presence of solitary non-encapsulated lymphatic nodules were regarded as normal (King and McLelland, 1975). In Pati duck the periductal connective tissue of the epididymis consisted of blood vessels, numerous collagen and reticular fibres (Fig. 4.66 to 4.75). The amount of reticular fibres in the periductal connective tissue increased with age of the ducks. A small amount of elastic fibres and nerve fibres were also observed (Fig. 4.76 to 4.85).

Histological observation of the epididymis showed that the ducts were empty and were devoid of spermatozoa at 1 month, 6-8 weeks and 20 weeks. The ducts of the epididymis of 30 and 40 weeks age group were observed to be filled with spermatozoa. It was also observed that the connecting ducts and epididymal duct were filled with spermatozoa. However some efferent duct had few spermatozoa while majority were empty. The observation was similar to the findings of Aire (1979) in the proximal efferent duct of Guinea fowl and Tingari (1971) in domestic fowl. According to Tingari (1971) the high density of spermatozoa in the lumen of the connecting ducts could be due to fluid vehicle being absorbed in the efferent duct or to a mechanical accumulation of spermatozoa, as they moved from the wide efferent ducts into the narrower connecting

ducts. In the present study it was observed that the efferent duct and collecting duct were almost same in diameter, however in few cases the efferent duct was slightly larger than the connecting duct in 1 month, 6-8 weeks and 20 weeks. There was greater difference in the diameter of the efferent and connecting duct in 30 and 40 weeks of age (Fig. 4.140).

The efferent duct was larger of two ducts and was tortuous and had many folds while the smaller collecting duct was circular duct without folded (Fig 4.56 to 4.60.). The epithelial lining of the efferent duct were folded villi-like lining which project into the lumen and were lined by ciliated pseudostratified columnar epithelium. The finding was in accordance with the reports of Aire *et al.* (1979) in Guinea fowl, Razi *et al.* (2010) in White Rooster and Clulow and Jones (1988) in Japanese quail. In the present study some of the efferent duct had no folding on the epithelial lining (Fig. 4.58 and 4.59). According to Abd-Elmaksoud *et al.* (2009) the smooth walled efferent duct was the distal part of efferent duct while the folded epithelial was proximal part of efferent duct The diameter of the efferent duct of epididymis in Pati duck of 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 18.50±0.67 µm, 37.50±1.28 µm, 43.50±5.49 µm, 91.33±5.04 µm and 122.83±11.66 µm respectively (Table 4.3). The finding in the present study was comparable to the observation of Razi *et al.* (2010) in White Rooster with efferent duct diameter 98 -103 µm. However, Tingari (1971) observed efferent duct diameter of 500 µm initially and reduced to 100µm towards the distal part in domestic fowl.

The collecting ducts were lined by non-ciliated pseudostratified columnar epithelium which had a few micro villi (Fig. 4.56 to 4.60). The finding was similar to the findings by Tingari (1971) in domestic fowl, Abd-Elmaksoud *et al.* (2009) in Sudani duck and Razi *et al.* (2010) in white Rooster. There were occasional basal cells in the basal lamina which had elongated oval nucleus. Similar to the collecting duct, the epididymal duct had pseudostratified columnar epithelial lining except the later was larger in diameter. The finding was in accordance with the reports of Abd-Elmaksoud *et al.* (2009) in Sudani duck. In the present study the diameter of the connecting duct of epididymis in Pati duck of 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 15.33±1.56 µm, 30.83±1.01 µm, 35.33±2.62 µm, 69.83±2.21 µm and 79.50±4.92 µm, respectively (Table 4.3). The diameter increased with age as the size of the epididymis

became more prominent with increased in age (Fig. 4.140). The diameter of collecting duct in domestic fowl as per Tingari (1971) was 60 µm.

4.2.3. Vas deferens

The diameter of vas deferens of Pati duck in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks was 36.50±1.98 µm, 42.00±1.71 µm, 61.50±2.75 µm, 158.00±17.39 µm and 243.67 ±22.76 µm, respectively (Table 4.3). There was slight increase in the diameter of vas deferens from 1 month to 20 weeks without any significant difference. However, there was significant difference in the diameter of vas deferens when the bird attained 30 weeks to 40 weeks of age (Fig. 4.141). Tingari (1971) reported that diameter of domestic fowl was 400µm at its cranial end.

The thickness of the vas deferens at 1 month, 6-8 weeks, 20 weeks, 30 week and 40 weeks of Pati duck were 2.17± 0.31, 2.67±0.42, 4.67±0.56, 9.50±1.06 and 14.67±1.61 µm, respectively (Table 4.3). The thickness of wall of vas deferens increased with age but the difference was less and non-significant up to 20 weeks. However there was significant increase in thickness of vas deferens at 30 weeks and 40 weeks.

Histologically, the vas deferens was lined by pseudostratified columnar epithelium (Fig. 4.86 to 4.90.). The finding was in accordance with the reports of Hess *et al.* (1976) in turkey, Razi *et al.* (2010) in White Rooster. In Pati duck the epithelium at the cranial part of vas deferens was smooth while the caudal part had folded epithelium along with the basal cells (Fig. 4.89). Similar findings were reported by Tingari *et al.* (1971) in domestic fowl, Hess *et al.* (1976) in Turkey, Aire *et al.* (1979) in guinea fowl. In the present investigation, a smooth muscle layer was observed after the lining epithelium where collagen fiber (Fig. 4.91 to 4.95), reticular fibers (Fig. 4.96 to 4.100), nerve fibers (Fig. 4.106 to 4.110) and few elastic fibers (Fig. 4.101 to 4.105) were observed. It was followed by loose connective tissue which had some blood vessels in it. The finding was similar to the findings of Tingari (1971) in domestic fowl, Aire (1979) in Japanese quail.

4.2.4. Phallus

The tubular phallus of Pati duck of Assam had a narrow lumen (Fig. 4.111) with its mucosal wall lined by non-keratinized stratified squamous epithelium (Fig. 4.116). The sub epithelial layer of lamina propria was a dense connective tissue which was a continued by a layer of loose connective tissue and consisted of abundant fibroblast, reticular fibres (Fig. 4.126), collagen fibres (Fig. 4.117 and 4.118), and few elastic (Fig. 4.127 and 4.128) and nerve fibres (Fig. 4.132 and 4.133). The finding was in accordance with the observation by Olivera and Mahecha (2000) in *Nothura maculosa* and Previatto *et al.* (2017) in *Ortalis canicollis.*

A layer of connective tissue consisted of large lymphatic spaces was observed in the entire circumference in the phallus of Pati duck (Fig. 4.113). The lymphatic space was lined by endothelium and crossed by trabeculae. The connective tissue around the lymphatic space and trabeculae were rich in reticular fibres (Fig 4.122 to 4.124) and collagen fibres (Fig. 4.117 and 4.118). Few elastic (Fig. 4.129 and 4.131) and nerve fibres (Fig. 4.134 and 4.136) were also observed. Blood vessels were mainly present in this layer. The finding was in accordance with Olivera and Mahecha (2000) in *Nothura maculosa.* According to Kirby and Froman (2000) presence of large lymphatic space might be the cause of the avian phallus was solely reproductive and became engorged by lymph fluid instead of blood during erection. In the present study few smooth muscle cells were observed along with other connective tissue in this layer.

The outer surface of the Phallus was lined by keratinized stratified squamous epithelium with numerous fibroblasts (Fig. 4.112 and 4.114). The finding was in accordance with Olivera and Mahecha (2000) in *Nothura maculosa* and Brennan *et al.* (2010) in duck. In the phallus of Pati duck the keratinized epithelium formed a spiked and ridges on its surface. Several layer of the epithelial cells projected into the underlying connective tissue forming pegs (Fig. 4.112). The connective tissue beneath the keratinized epithelium consisted of numerous tubuloacinar glands along with ducts (Fig. 4.114) and the connective tissue was rich in collagen (Fig. 4.119 to 4.121) and reticular fibres (Fig. 4.123 to 4.125). The ducts were lined by cuboidal cells. According

to Previatto *et al.* (2017) the presence of tubuloacinar glands gave a secretory aspect to these regions.

In the wall of the shaft of the phallus there was an open groove which is the sulcus of the phallus (Fig. 4.115). The finding was in contrast to the observation of Oliveira and Mahecha (2000) in *Nothura maculosa* where the phallic groove or sulci were found in the interior of tubular phallus. The difference in findings might be due to the difference in species. The diameter of the lumen of tubular phallus was increased with the advancement of age of the Pati duck.

TABLE 4.3. THE AVERAGE THICKNESS OF THE CAPSULE, THE DIAMETER OF THE SEMINIFEROUS TUBULES AND THE HEIGHT OF THE CELLULAR LAYER OF THE SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK DURING POSTNATAL DEVELOPMENT.

Histology micrometry	AGE				
	1 Month	6--8 Weeks	20 Weeks	30 Weeks	40 Weeks
Capsular Thickness (µm)	9.17± 0.98	12.67±1.26	11.67±1.43	12.17±0.60	14.00±3.39
Seminiferous tubules diameter (µm)	16.50±1.20^{B}	29.50±2.81^{B}	36.33±1.87^{B}	104.17±7.00^{A}	124.33±13.37^{A}
Thickness of cellular layers in ST (µm)	6.00±0.37^{B}	7.67±0.49^{B}	8.50±0.76^{B}	46.33±4.35^{A}	48.83±2.98^{A}
Efferent duct diameter (µm)	18.50±0.67	37.50±1.28	43.50±5.49	91.33±5.04	122.83±11.66
Connecting duct diameter (µm)	15.33±1.56^{D}	30.83±1.01^{C}	35.33±2.62^{C}	69.83±2.21^{B}	79.50±4.92^{A}
VD Diameter (µm)	36.50±1.98^{C}	42.00±1.71^{C}	61.50±2.75^{C}	158.0±17.39^{B}	243.67±22.76^{A}
VD Thickness (µm)	2.17±0.31^{C}	2.67±0.42^{C}	4.67±0.56^{C}	9.50±1.06^{B}	14.67±1.61^{A}

*Means with different superscript are significantly different.

Dependent Variable: Seminiferous tubules diameter (µm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Week	4	56691.66667	14172.91667	49.06	<.0001
Error	25	7222.50000	288.90000		
Total	29	63914.16667			

Dependent Variable: Height of tubules (µm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Week	4	11670.46667	2917.61667	84.52	<.0001
Error	25	863.00000	34.52000		
Total	29	12533.46667			

Dependent Variable: Connecting duct (µm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Week	4	17846.33333	4461.58333	94.38	<.0001
Error	25	1181.83333	47.27333		
Corrected Total	29	19028.16667			

Dependent Variable: VD Diameter (µm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Week	4	195212.3333	48803.0833	48.70	<.0001
Error	25	25054.3333	1002.1733		
Total	29	220266.6667			

Dependent Variable: VD Thickness (µm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Week	4	673.5333333	168.3833333	32.80	<.0001
Error	25	128.3333333	5.1333333		
Total	29	801.8666667			

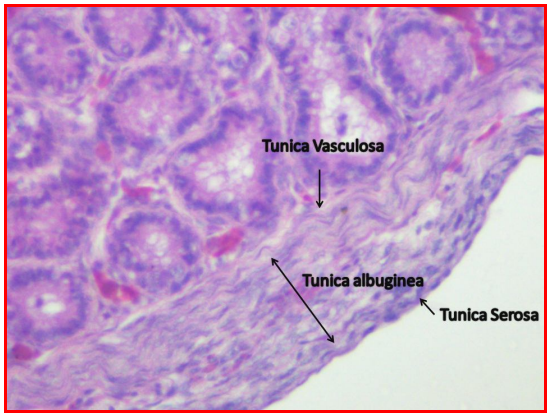

Fig. 4.25. PHOTOMICROGRAPH SHOWING THE DIFFERENT LAYES OF CAPSULE OF TESTIS OF PATI DUCK OF 20 WEEKS (H&E, 40X)

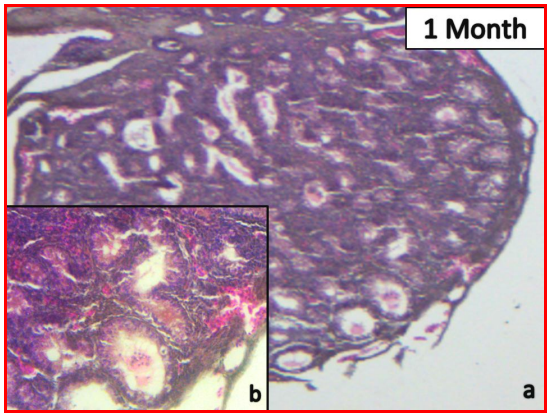

Fig. 4.26. PHOTOMICROGRAPH SHOWING THE TESTIS OF PATI DUCK OF 1 MONTH OF AGE.(H & E , 10X, 100X)

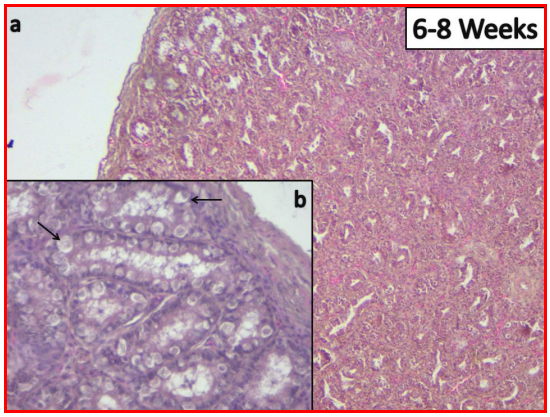

Fig. 4.27. PHOTOMICROGRAPH SHOWING THE TESTIS OF PATI DUCK OF 6-8 WEEKS OF AGE. SHOWING VACOULATED CELLS (→)(H & E , 10X, 100X)

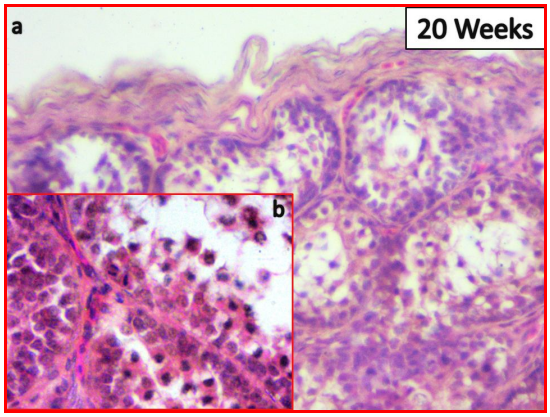

Fig. 4.28. PHOTOMICROGRAPH SHOWING THE TESTIS OF PATI DUCK OF 20 WEEKS OF AGE.(H & E , 10X, 100X)

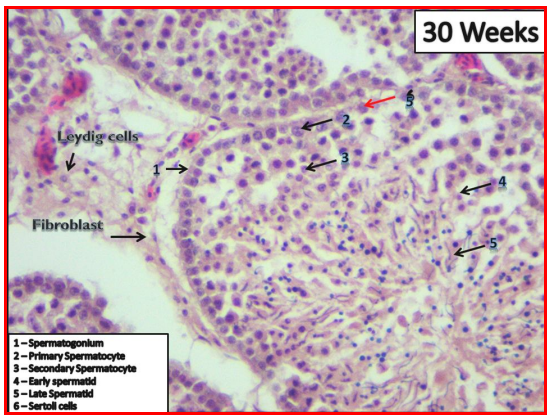

Fig. 4.29. PHOTOMICROGRAPH SHOWING THE TESTIS OF PATI DUCK OF 30 WEEKS OF AGE.(H & E, 10X, 100X)

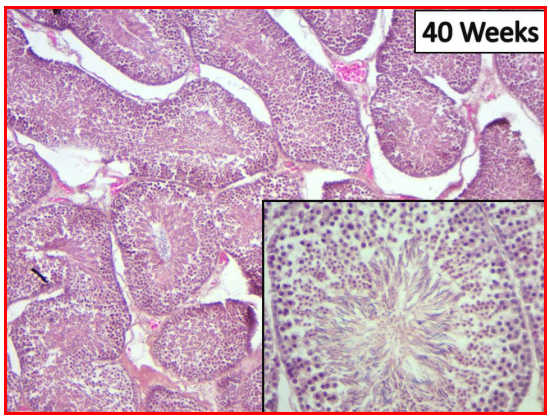

Fig. 4.30. PHOTOMICROGRAPH SHOWING THE TESTIS OF PATI DUCK OF 40 WEEKS OF AGE. (H & E, 10X, 40X)

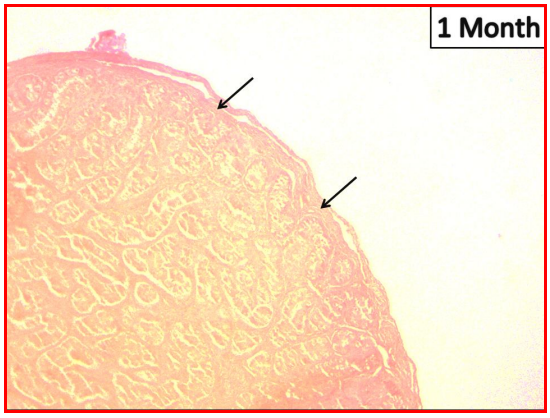

Fig. 4.31. PHOTOMICROGRAPH OF SHOWING THE COLLAGEN FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 1 MONTH. (40X, VAN GIESON'S)

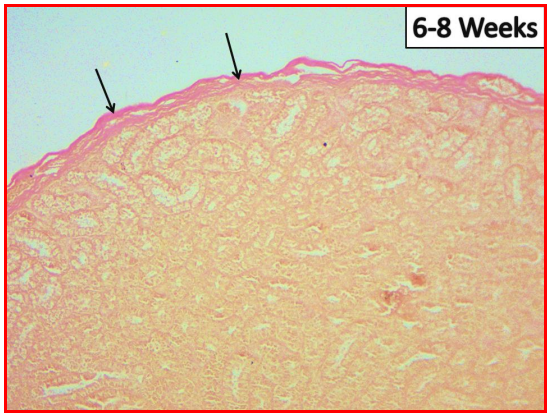

Fig. 4.32. PHOTOMICROGRAPH OF SHOWING THE COLLAGEN FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 6-8 WEEKS (40X, VAN GIESON'S)

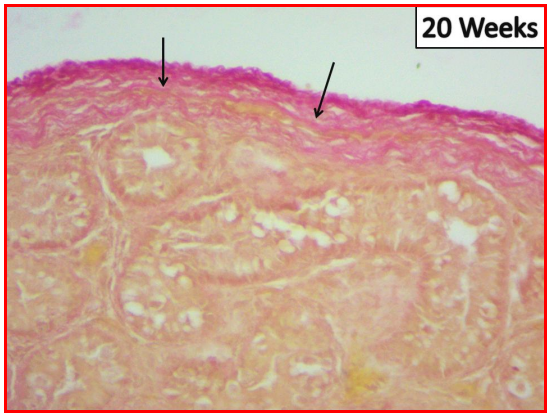

Fig. 4.33. PHOTOMICROGRAPH OF SHOWING THE COLLAGEN FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 20 WEEKS (40X, VAN GIESON'S)

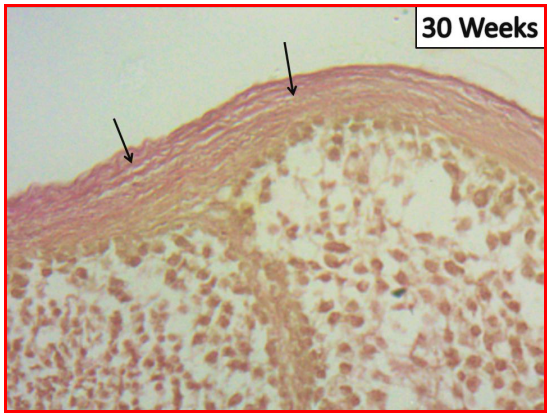

Fig. 4.34. PHOTOMICROGRAPH OF SHOWING THE COLLAGEN FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 30 WEEKS (40X, VAN GIESON'S)

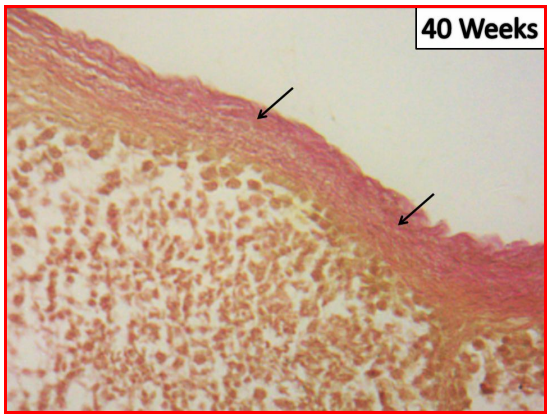

Fig. 4.35. PHOTOMICROGRAPH OF SHOWING THE COLLAGEN FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 40 WEEKS (40X, VAN GIESON'S)

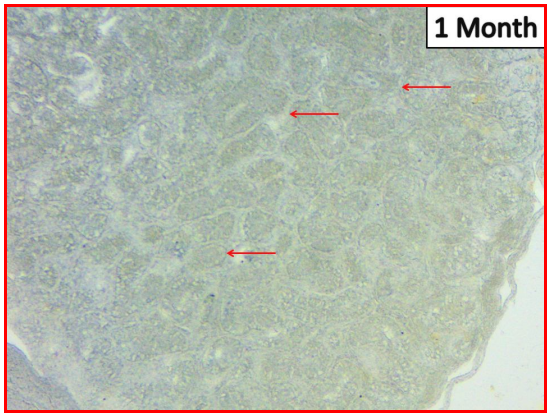

Fig. 4.36. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 1 MONTH (10X, GOMORI)

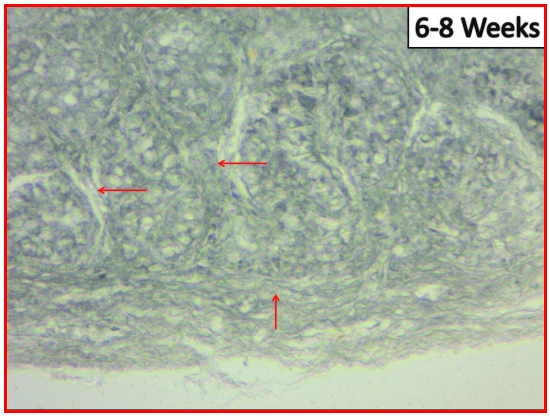

Fig. 4.37. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 6-8 WEEKS (40X, GOMORI)

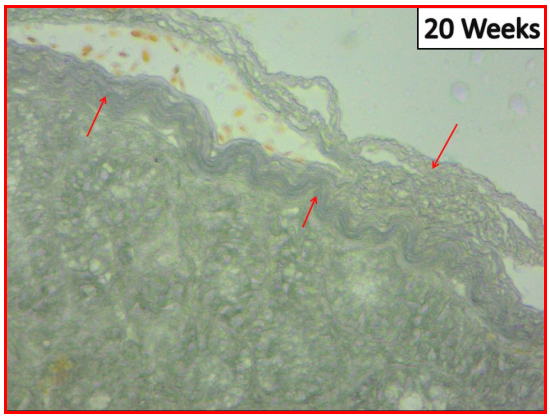

Fig. 4.38. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 20 WEEKS (40X, GOMORI)

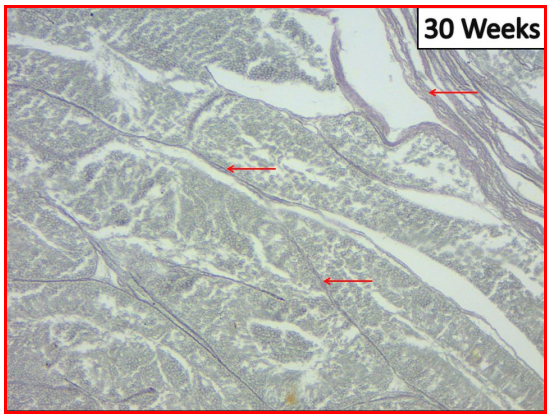

Fig. 4.39. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 30 WEEKS (10X, GOMORI)

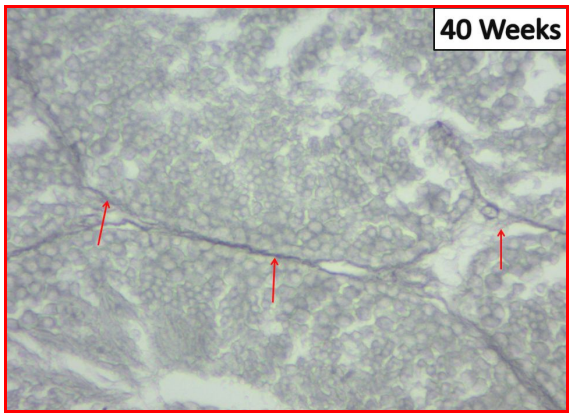

Fig. 4.40. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE TESTIS OF PATI DUCK 40 WEEKS (40X, GOMORI)

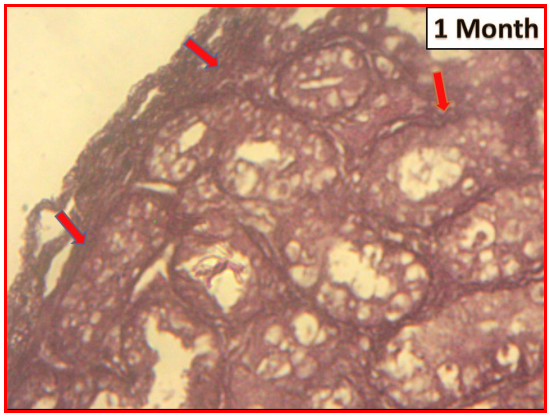

Fig. 4.41. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 1 MONTH AGE GROUP (HART'S, 40X)

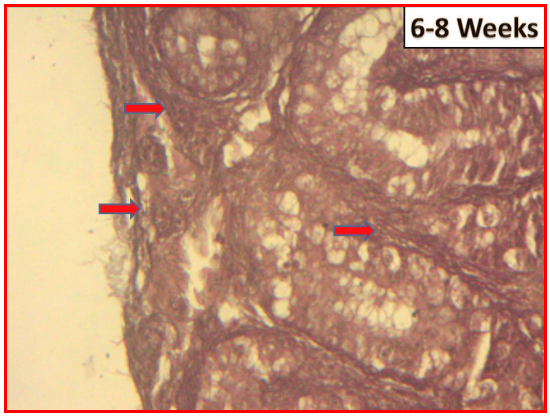

Fig. 4.42. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (HART'S, 40X)

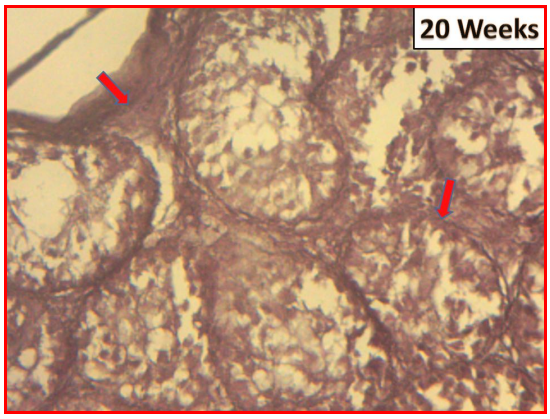

Fig. 4.43. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 20 WEEKS AGE GROUP (HART'S, 40X)

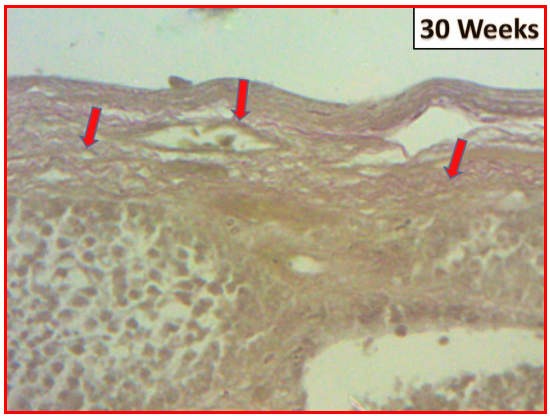

Fig. 4.44. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 30 WEEKS AGE GROUP (HART'S, 40X)

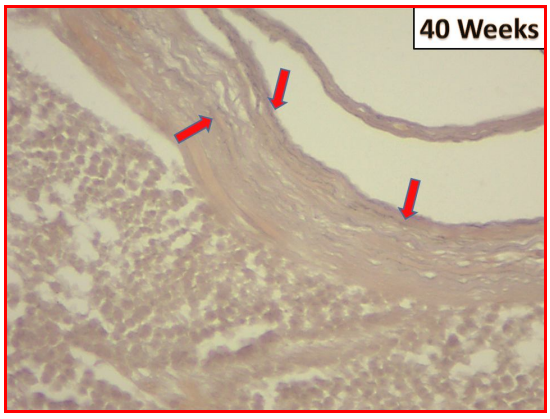

Fig. 4.45. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 40 WEEKS AGE GROUP (HART'S, 40X)

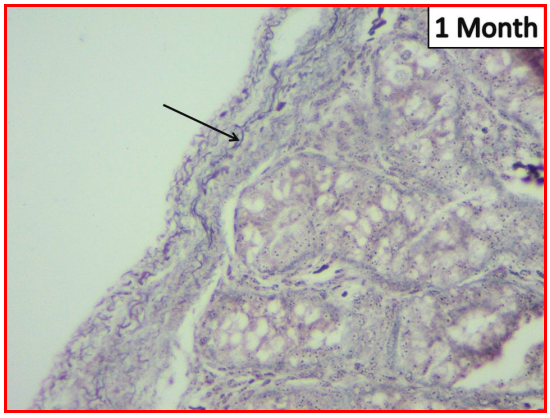

Fig. 4.46. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 1 MONTH AGE GROUP (BIELSCHOWSKY'S, 40X)

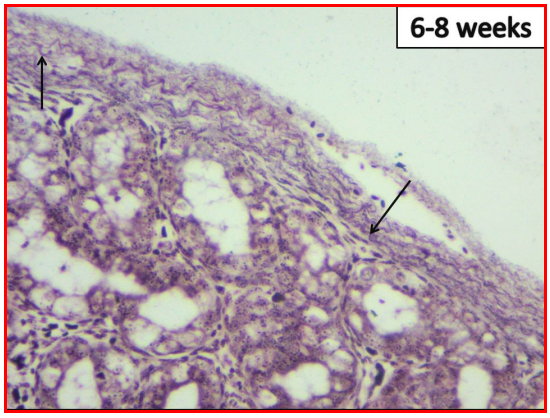

Fig. 4.47. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

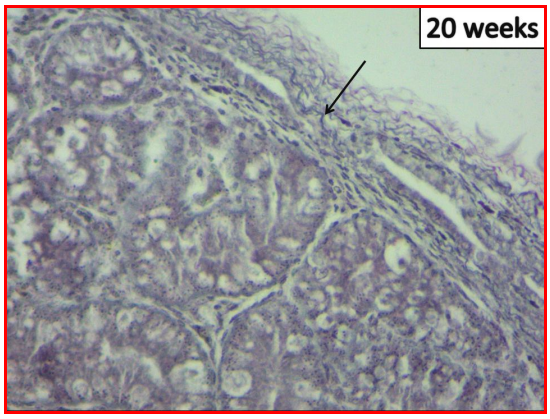

Fig. 4.48. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 20 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

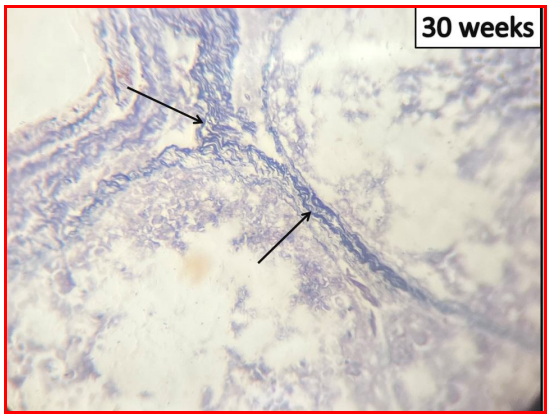

Fig. 4.49. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 30 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

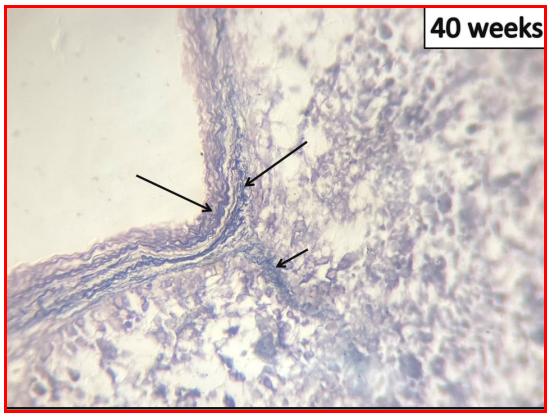

Fig. 4.50. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE TESTIS OF PATI DUCK OF 40 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

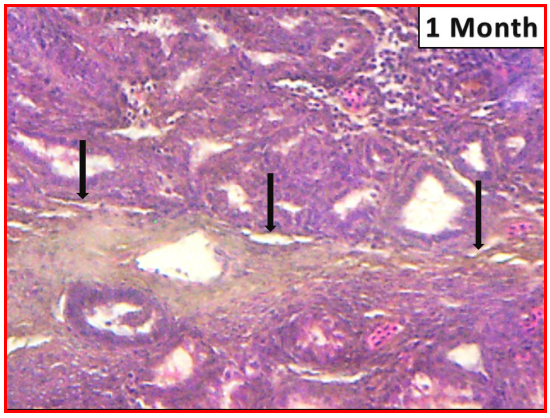

Fig. 4.51. PHOTOMICROGRAPH SHOWING THE RETE TESTIS (ARROW) OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , 40X)

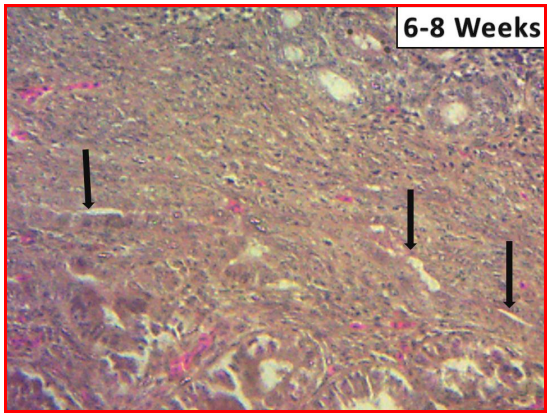

Fig. 4.52. PHOTOMICROGRAPH SHOWING THE RETE TESTIS (ARROW) OF PATI DUCK OF 6-8 WEEKS AGE GROUP (H & E , 40X)

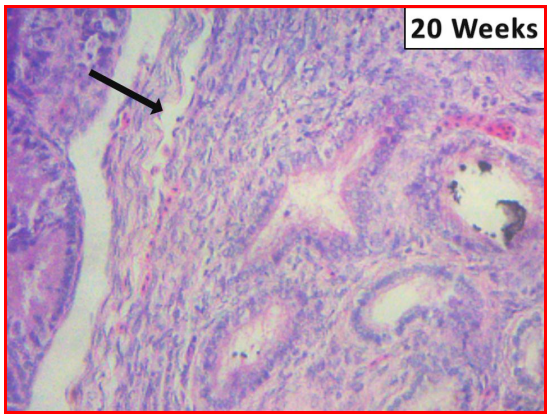

Fig. 4.53. PHOTOMICROGRAPH SHOWING THE RETE TESTIS (ARROW) OF PATI DUCK OF 20 WEEKS AGE GROUP (H & E , 40X)

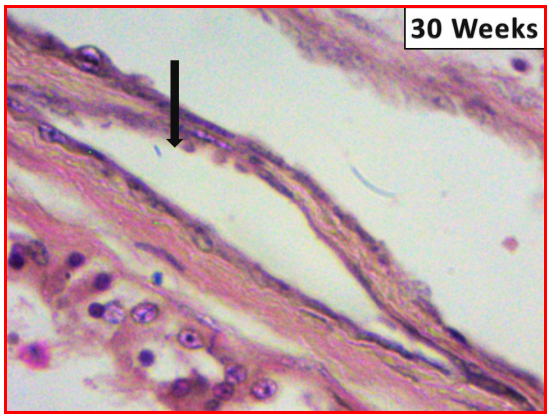

Fig. 4.54. PHOTOMICROGRAPH SHOWING THE RETE TESTIS (ARROW) OF PATI DUCK OF 30 WEEKS AGE GROUP (H & E , 40X)

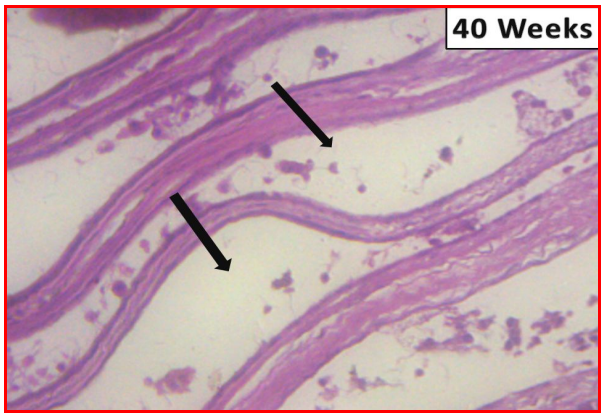

Fig. 4.55. PHOTOMICROGRAPH SHOWING THE RETE TESTIS (ARROW) OF PATI DUCK OF 40 WEEKS AGE GROUP (H & E , 40X)

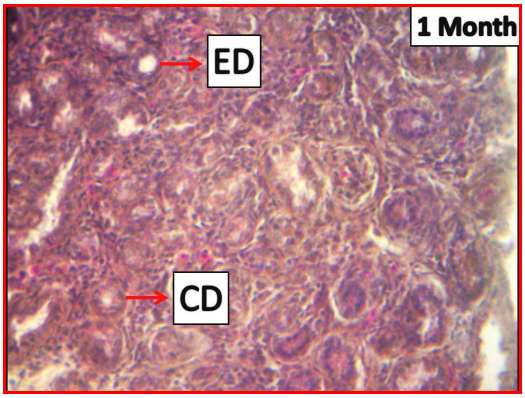

Fig. 4.56. PHOTOMICROGRAPH SHOWING THE EFFERENT DUCT AND CONNECTING DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , 40X)

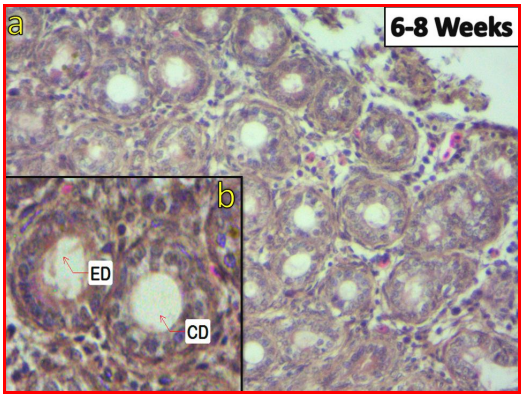

Fig. 4.57. PHOTOMICROGRAPH SHOWING THE EFFERENT DUCT AND CONNECTING DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (H & E , a - 40X, b- 100X)

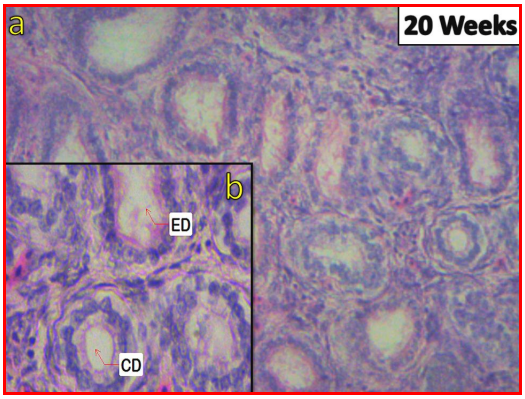

Fig. 4.58. PHOTOMICROGRAPH SHOWING THE EFFERENT DUCT AND CONNECTING DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 20 WEEKS AGE GROUP (H & E , a - 40X, b- 100X)

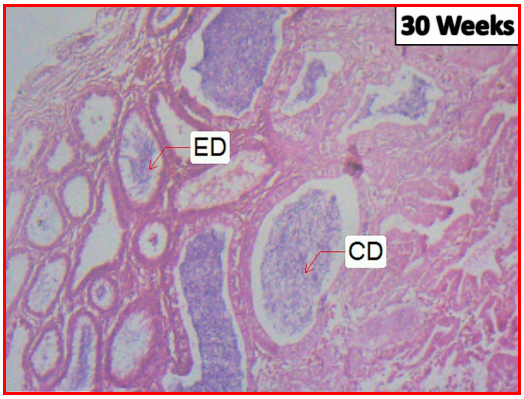

Fig. 4.59. PHOTOMICROGRAPH SHOWING THE EFFERENT DUCT AND CONNECTING DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 30 WEEKS AGE GROUP (H & E , 40X)

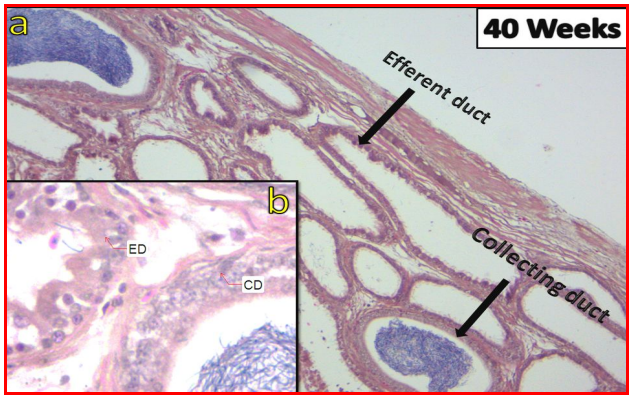

Fig. 4.60. PHOTOMICROGRAPH SHOWING THE EFFERENT DUCT AND CONNECTING DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 40 WEEKS AGE GROUP (H & E , a - 40X, b- 100X)

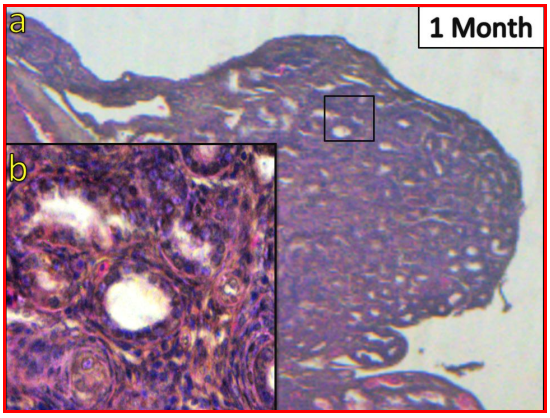

Fig. 4.61. PHOTOMICROGRAPH SHOWING THE EPIDIDYMAL DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , a - 10X, b- 100X)

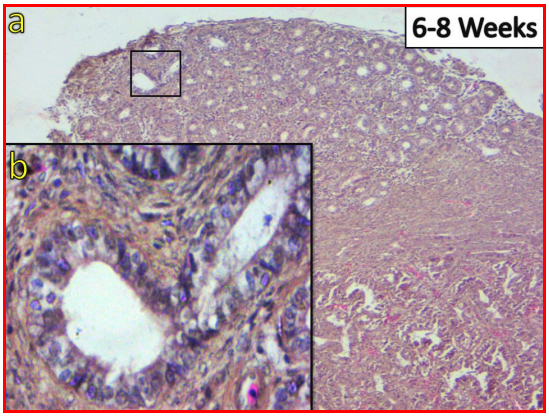

Fig. 4.62. PHOTOMICROGRAPH SHOWING THE EPIDIDYMAL DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (H & E , a - 10X, b- 100X)

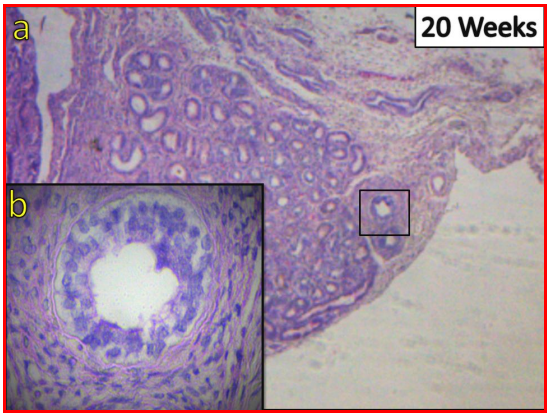

Fig. 4.63. PHOTOMICROGRAPH SHOWING THE EPIDIDYMAL DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 20 WEEKS AGE GROUP (H& E , a - 10X, b- 100X)

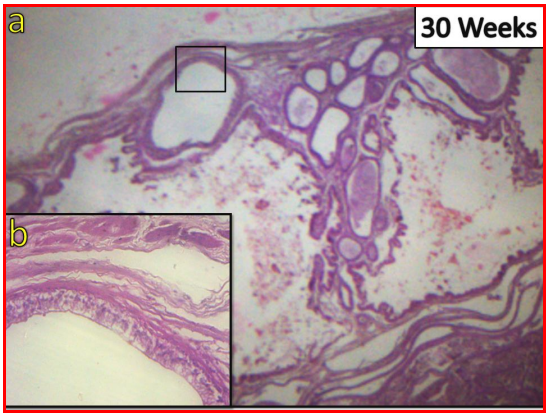

Fig. 4.64. PHOTOMICROGRAPH SHOWING THE EPIDIDYMAL DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 30 WEEKS AGE GROUP (H & E , a - 40X, b- 100X)

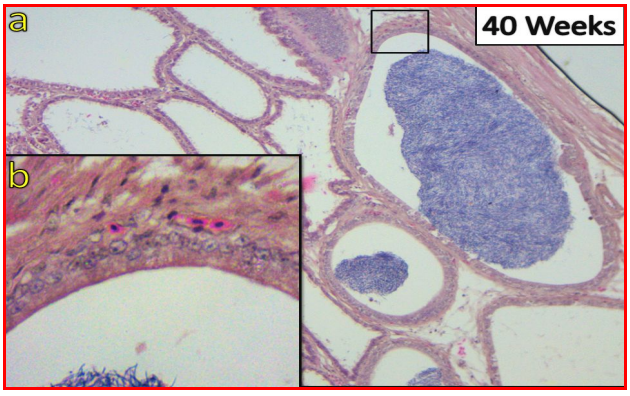

Fig. 4.65. PHOTOMICROGRAPH SHOWING THE EPIDIDYMAL DUCT OF THE EPIDIDYMIS OF PATI DUCK OF 40 WEEKS AGE GROUP (H & E , a - 40X, b- 100X)

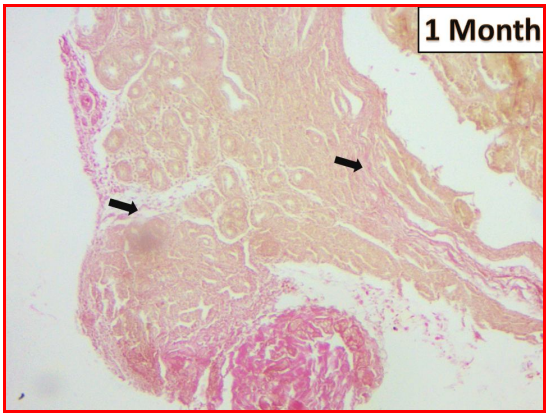

Fig. 4.66. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES OF EPIDIDYMIS OF PATI DUCK OF 1 MONTH AGE GROUP (VAN GIESON'S, 10X)

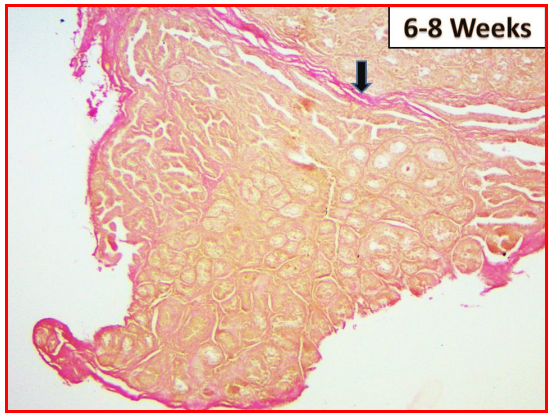

Fig. 4.67. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES OF EPIDIDYMIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (VAN GIESON'S, 10X)

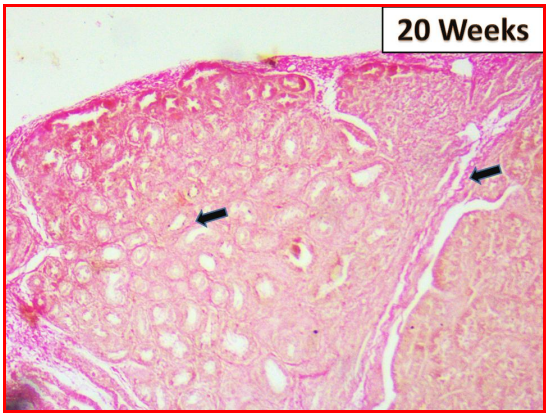

Fig. 4.68. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROW) OF EPIDIDYMIS OF PATI DUCK OF 20 WEEKS AGE GROUP (VAN GIESON'S, 10X)

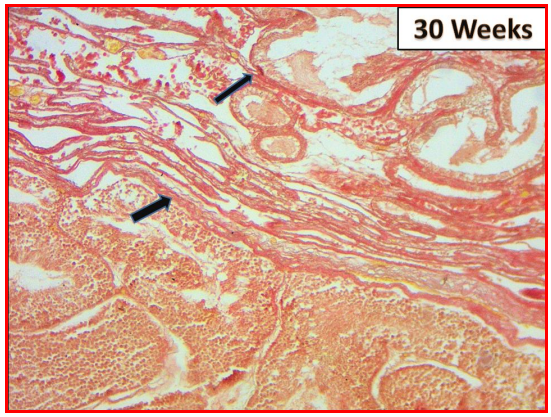

Fig. 4.69. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROW) OF EPIDIDYMIS OF PATI DUCK OF 30 WEEKS AGE GROUP (VAN GIESON'S, 40X)

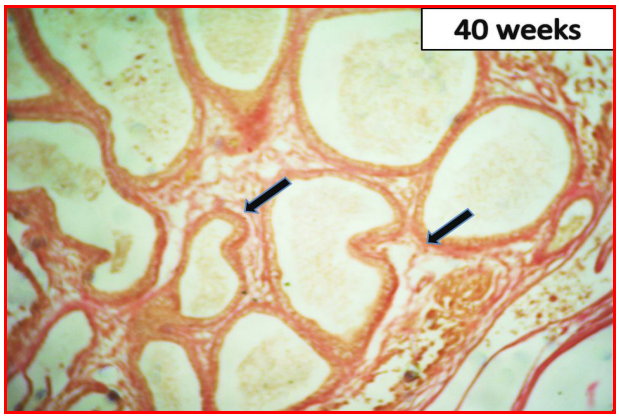

Fig. 4.70. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROW) OF EPIDIDYMIS OF PATI DUCK OF 40 WEEKS AGE GROUP (VAN GIESON'S , 40X)

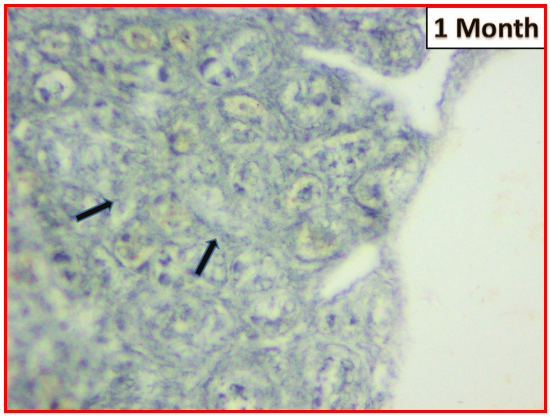

Fig. 4.71. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS(ARROW) OF THE EPIDIDYMIS OF PATI DUCK 1 MONTH (40X, GOMORI)

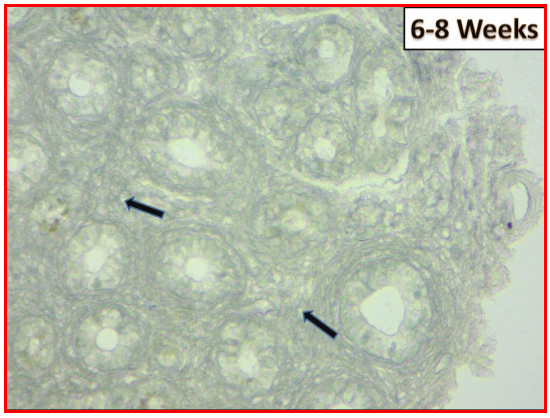

Fig. 4.72. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS(ARROW) OF THE EPIDIDYMIS OF PATI DUCK 6-8 WEEKS (40X, GOMORI)

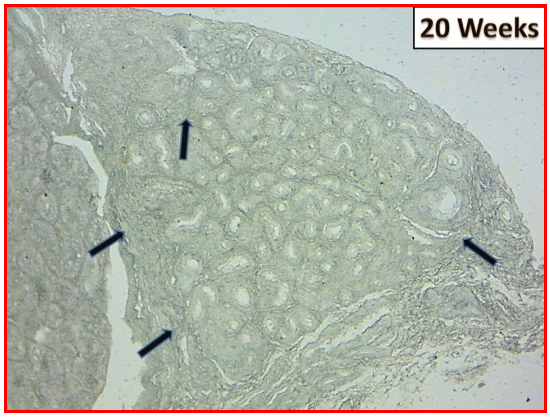

Fig. 4.73. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS(ARROW) OF THE EPIDIDYMIS OF PATI DUCK 20 WEEKS (10X, GOMORI)

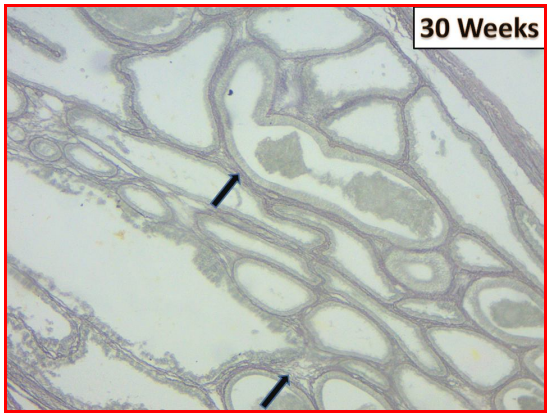

Fig. 4.74. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS(ARROW) OF THE EPIDIDYMIS OF PATI DUCK 30 WEEKS (40X, GOMORI)

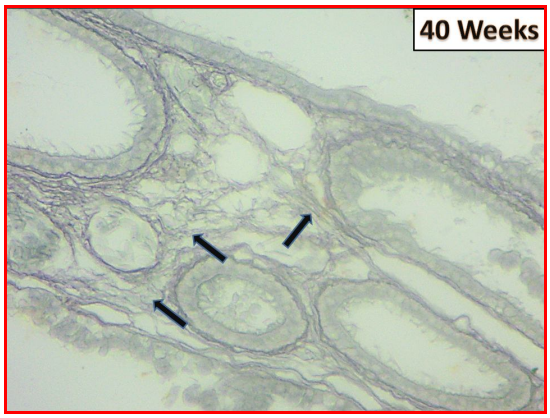

Fig. 4.75. PHOTOMICROGRAPH OF SHOWING THE RETICULAR FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK 40 WEEKS (40X, GOMORI)

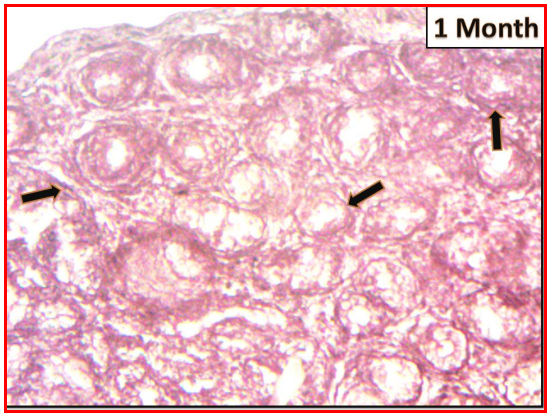

Fig. 4.76. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 1 MONTH AGE GROUP (HART'S, 40X)

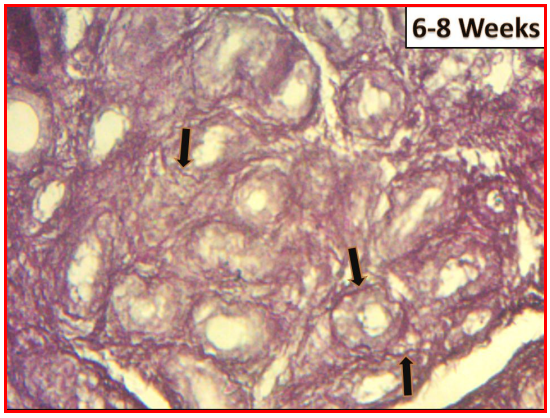

Fig. 4.77. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (HART'S, 40X)

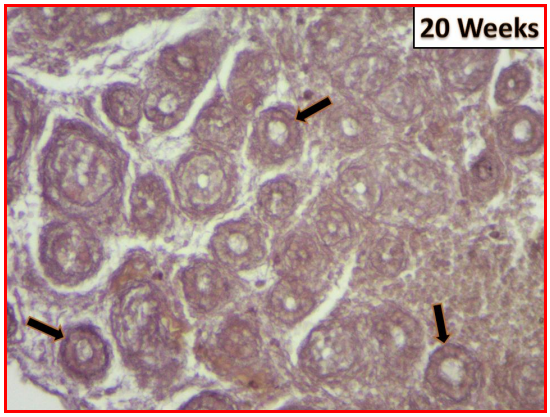

Fig. 4.78. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 20 WEEKS AGE GROUP (HART'S, 40X)

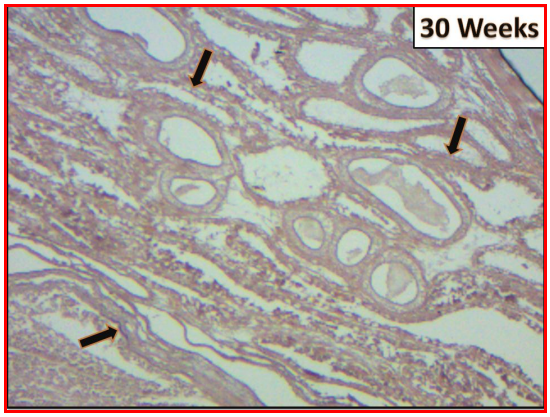

Fig. 4.79. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 30 WEEKS AGE GROUP (HART'S, 40X)

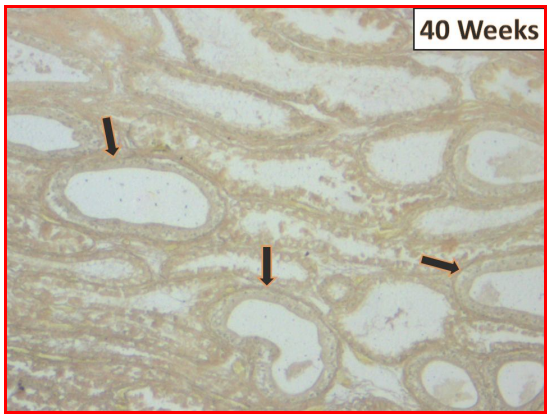

Fig. 4.80. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 40 WEEKS AGE GROUP (HART'S, 40X)

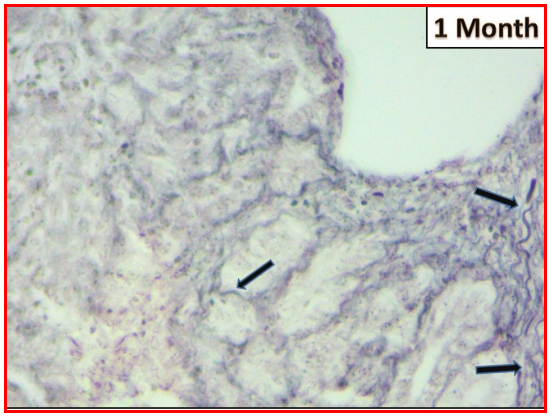

Fig. 4.81. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 1 MONTH AGE GROUP. (BIELSCHOWSKY'S , 40X)

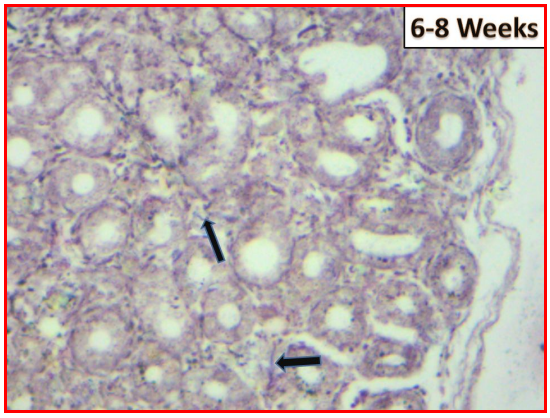

Fig. 4.82. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 6-8 WEEKS AGE GROUP. (BIELSCHOWSKY'S , 40X)

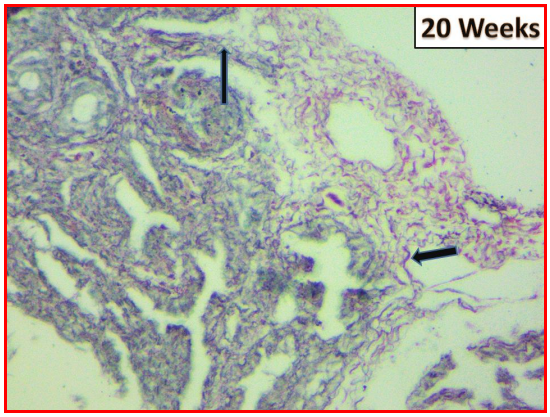

Fig. 4.83. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 20 WEEKS AGE GROUP. (BIELSCHOWSKY'S , 40X)

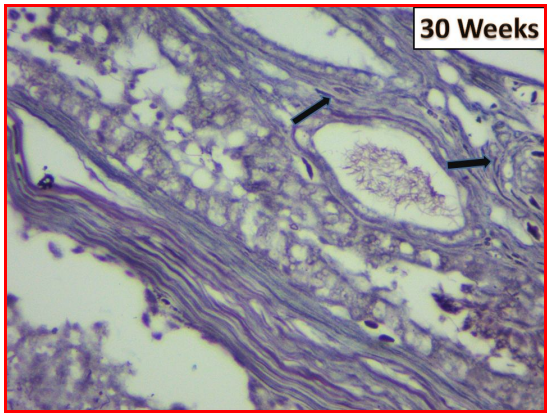

Fig. 4.84. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 30 WEEKS AGE GROUP. (BIELSCHOWSKY'S , 40X)

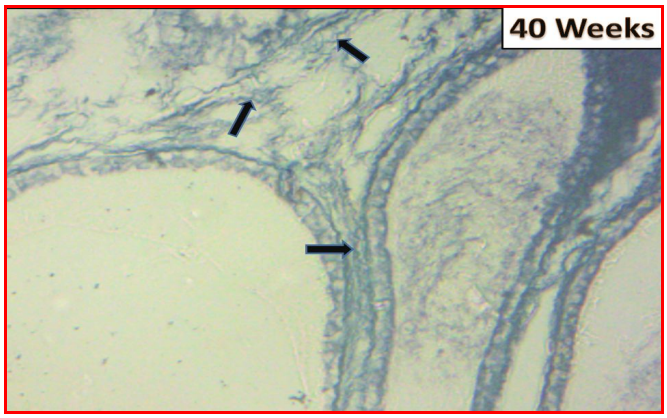

Fig. 4.85. PHOTOMICROGRAPH SHOWING THE NERVE FIBERS (ARROW) OF THE EPIDIDYMIS OF PATI DUCK OF 40 WEEKS AGE GROUP. (BIELSCHOWSKY'S , 40X)

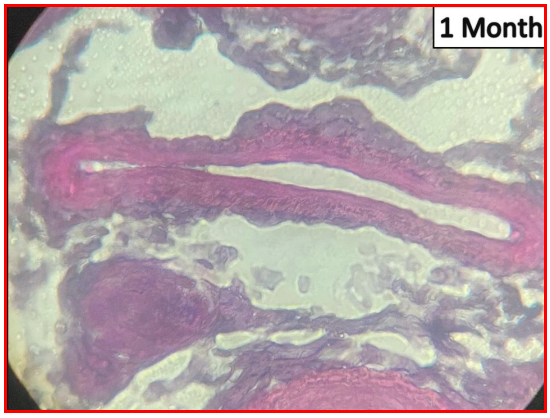

Fig. 4.86. PHOTOMICROGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , 40X)

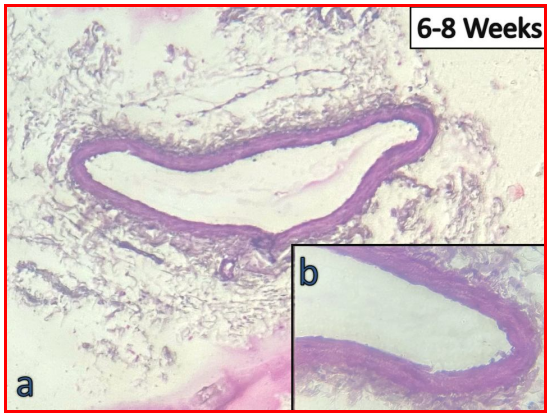

Fig. 4.87. PHOTOMICROGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (H & E , a- 10X b- 40X)

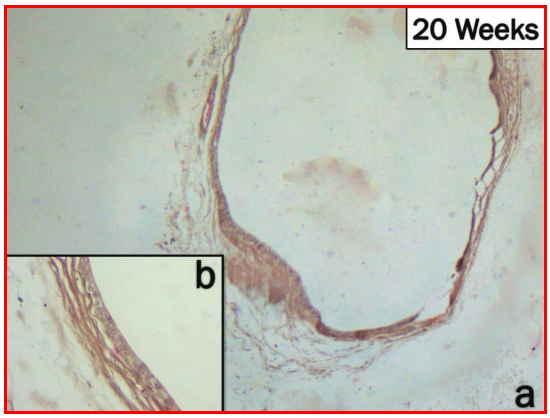

Fig. 4.88. PHOTOMICROGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK OF 20 WEEKS AGE GROUP (H & E , a- 10X b- 40X)

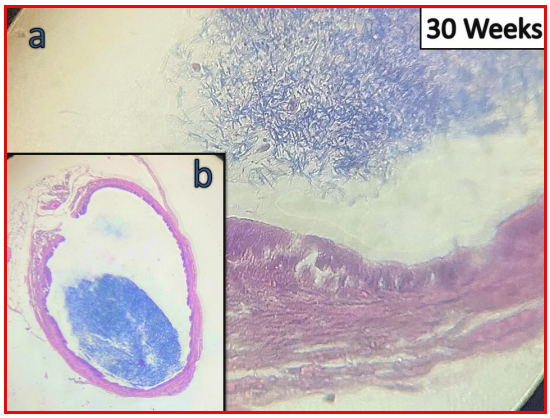

Fig. 4.89. PHOTOMICROGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK OF 30 WEEKS AGE GROUP (H & E , a- 10X b- 40X)

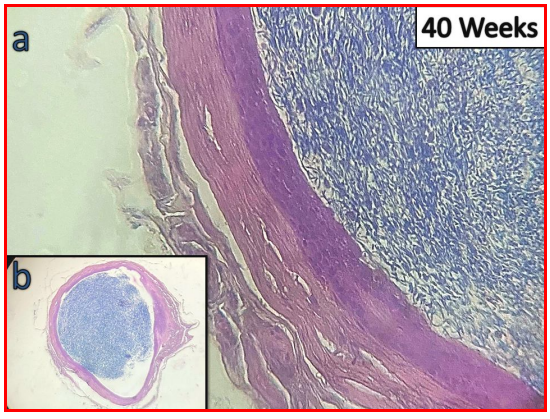

Fig. 4.90. PHOTOMICROGRAPH SHOWING THE VAS DEFERENS OF PATI DUCK OF 40 WEEKS AGE GROUP (H & E , a- 40X b- 10X)

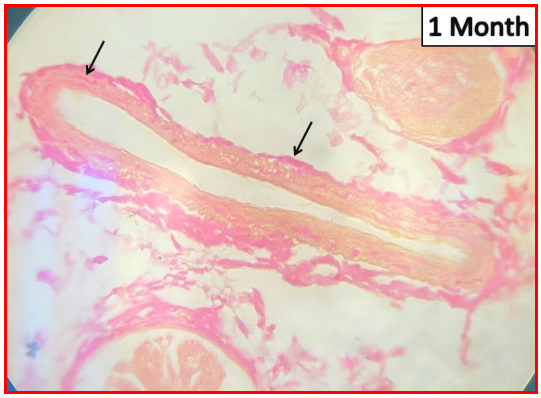

Fig. 4.91. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 1 MONTH AGE GROUP (VAN GIESON'S, 40X)

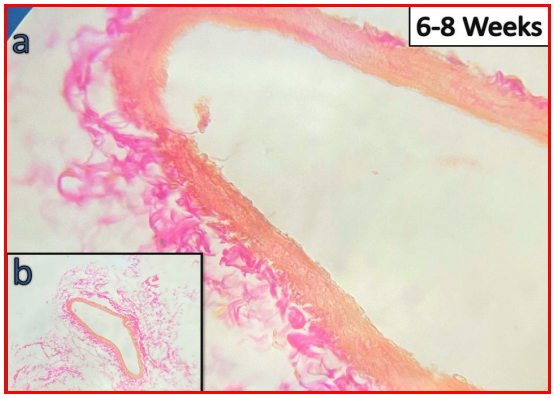

Fig. 4.92. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (VAN GIESON'S, a- 40X, b- 10X)

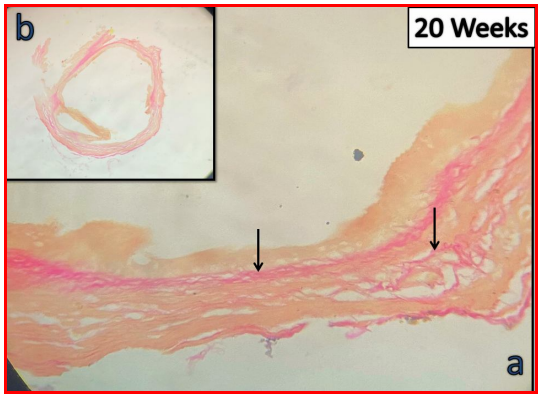

Fig. 4.93. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES(ARROWS) OF VAS DEFERENS OF PATI DUCK OF 20 WEEKS AGE GROUP (VAN GIESON'S , a- 40X, b- 10X)

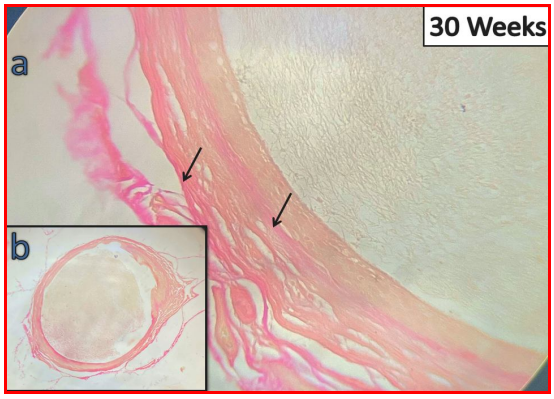

Fig. 4.94. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 30 WEEKS AGE GROUP (VAN GIESON'S, a- 40X, b- 10X)

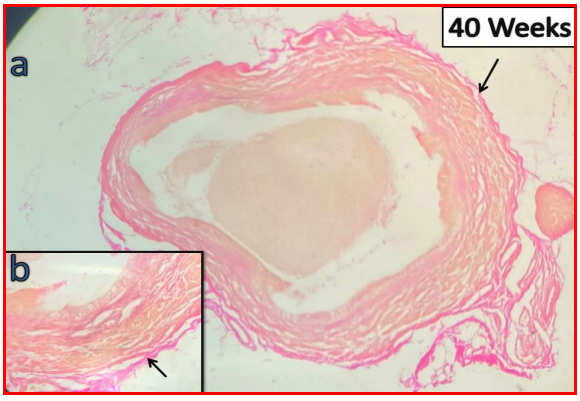

Fig. 4.95. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 40 WEEKS AGE GROUP (VAN GIESON'S , a- 10X, b- 40X)

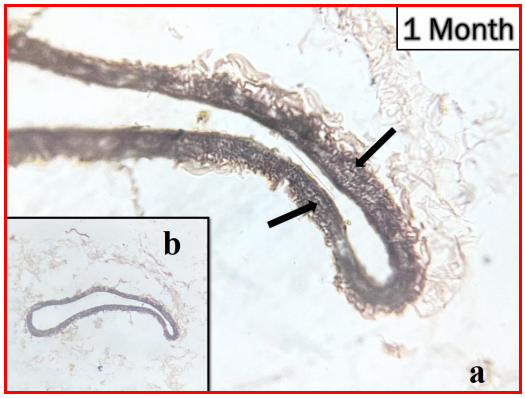

Fig. 4.96. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 1 MONTH AGE GROUP (GOMORI, a-40X, b-10X)

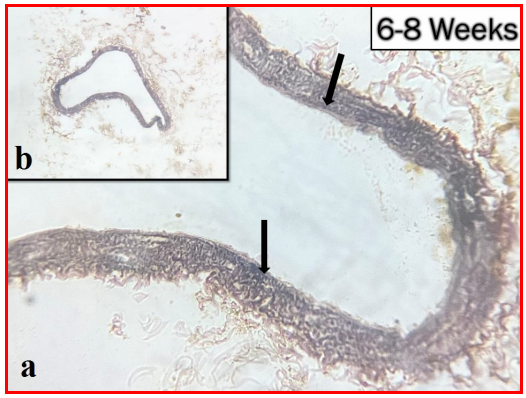

Fig. 4.97. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARRWS) OF VAS DEFERENS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (GOMORI, a-40X, b-10X)

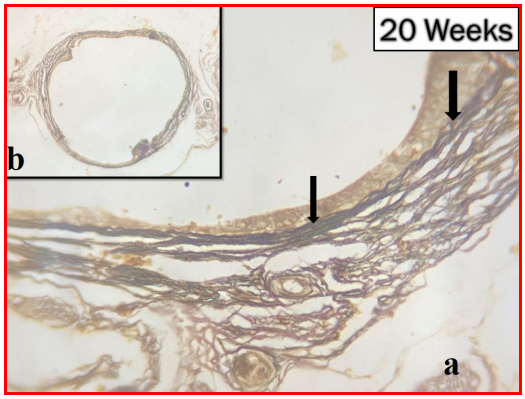

Fig. 4.98. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 20 WEEKS AGE GROUP (GOMORI, a-40X, b-10X)

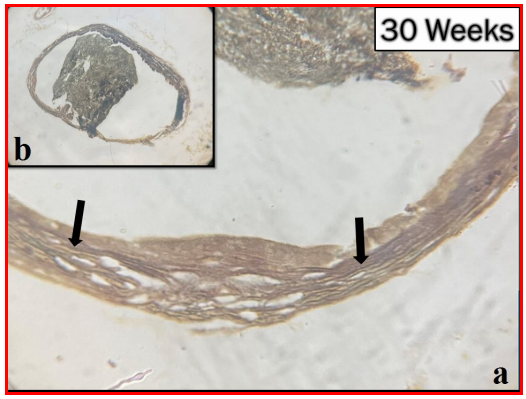

Fig. 4.99. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 30 WEEKS AGE GROUP (GOMORI, a-40X, b-10X)

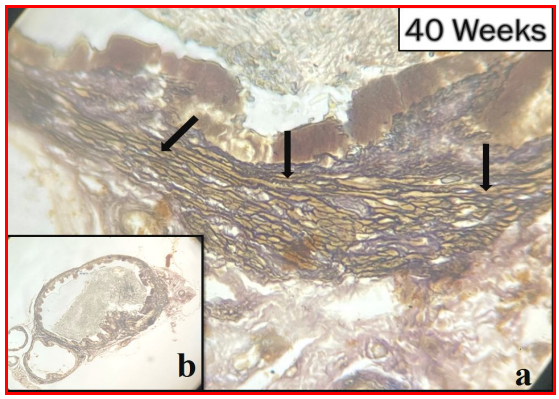

Fig. 4.100. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 40 WEEKS AGE GROUP (GOMORI , a-40X, b-10X)

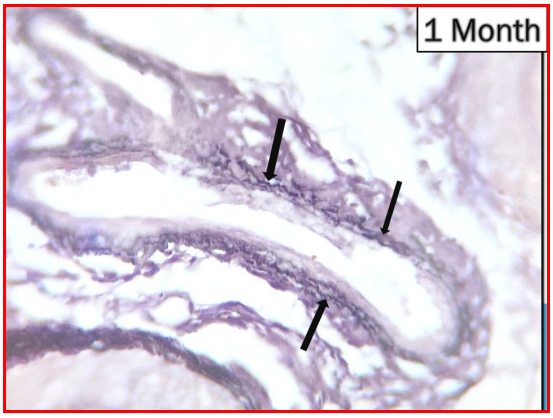

Fig. 4.101. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 1 MONTH AGE GROUP (HART'S, 40X)

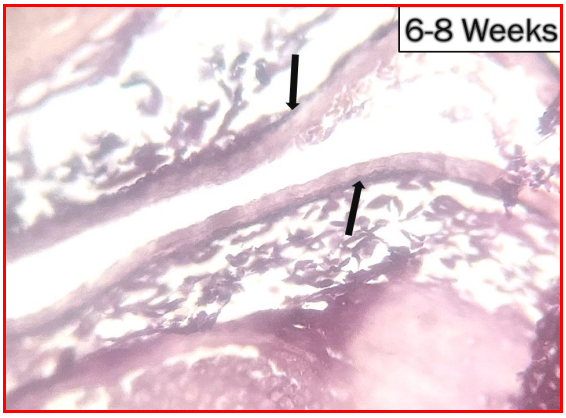

Fig. 4.102. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (HART'S, 40X)

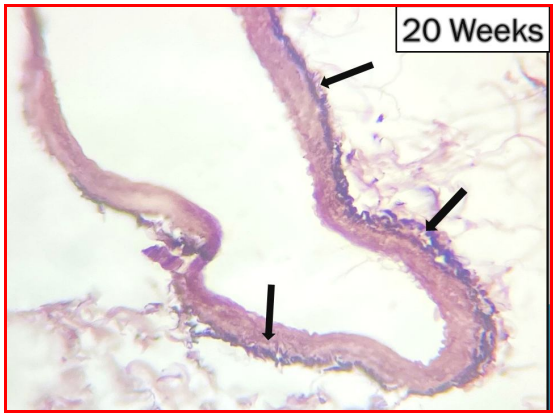

Fig. 4.103. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 20 WEEKS AGE GROUP (HART'S, 10X)

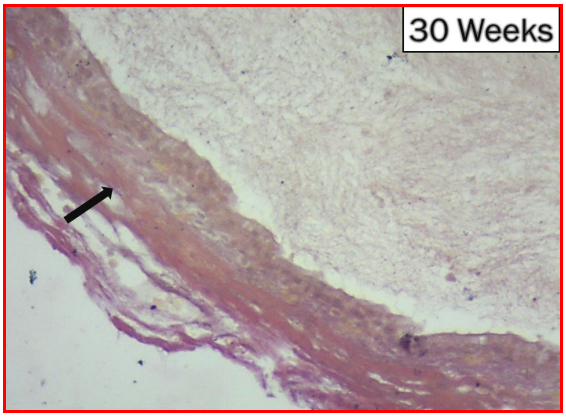

Fig. 4.104. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 30 WEEKS AGE GROUP (HART'S, 40X)

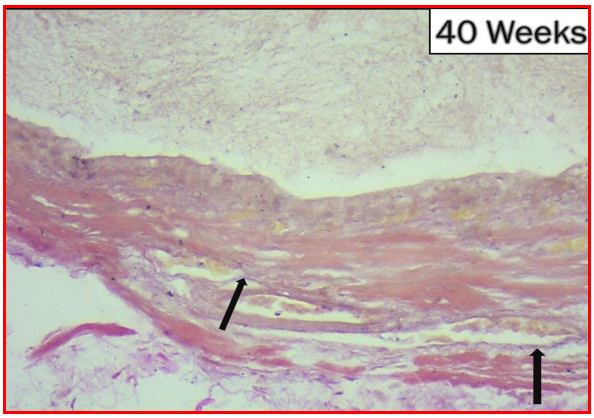

Fig. 4.105. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 40 WEEKS AGE GROUP (HART'S, 40X)

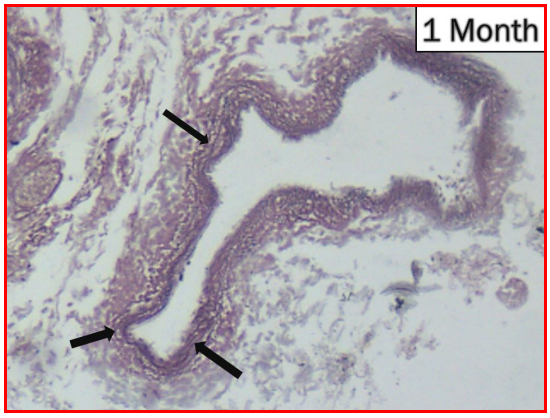

Fig. 4.106. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 1 MONTH AGE GROUP (BIELSCHOWSKY'S, 40X)

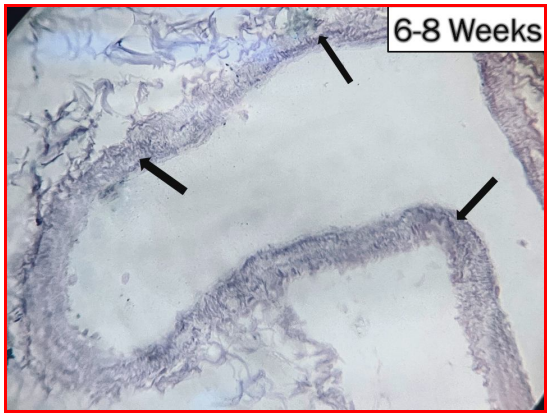

Fig. 4.107. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

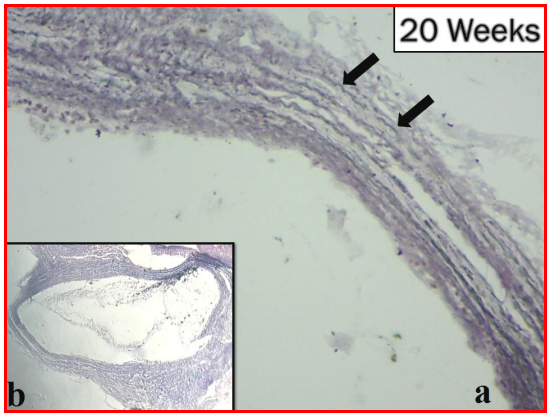

Fig. 4.109. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 20 WEEKS AGE GROUP (BIELSCHOWSKY'S, a-40X, b-10X)

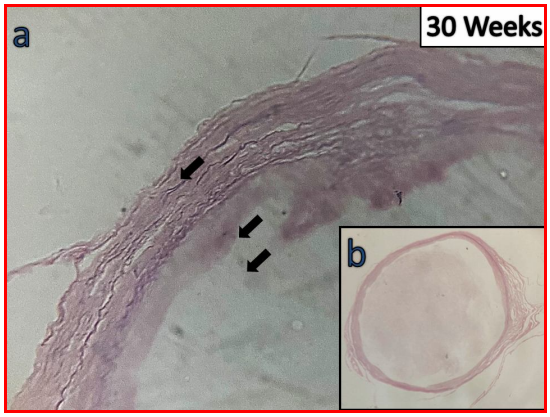

Fig. 4.109. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 30 WEEKS AGE GROUP (BIELSCHOWSKY'S, a-40X, b-10X)

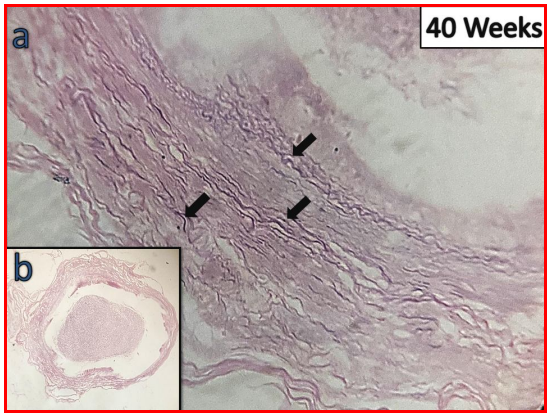

Fig. 4.110. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF VAS DEFERENS OF PATI DUCK OF 40 WEEKS AGE GROUP (BIELSCHOWSKY'S, a-40X, b-10X)

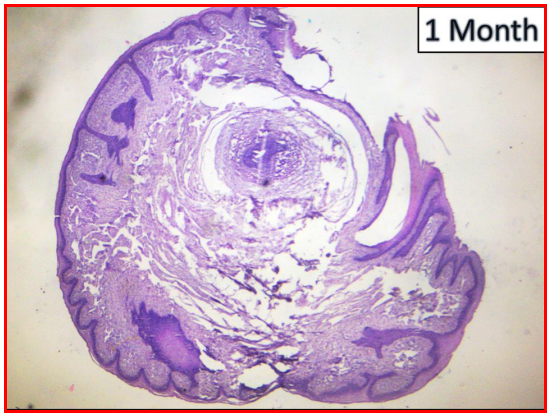

Fig. 4.111. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , 10X)

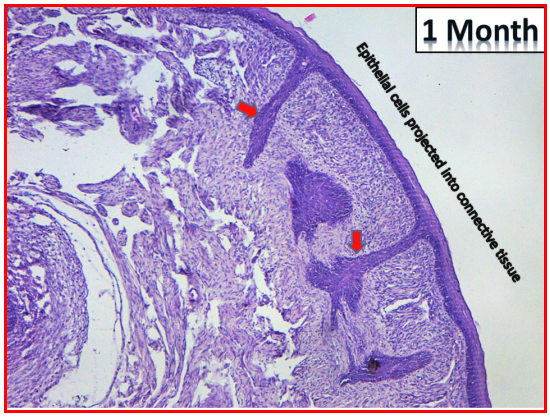

Fig. 4.112. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (H & E , 40X)

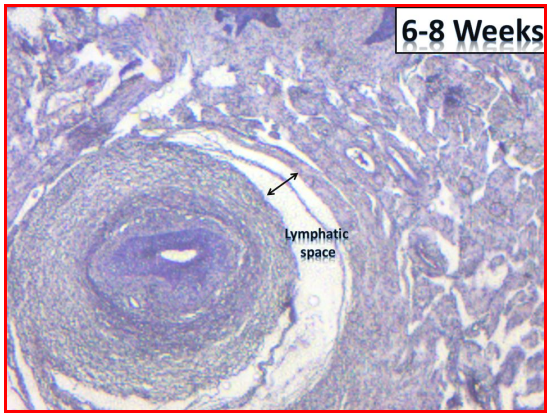

Fig. 4.113. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (H & E, 40X)

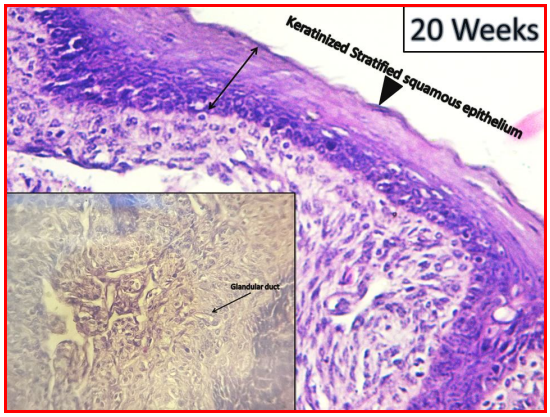

Fig. 4.114. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 20 WEEKS AGE GROUP (H & E, 40X)

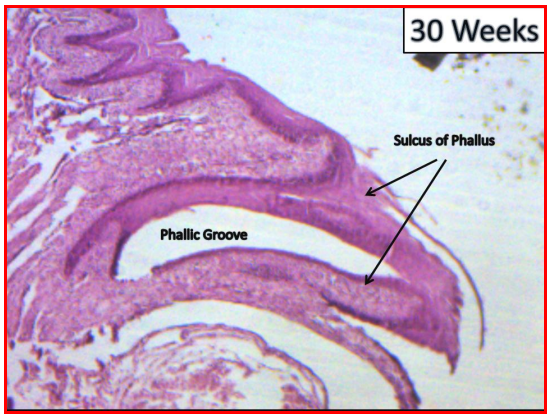

Fig. 4.115. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 30 WEEKS AGE GROUP (H & E, 40X)

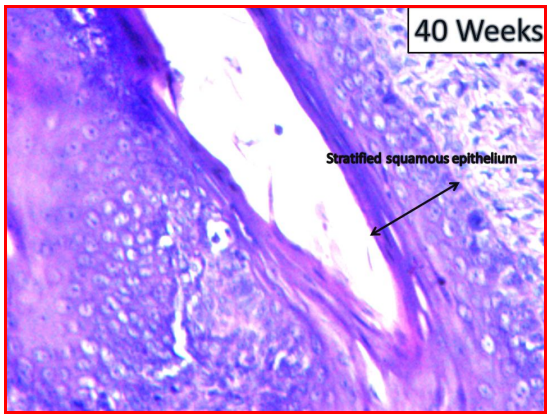

Fig. 4.116. PHOTOMICROGRAPH SHOWING THE PHALLUS OF PATI DUCK OF 40 WEEKS AGE GROUP (H & E, 40X)

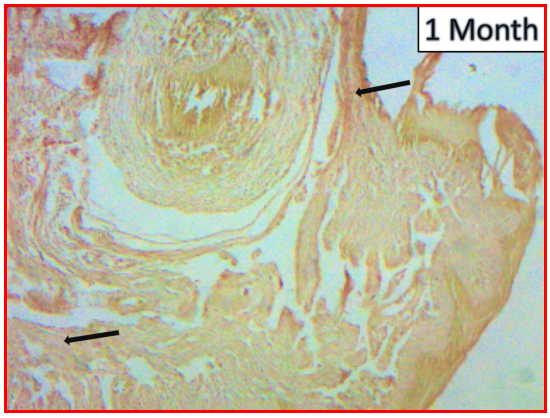

Fig. 4.117. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (VAN GIESON'S, 10X)

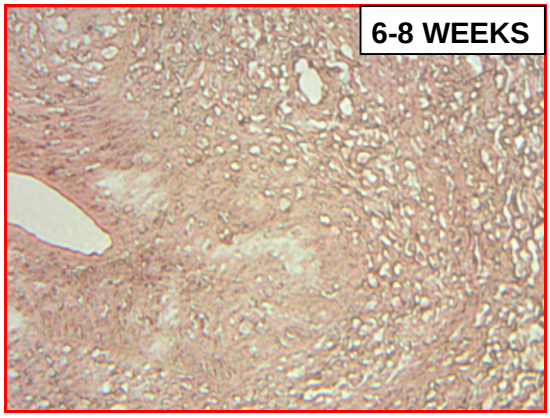

Fig. 4.118. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (VAN GIESON'S, 40X)

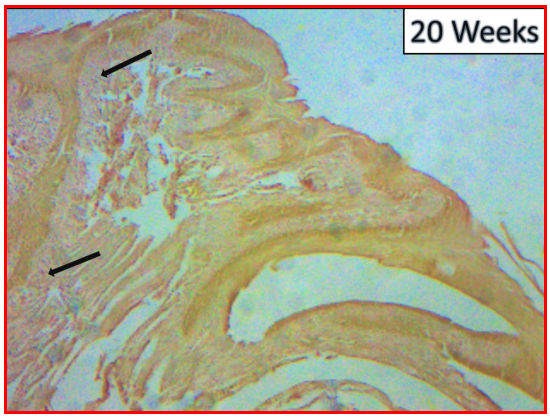

Fig. 4.119. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 20 WEEKS AGE GROUP (VAN GIESON'S, 40X)

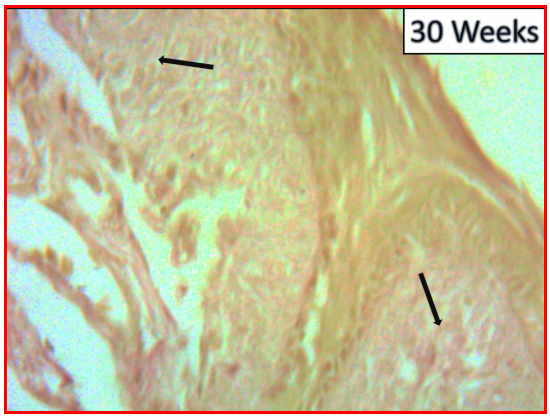

Fig. 4.120. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 30 WEEKS AGE GROUP (VAN GIESON'S, 40X)

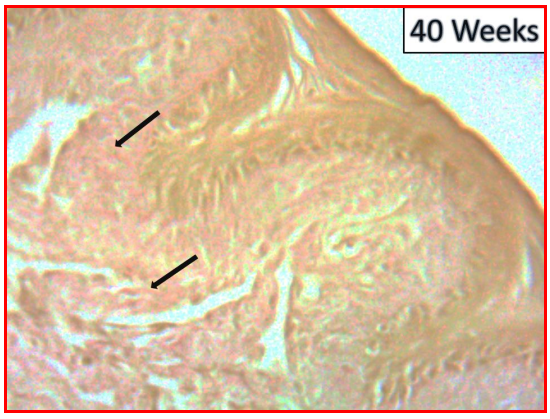

Fig. 4.121. PHOTOMICROGRAPH SHOWING THE COLLAGEN FIBRES (ARROWS) OF PHALLUS OF PATI DUCK OF 40 WEEKS AGE GROUP (VAN GIESON'S, 40X)

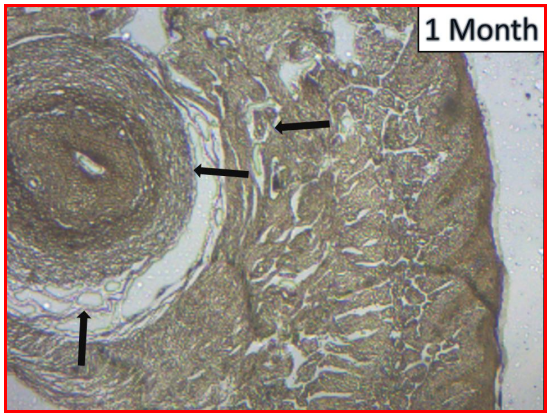

Fig. 4.122. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (GOMORI , 10X)

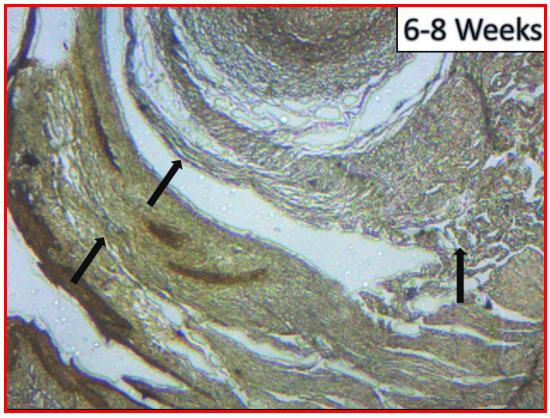

Fig. 4.123. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 6-8WEEKS AGE GROUP (GOMORI , 10X)

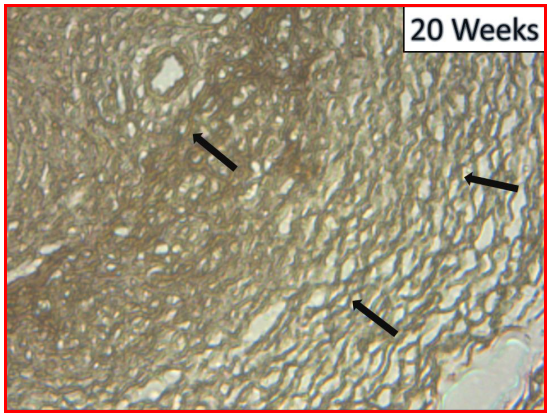

Fig. 4.124. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 20 WEEKS AGE GROUP (GOMORI , 40X)

Fig. 4.125. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 30 WEEKS AGE GROUP (GOMORI , 40X)

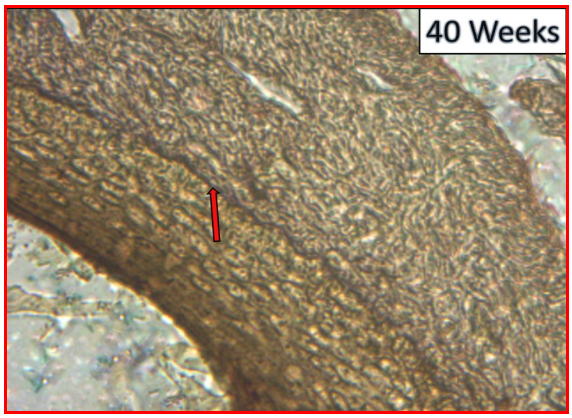

Fig. 4.126. PHOTOMICROGRAPH SHOWING THE RETICULAR FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 40 WEEKS AGE GROUP (GOMORI , 40X)

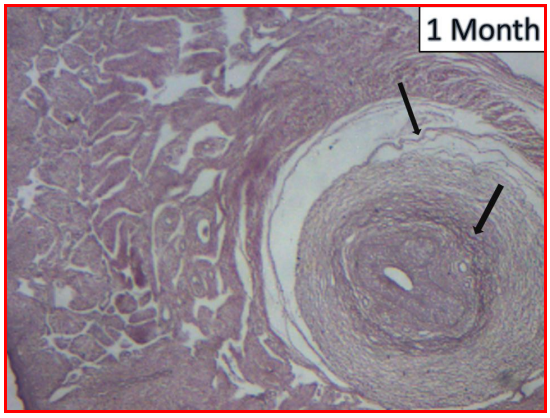

Fig. 4.127. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (HART'S , 10X)

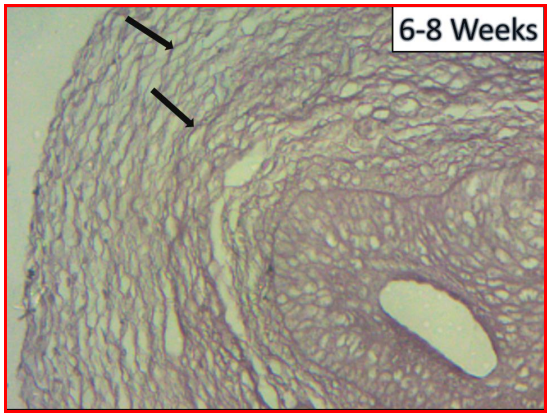

Fig. 4.128. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (HART'S , 40X)

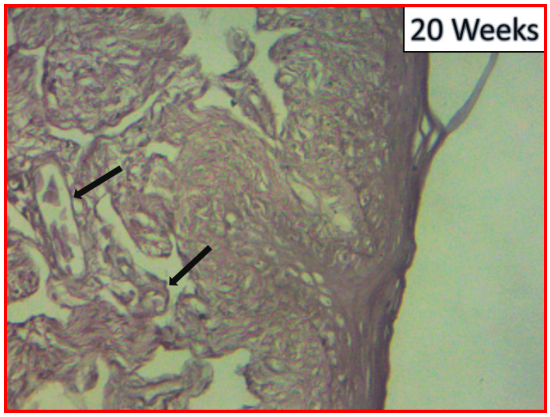

Fig. 4.129. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 20 WEEKS AGE GROUP (HART'S , 10X)

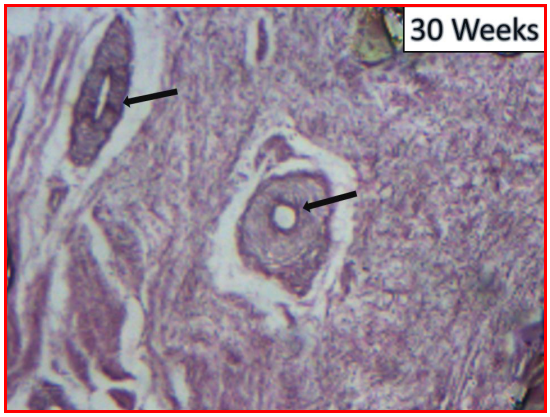

Fig. 4.130. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 30 WEEKS AGE GROUP (HART'S , 40X)

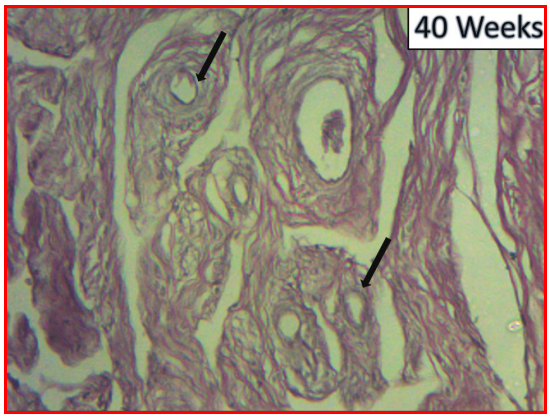

Fig. 4.131. PHOTOMICROGRAPH SHOWING THE ELASTIC FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 40 WEEKS AGE GROUP (HART'S , 40X)

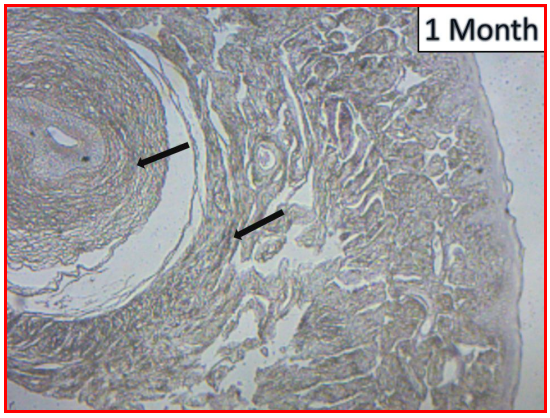

Fig. 4.132. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 1 MONTH AGE GROUP (BIELSCHOWSKY'S, 40X)

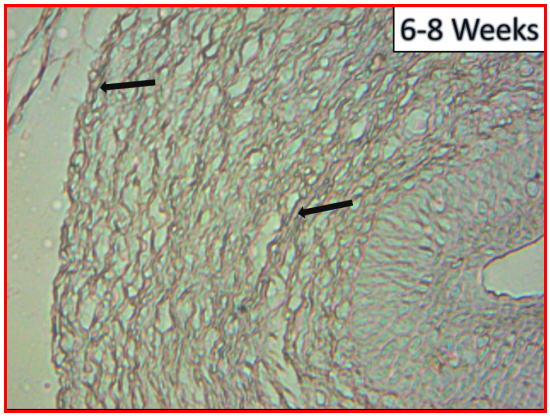

Fig. 4.133. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 6-8 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

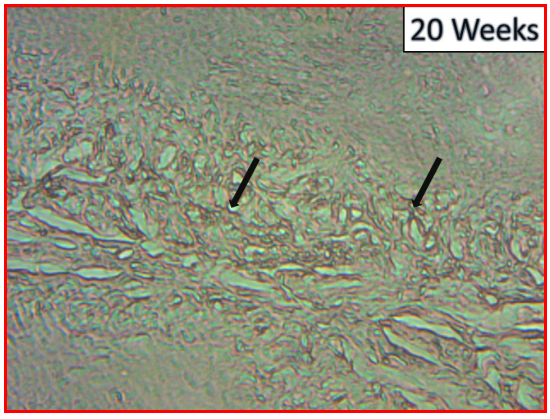

Fig. 4.134. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 20 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

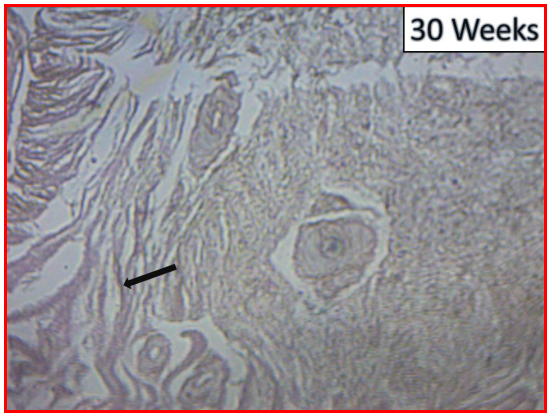

Fig. 4.135. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 30 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

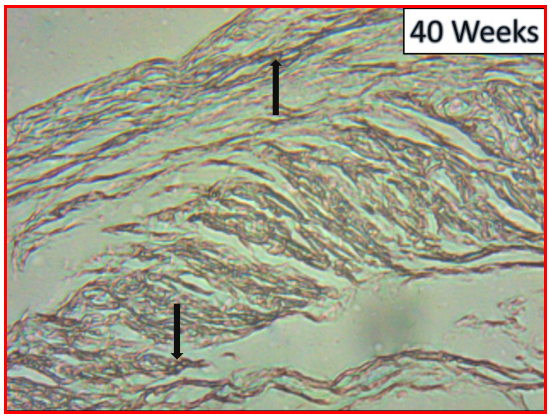

Fig. 4.136. PHOTOMICROGRAPH SHOWING THE NERVE FIBRES (ARROWS) OF THE PHALLUS OF PATI DUCK OF 40 WEEKS AGE GROUP (BIELSCHOWSKY'S, 40X)

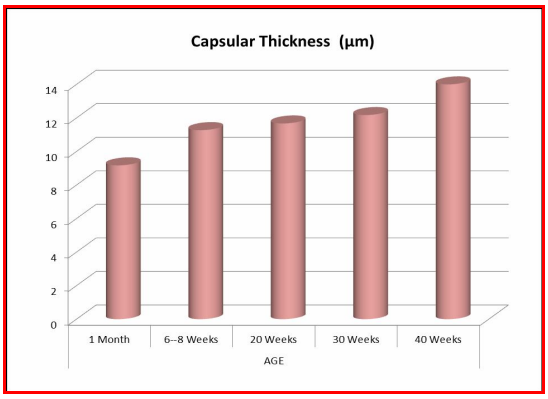

Fig. 4.137. GRAPHICAL REPRESENTATION OF THE AVERAGE CAPSULAR THICKNESS OF THE TESTIS OF PATI DUCK

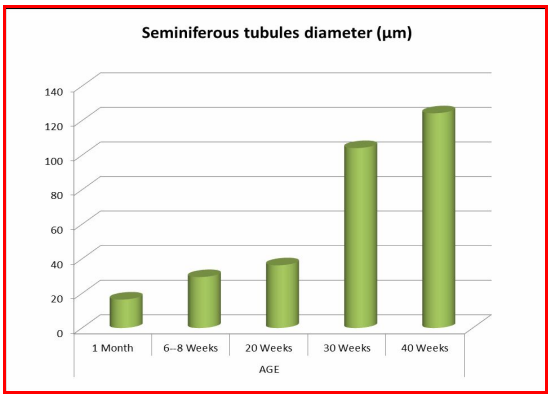

Fig. 4.138. GRAPHICAL REPRESENTATION OF THE AVERAGE SEMINIFEROUS TUBULES DIAMETER OF THE TESTIS OF PATI DUCK

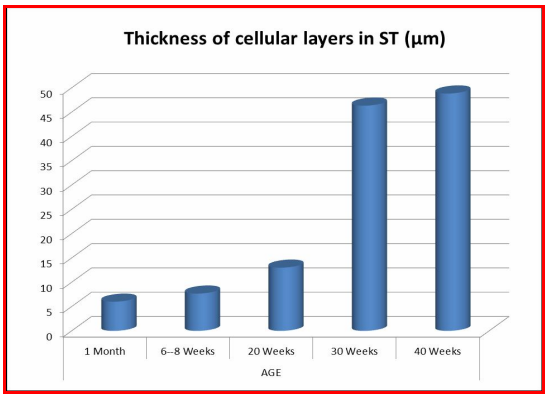

Fig. 4.139. GRAPHICAL REPRESENTATION OF THE AVERAGE THICKNESS OF THE CELLULAR LAYER IN THE SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK

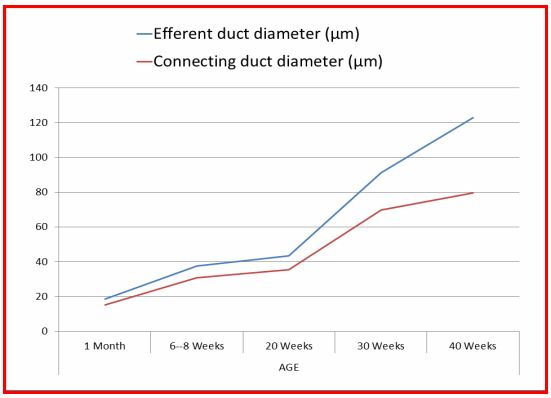

Fig. 4.140. GRAPHICAL REPRESENTATION OF THE AVERAGE EFFERENT DUCT AND CONNECTING DUCT DIAMETER OF THE EPIDIDYMIS OF PATI DUCK

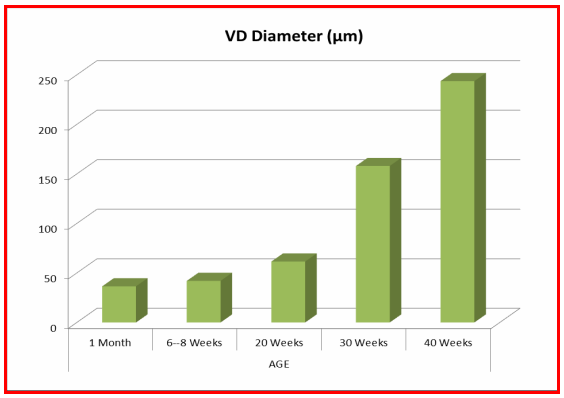

Fig. 4.141. GRAPHICAL REPRESENTATION OF THE AVERAGE VAS DEFERENS DIAMETER OF PATI DUCK AT DIFFERENT AGE GROUPS

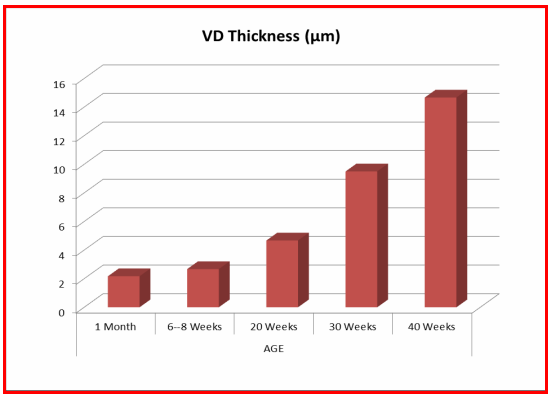

Fig. 4.142. GRAPHICAL REPRESENTATION OF THE AVERAGE THICKNESS OF VAS DEFERENS OF PATI DUCK AT DIFFERENT AGE GROUPS

4.3. HISTOCHEMISTRY

4.3.1. Alkaline Phosphatase

In the present histochemical study of the testis of Pati duck, the activity of alkaline phosphatase was strong in testis of 1 month of age, moderate in the testis of 6-8 weeks and 20 weeks of age, while there was weak reaction in the testis of 30 weeks and 40 weeks of age. It was also observed that the enzyme activity was weak in vas deferens and intense in the phallus (Fig. 4.143 to 4.147 and Table 4.4).

The activity of the enzyme was observed in peri-tubular connective tissue cells of seminiferous tubules was strong in 1 month and moderate in 6-8 weeks (Fig. 4.143 and 4.144). The finding was in accordance with the findings of Kugler (1975) in Rooster. A weak reaction was also found in the interstitial cells. Germs cells in the seminiferous tubules also showed moderate reaction including the vacuolated cells in testis of younger age group. A weak reaction was observed on the lumen surface of the seminiferous tubules (Fig. 4.143 to 4.147). The basal cells and epithelial lining of the ducts of the epididymis also showed moderate activity of the enzyme. The findings in Pati duck of Assam was in accordance with the reports by Gunawardhana (1985) in testis of domestic fowl. However, Bakst (2007) reported that only Leydig cells were alkaline phosphatase reactive in the testis of mature turkey. According to Gunawardhana (1985), alkaline phosphatase was not secreted into the lumen and therefore unlikely to contribute to the seminal alkaline phosphatase. In the present study the intensity of the enzyme activity was stronger in the spermatozoa than other germinal cells in the ST. There was a weak activity of the enzyme in the peri-ductal connective tissue of the epididymis.

Vas deferens of different age groups of Pati duck showed moderate reactions for alkaline phosphatases in the cells of the epithelial lining and some isolated areas (Fig. 4.148 to 4.152 and Table 4.4). The findings were in accordance with the observation by Silverin (1978) in Pied flycatcher. Lake (1957) also observed a positive reaction of alkaline phosphatase enzyme in the epithelial cells.

The phallus of Pati duck showed moderate activity of alkaline phosphatase in the keratinized stratified squamous epithelium. The intensity increased in the connective tissue beneath the surface epithelium where glands and blood vessels were present. The mucosal lining and connective tissue around the lymphatic space also showed an intense

activity of the enzyme (Fig. 4.153 to 4.157 and Table 4.4). The literatures regarding activities of Alkaline Phosphatase in the Phallus of avian species were very scanty.

A strong reaction of alkaline phosphatase in the peritubular cells of seminiferous tubules might play a part in energy disposition for contractions as stated by Kugler (1975). Alkaline phosphatase was a hydrolase enzyme which removes phosphate group from many molecules (Tamas, 2002).

4.3.2. Acid Phosphatase

The activity of the Acid Phosphatase was moderate in the peritubular area, lumen of the seminiferous tubules, interstitial connective tissue and capsule of the testis in all age groups (Fig. 4.158 to 4.162 and Table 4.4). The germs cells showed the moderate activity of the enzyme. Kugler (1975) also observed an extraordinarily high activity of acid phosphatase in testicular interstitial cells of adult fowl. The difference in findings might be due to difference in species.

Acid phosphatase had been used as a cytochemical marker for the identification of lysosomes in the seminiferous tubules of the domestic fowl (Gunawardhana, 1982) which gave an idea about cells undergoing extensive remodeling especially the germinal cell.

In the Vas deferens a moderate reaction was observed in the surface of the epithelial lining and few isolated layers in all the age groups (Fig. 4.163 to 4.167 and Table 4.4). Similar finding was observed by Silverin (1978) in Pied Flycatcher. However, Angulo and Bosch (1964) observed a high degree of activity of acid phosphatase in vas deferens of male fowl. The difference in observation might be due to difference in species.

Phallus showed strong reaction of acid phosphatase in the loose connective tissue just beneath the outer surface epithelium and moderate reaction in the connective tissue surrounding the lymphatic channel. There was intense reaction on the keratinized part of epithelium while the underlying stratified squamous cells showed no reaction (Fig. 4.168 to 1.172 and Table 4.4). There was scanty literature for comparison regarding the Acid phosphatase activity in the phallus of avian.

4.3.3. Adenosine Tri Phosphatase (ATPase)

In the testis of Pati duck of Assam ATPase activity was observed weak to moderate in the interstitial cells, peri-tubular cells of the seminiferous tubules and tunica serosa of the capsule. The finding was in accordance with the reports of Silverin (1978) in Pied flycatcher. In the present study the enzyme activity was seen in all the stages of germ cells in 30 weeks and 40 weeks. Vacuolated cells present in the seminiferous tubules of 1 month, 6-8 weeks and 20 weeks also showed a weak activity in the cell border. The activity of the ATPase enzyme increased with increased of age (Fig. 4.173 to 4.177 and Table 4.4). However Kugler (1975) reported a negative reaction in the lamina propria of seminiferous tubules and germinal cells, but positive in the Sertoli cells of cock. The difference in findings might be due to difference in species of avian studied.

The luminal surface of the efferent duct, connecting duct and epididymal duct showed a weak reaction to the ATPase enzyme in all age group. The weak activity was observed in the basal lamina of all the ducts in 30 and 40 weeks.

ATPase reaction was very week in the Vas deferens of Pati duck. Feeble reaction was observed in the lining epithelium. The spermatozoa in the lumen showed moderate reaction (Fig. 4.178 to 4.182 and Table 4.4).

In the Phallus the activity of ATPase was most intense in the keratinized epithelium. The trabeculae and connective tissue around the lymphatic space also showed a weak activity. The mucosal epithelium and lamina propria showed a moderate reaction (Fig. 4.183 to 4.187 and Table 4.4). There was scanty literature for comparison regarding the ATPase activity in the phallus of avian

According to Silverin (1978) the increased in ATPase activity in the testis might reflect an increased transfer of metabolites and electrolytes between the capillaries in the interstitium, sertoli cells and spermatogonium, they were the key enzyme involved in generating electrochemical gradients necessary for driving primary and secondary ion transport process (Tian and Xie, 2008).

TABLE 4.4. SHOWING THE ACTIVITY OF HISTOENZYMIC REACTION IN THE MALE GENITAL ORGANS OF PATI DUCK IN DIFFERENT AGE GROUP

Histoenzymic Reaction		**1 month**	**6-8 week**	**20 week**	**30 week**	**40 week**
Alkaline phosphatas	Testis	+++	+ +	+ +	+	+
	Vas deferens	++	++	++	++	++
	Phallus	++++	++++	++++	++++	++++
Acid phosphatase	Testis	++	++	++	++	++
	Vas deferens	++	++	++	++	++
	Phallus	+++	+++	+++	+++	+++
ATPase	Testis	+/++	+/++	++	++	++
	Vas deferens	+	+	++	++	++
	Phallus	+++	+++	+++	+++	+++

Gradation for intensity:
Absent = --
Weak = +
Moderate = + +
Strong = + + +
Intense= ++++

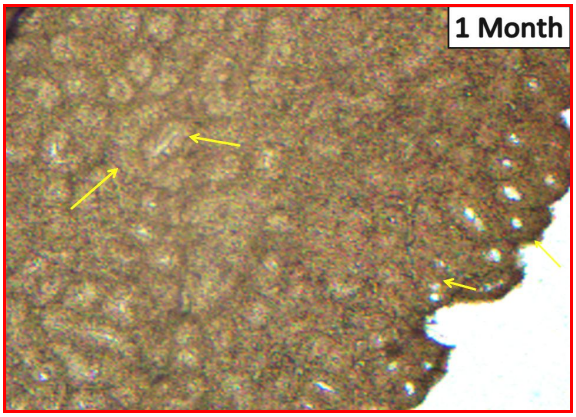

Fig. 4.143. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE TESTIS OF PATI DUCK, 1 MONTH

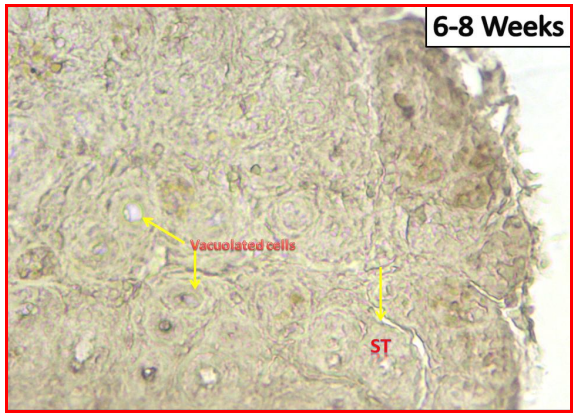

Fig. 4.144. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE TESTIS OF PATI DUCK, 6-8 WEEKS

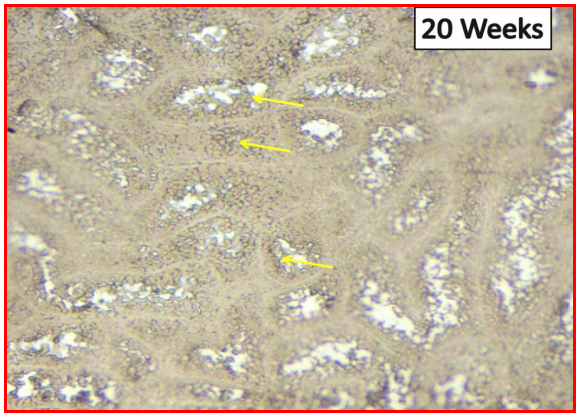

Fig. 4.145. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE TESTIS OF PATI DUCK, 20 WEEKS

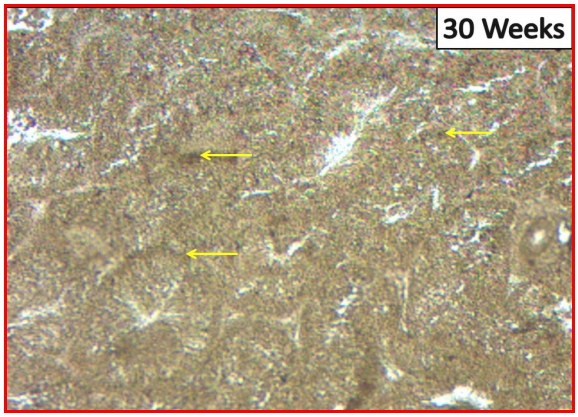

Fig. 4.146. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE TESTIS OF PATI DUCK, 30 WEEKS

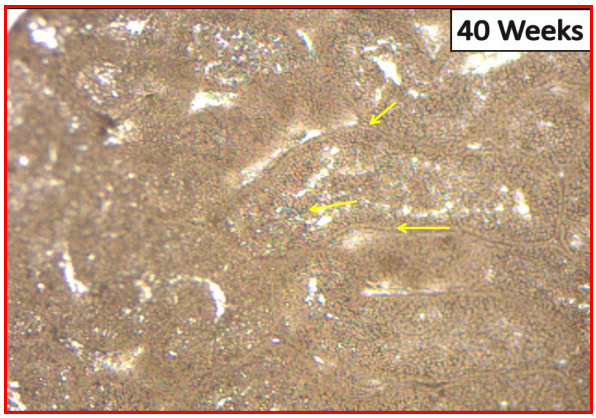

Fig. 4.147. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE TESTIS OF PATI DUCK, 40 WEEKS

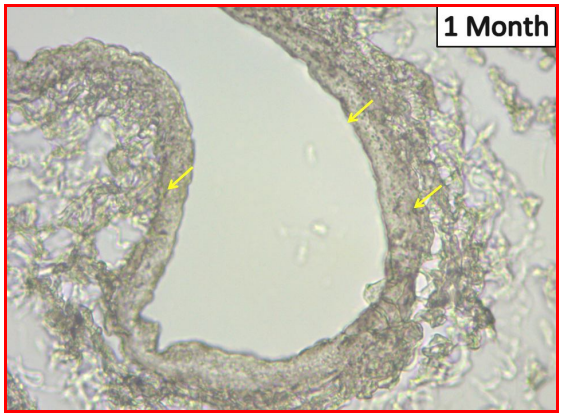

Fig. 4.148. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 1 MONTH

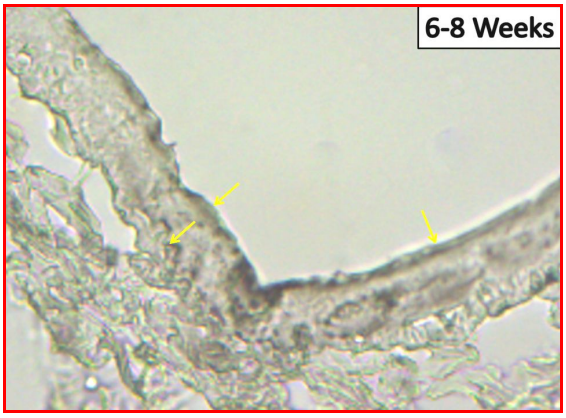

Fig. 4.149. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 6-8 WEEKS

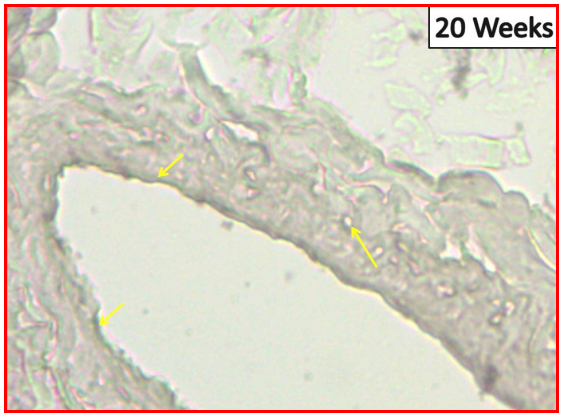

Fig. 4.150. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 20 WEEKS

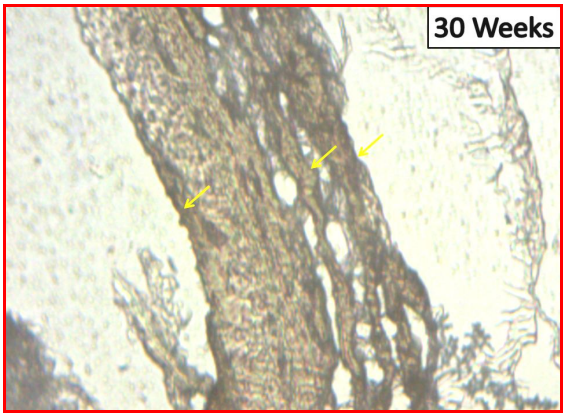

Fig. 4.151. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 30 WEEKS

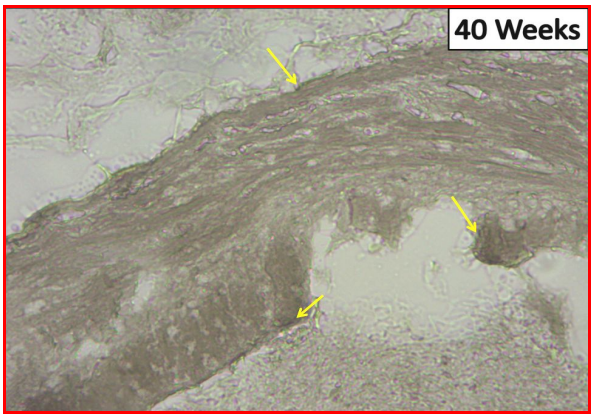

Fig. 4.152. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 40 WEEKS

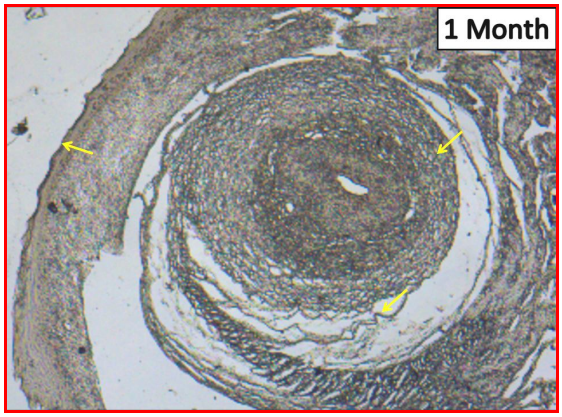

Fig. 4.153. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 1 MONTH

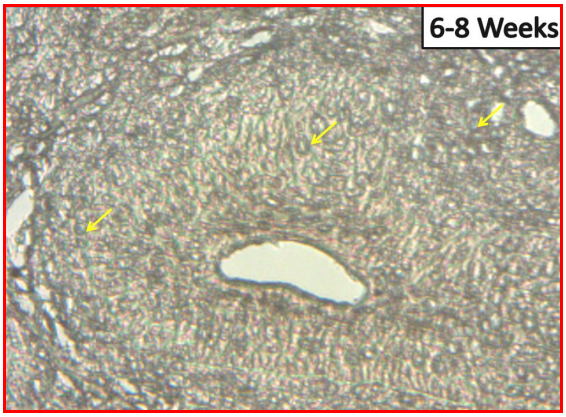

Fig. 4.154. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 6-8 WEEKS

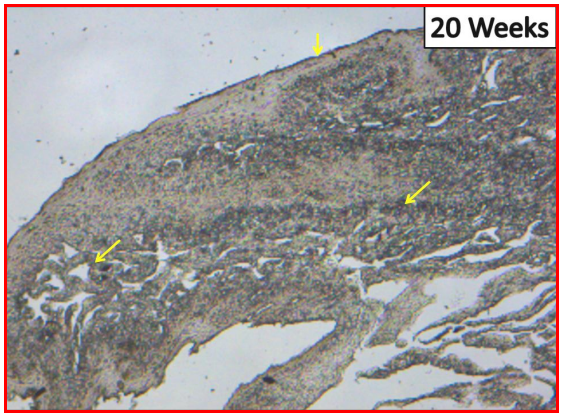

Fig. 4.155. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 20 WEEKS

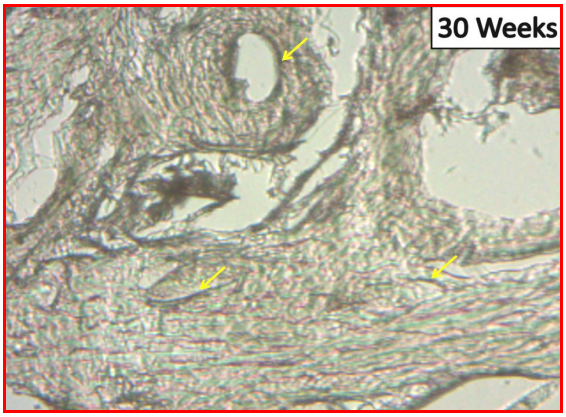

Fig. 4.156. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 30 WEEKS

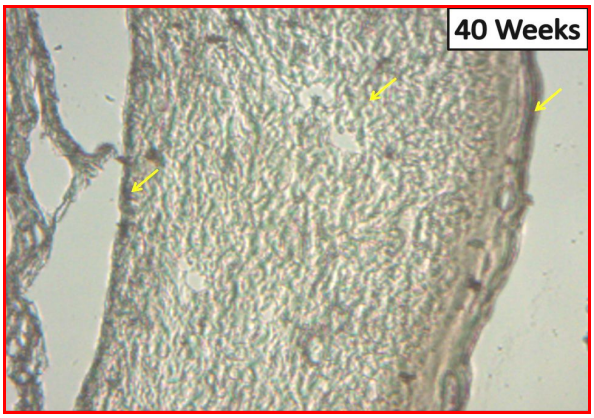

Fig. 4.157. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ALKALINE PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 40 WEEKS

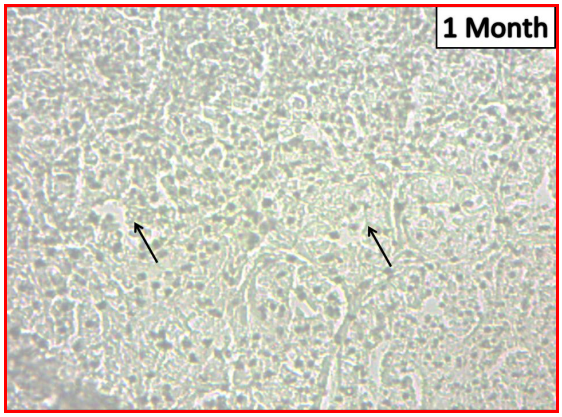

Fig. 4.158. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE TESTIS OF PATI DUCK, 1 MONTH

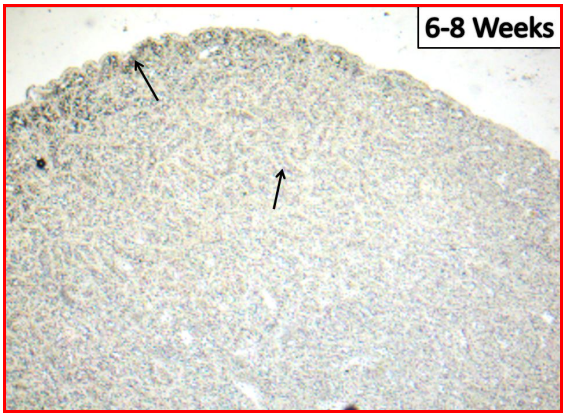

Fig. 4.159. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE TESTIS OF PATI DUCK, 6-8 WEEK

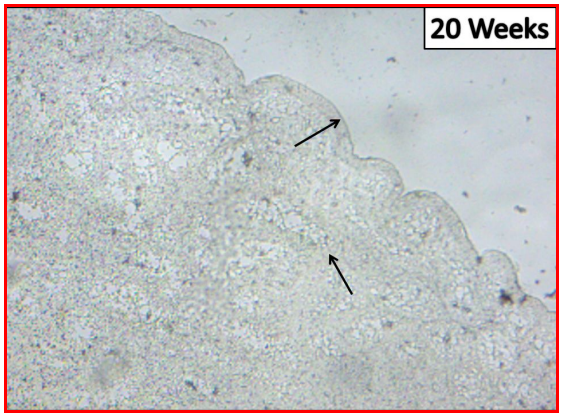

Fig. 4.160. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE TESTIS OF PATI DUCK, 20 WEEK

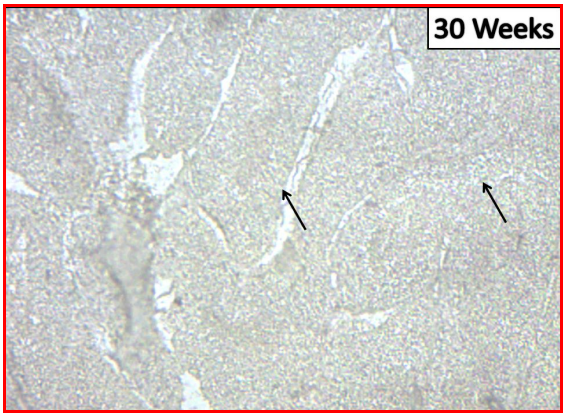

Fig. 4.161. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE TESTIS OF PATI DUCK, 30 WEEK

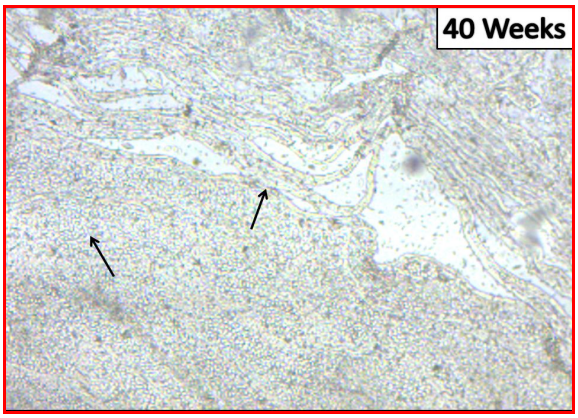

Fig. 4.162. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE TESTIS OF PATI DUCK, 40 WEEK

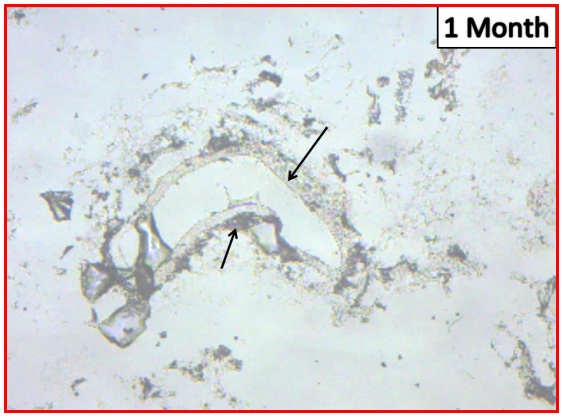

Fig. 4.163. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 1 MONTH

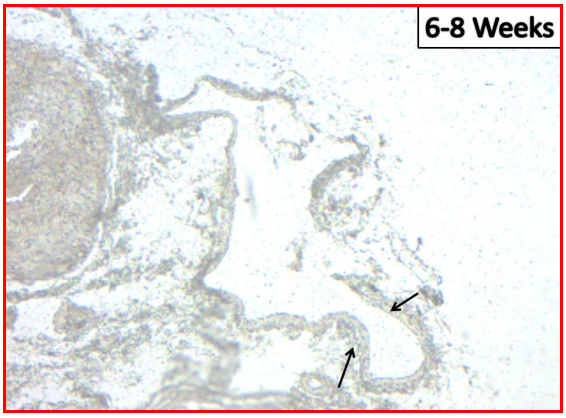

Fig. 4.164. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 6-8 WEEK

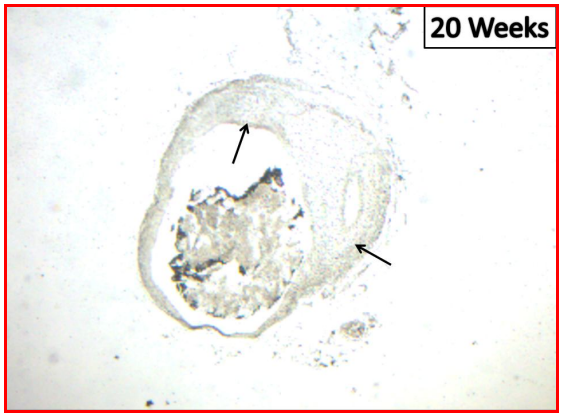

Fig. 4.165. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 20 WEEK

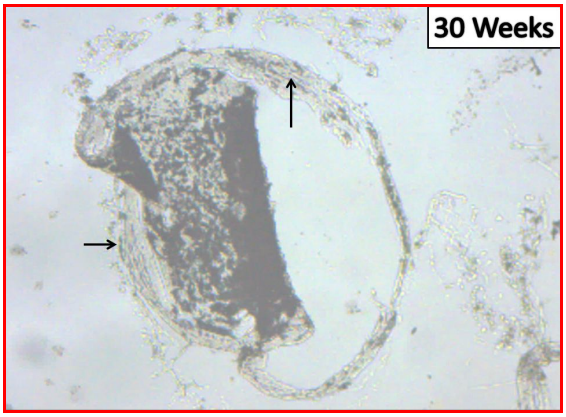

Fig. 4.166. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 30 WEEK

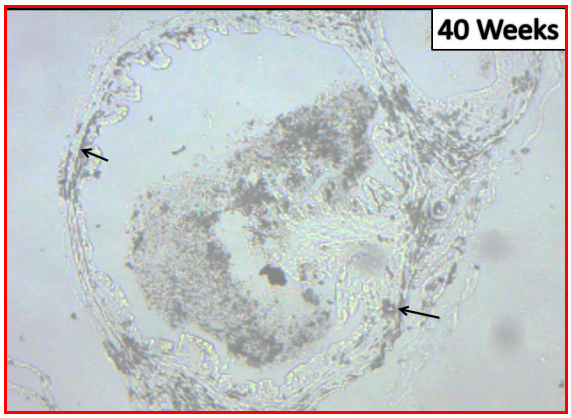

Fig. 4.167. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE VAS DEFERENS OF PATI DUCK, 40 WEEK

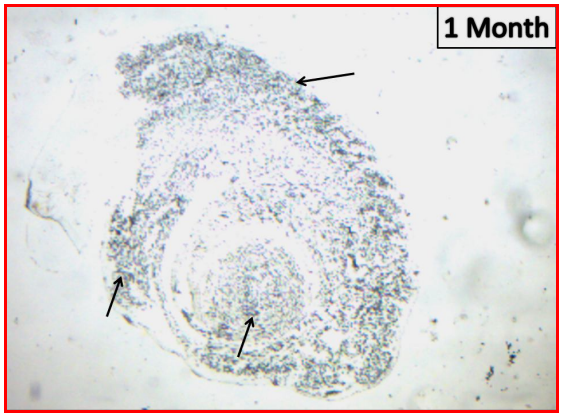

Fig. 4.168. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 1 MONTH

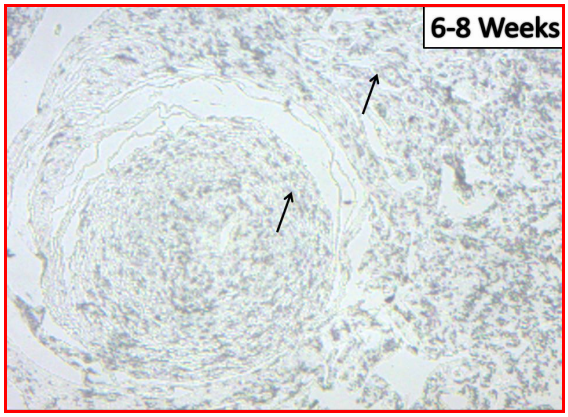

Fig. 4.169. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 6-8 WEEK

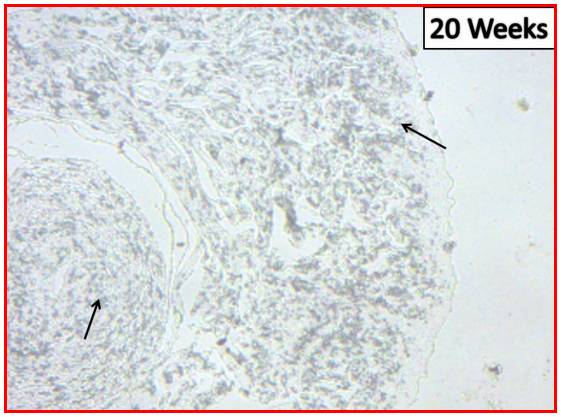

Fig. 4.170. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 20 WEEK

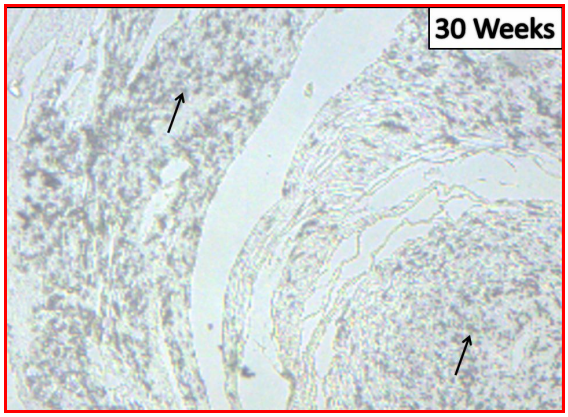

Fig. 4.171. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 30 WEEK

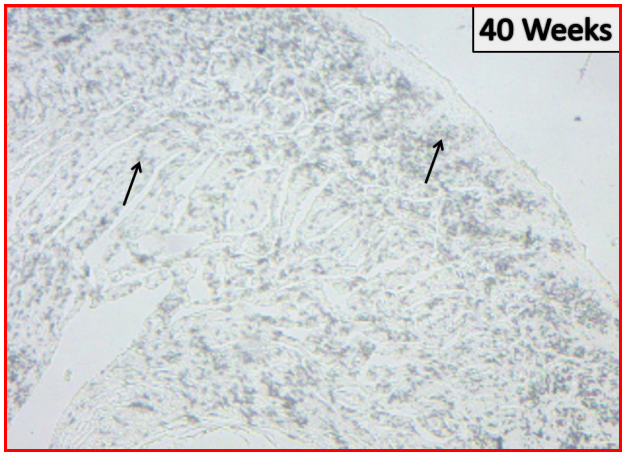

Fig. 4.172. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ACID PHOSPHATASE IN THE PHALLUS OF PATI DUCK, 40 WEEK

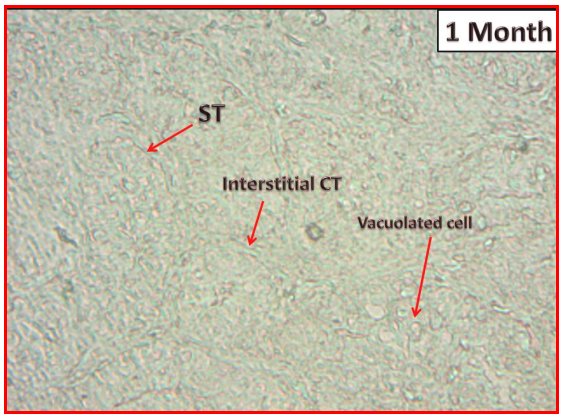

Fig. 4.173. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE TESTIS OF PATI DUCK, 1 MONTH

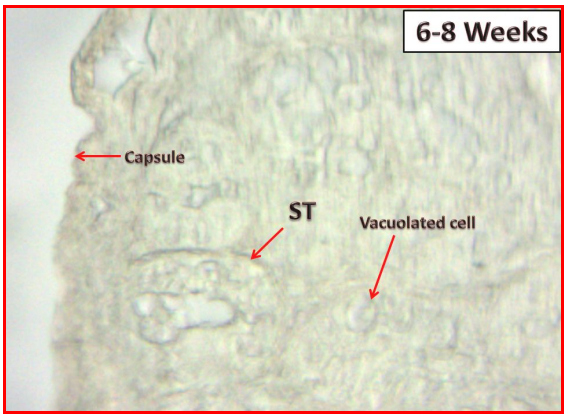

Fig. 4.174. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE TESTIS OF PATI DUCK, 6-8 WEEK

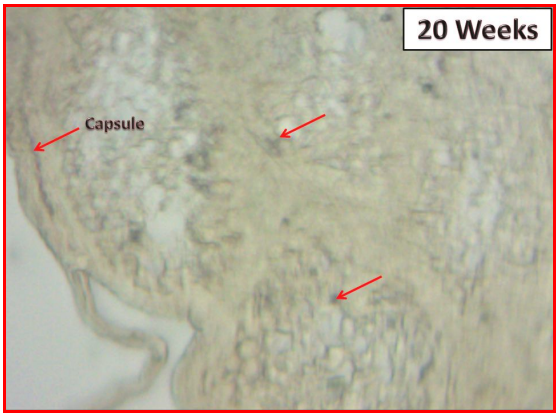

Fig. 4.175. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE TESTIS OF PATI DUCK, 20 WEEK

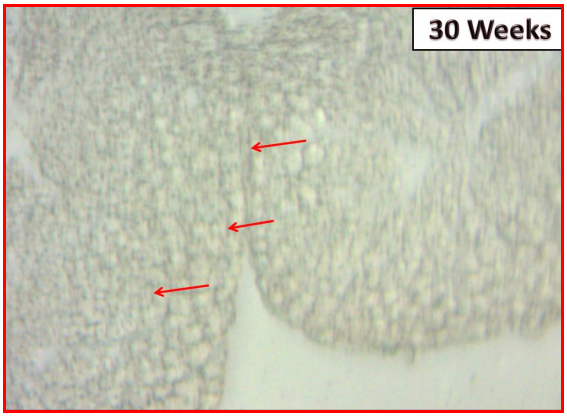

Fig. 4.176. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE TESTIS OF PATI DUCK, 30 WEEK

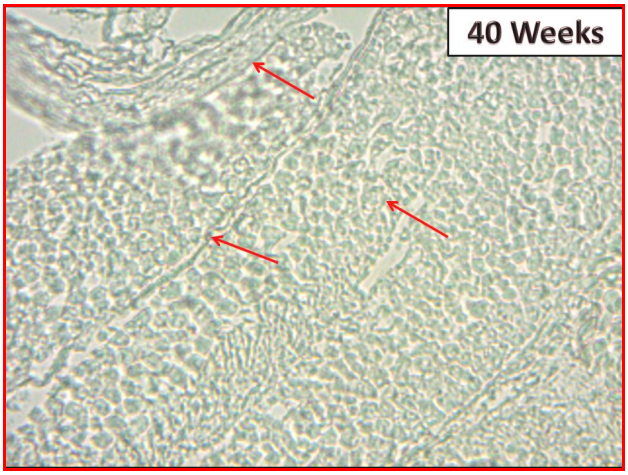

Fig. 4.177. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE TESTIS OF PATI DUCK, 40 WEEK

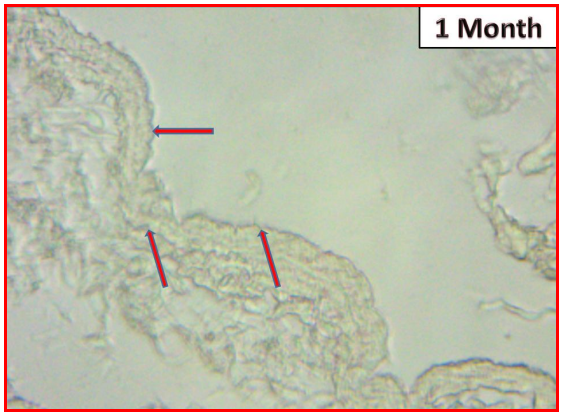

Fig. 4.178. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE VAS DEFERENS OF PATI DUCK, 1 MONTH

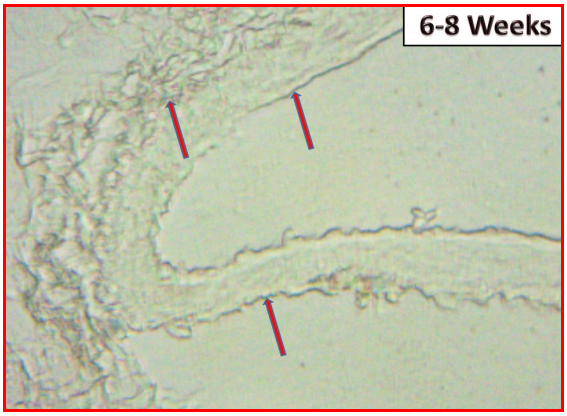

Fig. 4.179. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE VAS DEFERENS OF PATI DUCK, 6-8 WEEK

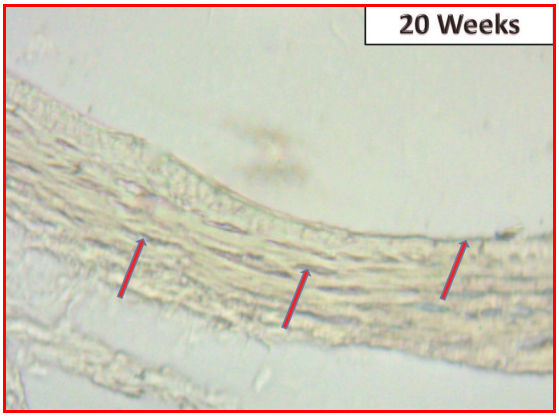

Fig. 4.180. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE VAS DEFERENS OF PATI DUCK, 20 WEEK

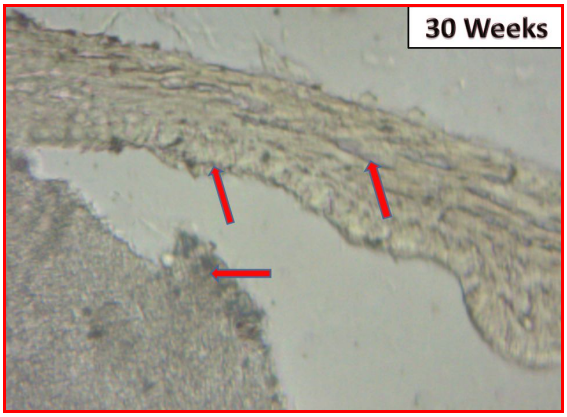

Fig. 4.181. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE VAS DEFERENS OF PATI DUCK, 30 WEEK

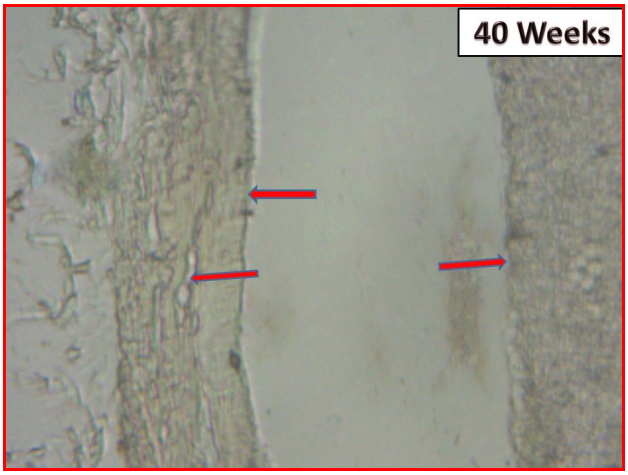

Fig. 4.182. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE VAS DEFERENS OF PATI DUCK, 40 WEEK

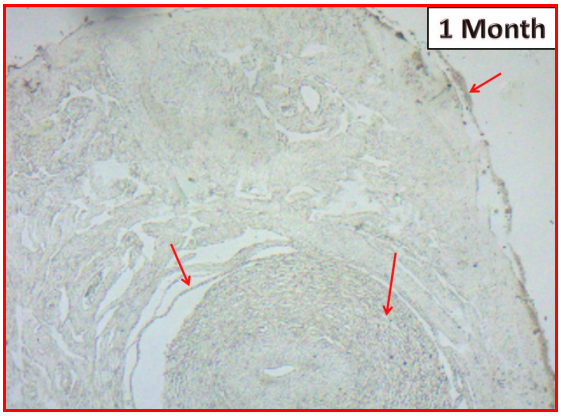

Fig. 4.183. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE PHALLUS OF PATI DUCK, 1 MONTH

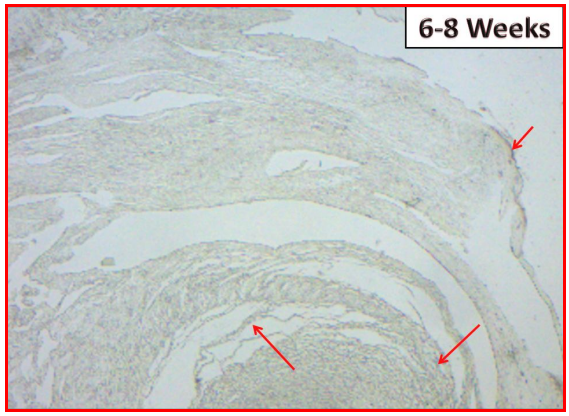

Fig. 4.184. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE PHALLUS OF PATI DUCK, 6-8 WEEK

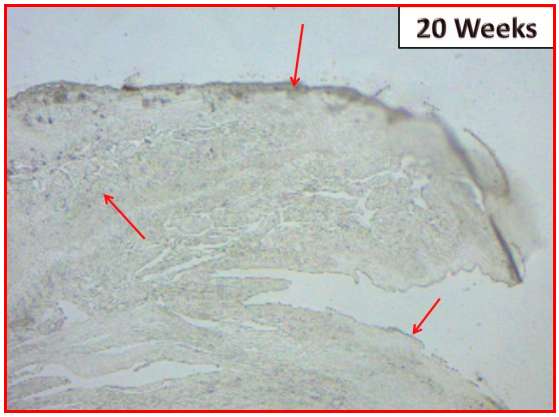

Fig. 4.185. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE PHALLUS OF PATI DUCK, 20 WEEK

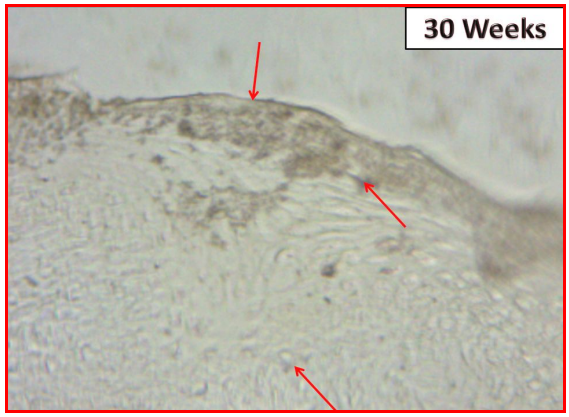

Fig. 4.186. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE PHALLUS OF PATI DUCK, 30 WEEK

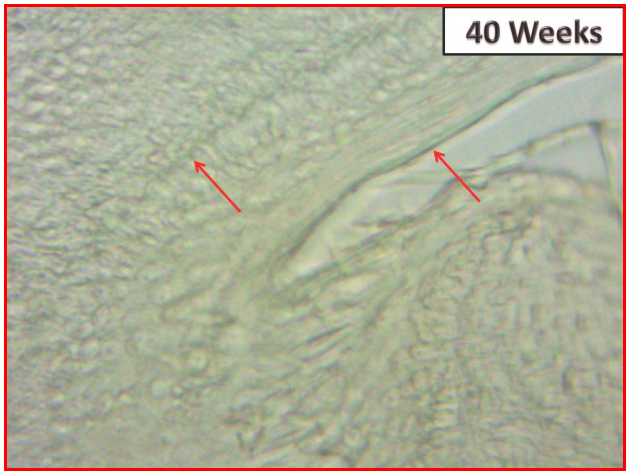

Fig. 4.187. PHOTOMICROGRAPH SHOWING THE ACTIVITY OF ATPASE IN THE PHALLUS OF PATI DUCK, 40 WEEK

4.4. ULTRASTRUCTURE

The seminiferous tubules of the testis of Pati duck were bounded by basal lamina. The space between two adjacent seminiferous tubules consisted of layers of cells which have an elongated shaped with different shaped nucleus (Fig. 4.188 to 4.190). Fibroblast-like cells were found within the interstitial space and in the peritubular area. Similar finding was observed by Nicholls and Graham (1972) in Japanese quail.

In the present study spermatogonium were resting on the basal membrane near the sertoli cells. They had a small nucleus and their cytoplasm consisted of mitochondria and ribosomes (Fig. 4.188 to 4.190). The absence of lipid droplets in spermatogonium differentiates the cells from sertoli cells. The finding was in accordance with Rothwell (1978) in domestic fowl. In the present study spermatocyte was observed towards the lumen (Fig. 4.189 and 4.192) having a prominent round nucleus with dense chromatin material; the cytoplasm consisted of numerous mitochondria (Fig. 4.192). A smaller early spermatid were also observed (Fig. 4.192) having a round nucleus with fine chromatin material. Longitudinal section of Midpeice of spermatozoa and cross section of midpiece and principal piece showed a central axoneme consisting of a paired microfilament surrounded by 9 pairs of microfilament which was surrounded by outer dense fibres. In mid piece the entire structure was covered by mitochondrial sheath which was absent in Principal piece (Fig. 4.192).

In Pati duck the sertoli cells were found in the basal membrane along with the spermatogonium as well as along with the spermatogenic cells towards the lumen with increased in layers of cells (Fig. 188 to 190). They were differentiated from germ cells by their irregularly shaped large nucleus. Some of the nucleus had intranuclear cleft (Fig. 4.193). The nucleolus of sertoli cell nucleus consists of a moderately dense and a very dense area. The cytoplasm was reduced by a larger nucleus. Endoplasmic reticulum, mitochondria and ribosomes were found in the cytoplasm (Fig 4.194). The finding was in accordance with Cooksey and Rothwell (1973) and Rothwell Tingari (1973) in domestic fowl. In the present study lipid droplets were found with the sertoli cells, some of them have large lipid bodies (Fig 4.188). Similar finding was reported by Rothwell (1978) in domestic fowl.

Under transmission electron microscope, different types of leydig cells were observed in the interstitial space of the testis of Pati duck. Elongated Leydig cells which appeared spindle shaped with elongated or round nucleus and prominent nucleolus. Numerous lipid droplets were found within the cells along with mitochondria and endoplasmic reticulum (Fig. 4.195). These elongated Leydig cells were transitional cell or primitive mesenchyme cells as per Connel (1972). In the present study the appearance of the elongated leydig cell was similar with fibroblast except the presence of lipid droplets and smooth endoplasmic reticulum in Leydig cells. The finding was in accordance with the reports by Rothwell (1973) in domestic fowl and Nicholls and Graham (1972) in Japanese quail. In Pati duck these types of cells were found in all age group in the interstitial space. Similar finding was reported by Rothwell (1973) in domestic fowl till sexual maturity.

Polygonal Leydig cells which were irregularly shaped cell with round nucleus and prominent nucleolus the cell surface were extended to form microvillus projection (Fig. 4.196 to 4.198). Similar finding was reported by Rothwell (1973) in domestic fowl. Connel (1972) reported that Polygonal shaped cells were also transitional or primitive mesenchymal cells. According to Bakst (2007) Leydig cells were characterized by their round nuclei and the numerous lipid droplets which were typical of steroid secreting cells.

In the present study some of the polygonal leydig cells had reduced amount of lipid droplets or only remnants of lipid droplets (Fig. 4.198). According to Rothwell (1973) a diminution of lipid droplets in leydig cells was considered an indication of Leydig cell functional maturation, as stored precursor were utilized in the production of new steroid. In Pati duck the cytoplasm of polygonal leydig cells consist of numerous mitochondria, smooth endoplasmic reticulum, ribosomes and cytoplasmic vesicles.

Under electron microscope the peritubular area of the seminiferous tubules was formed by layers of myoid cells which over lapped with each other (Fig. 4.199 to 4.201). The myoid cells were separated from the seminiferous tubules by basal lamina. The finding was in consonance with the reports by Aire and Ozegbe (2007) in Japanese quail where 1-6 layers of overlapping myoid cells were found. The finding was

contraindicated with the observations by Rothwell and Tingari (1973) in domestic fowl where fibroblast cells were observed in the peritubular area. The difference in findings can be attributed to the difference in species studied.

In Pati duck under electron microscope the peritubular myoid cells were observed to have a large and elongated nucleus with prominent nucleoli (Fig. 4.199 to 4.200). Some of the nucleus had smooth elongated shape while some have numerous invaginations and crooked shape. The cytoplasm which was reduced to a thin rim due to large nucleus contains mitochondria and endoplasmic reticulum (Fig. 4.201). The findings were in accordance with Aire and Ozegbe (2007) in Japanese quail. In Pati duck blood vessels and other connective tissue were found in between the adjacent peritubular boundary forming the peripheral limit to the peritubular layer (Fig). However Rothwel and Tingari (1973) reported that there were no interstitial elements intervening and the peritubular boundary layers run parallel in domestic fowl.

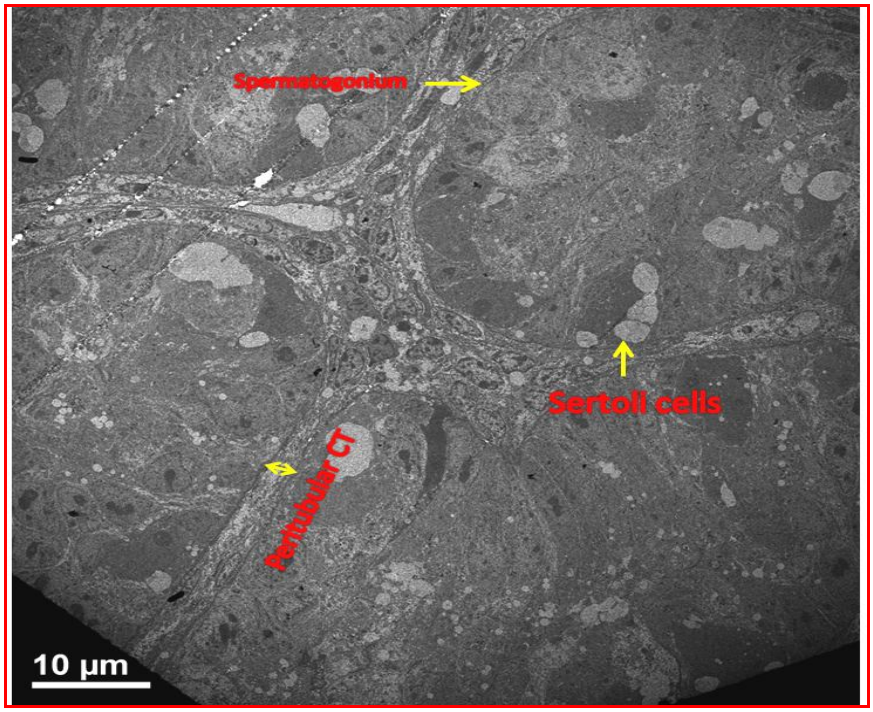

Fig. 4.188. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE PERITUBULAR CONNECTIVE TISSUE, SERTOLI CELLS AND SPERMATOGENIC CELLS OF SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK, 1 MONTH

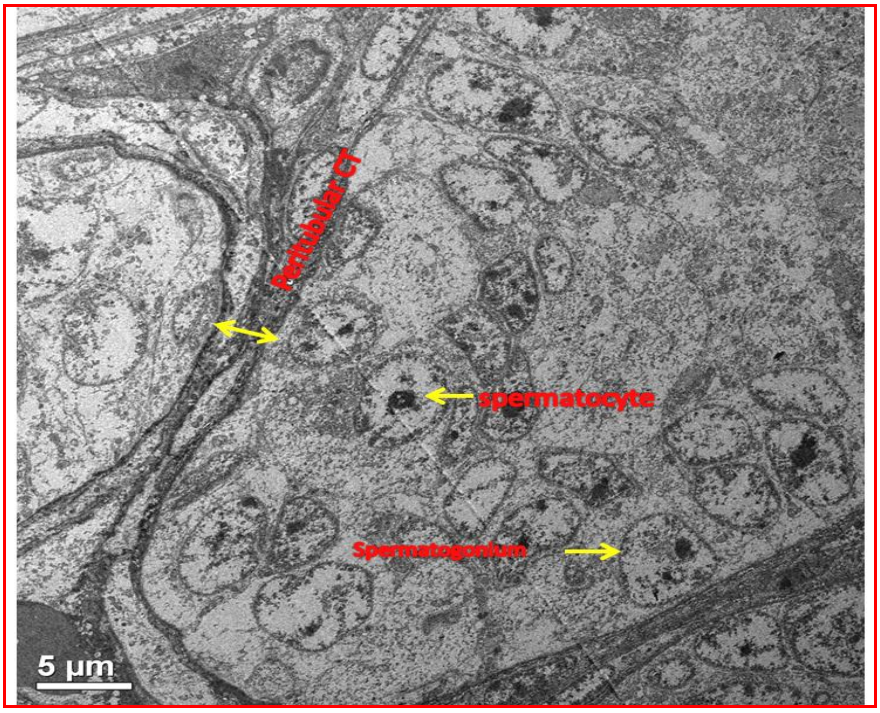

Fig. 4.189. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE PERITUBULAR CONNECTIVE TISSUE, SERTOLI CELLS AND SPERMATOGENIC CELLS OF SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK, 6-8 WEEKS

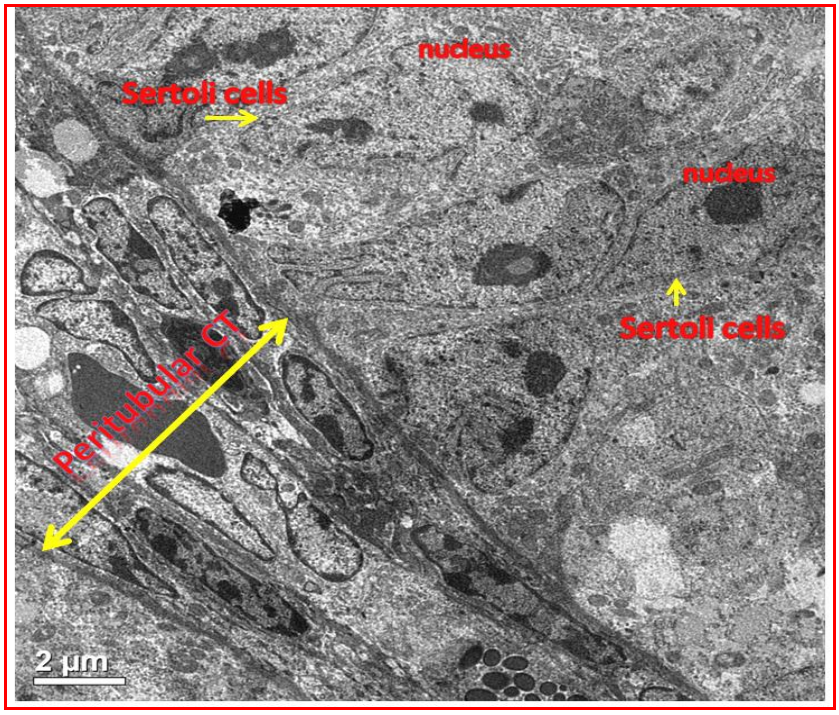

Fig. 4.190. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE PERITUBULAR CONNECTIVE TISSUE, SERTOLI CELLS AND SPERMATOGENIC CELLS OF SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK, 20 WEEKS

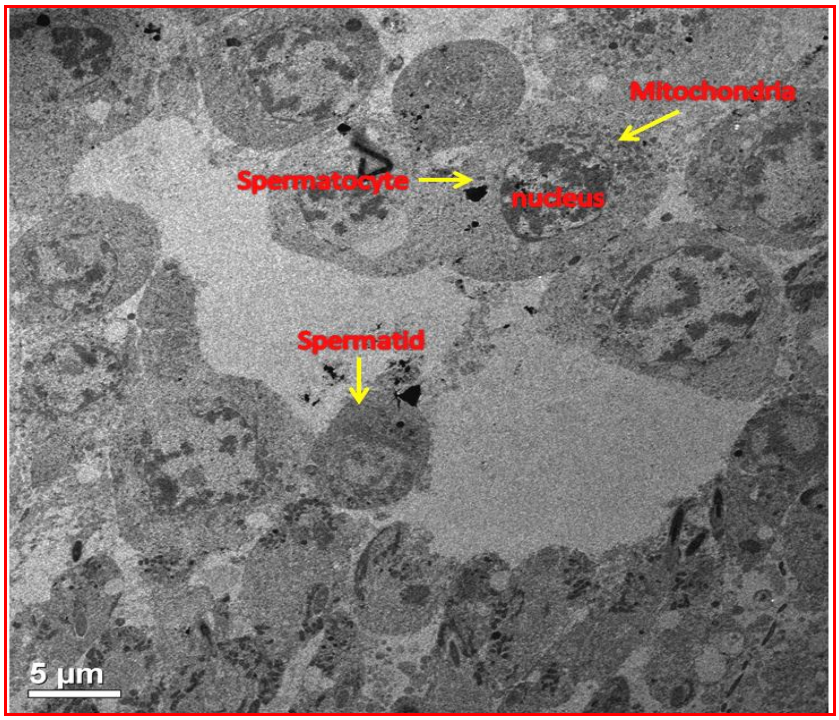

Fig. 4.191. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE SPERMATOGENIC CELLS OF SEMINIFEROUS TUBULES OF THE TESTIS OF PATI DUCK, 30 WEEKS

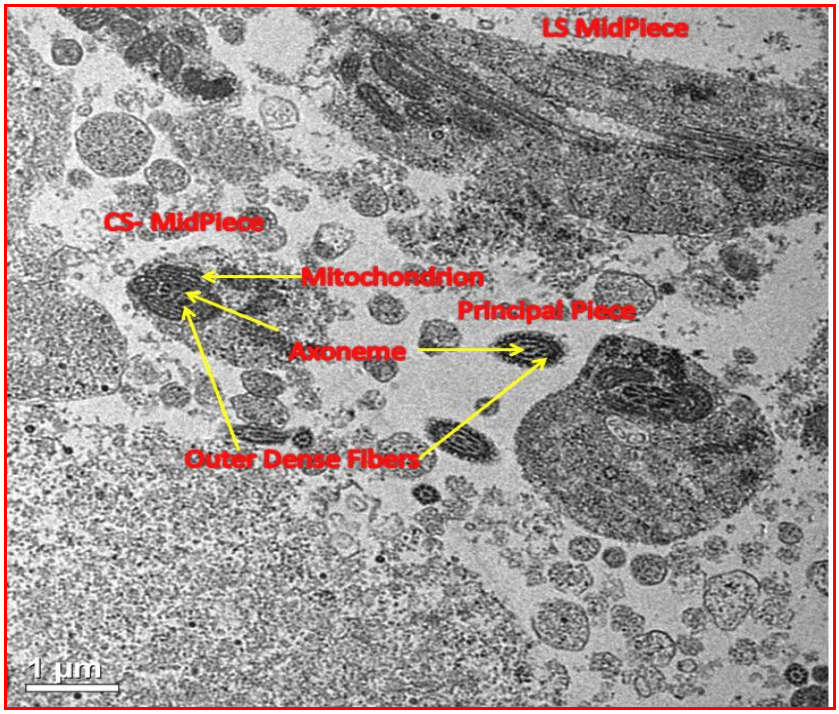

Fig. 4.192. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE LONGITUDINAL SECTION (LS) OF MIDPIECE, CROSS SECTION (CS) OF MIDPIECE AND PRINCIPAL PIECE OF SPERMATOZOA PATI DUCK, 40 WEEKS

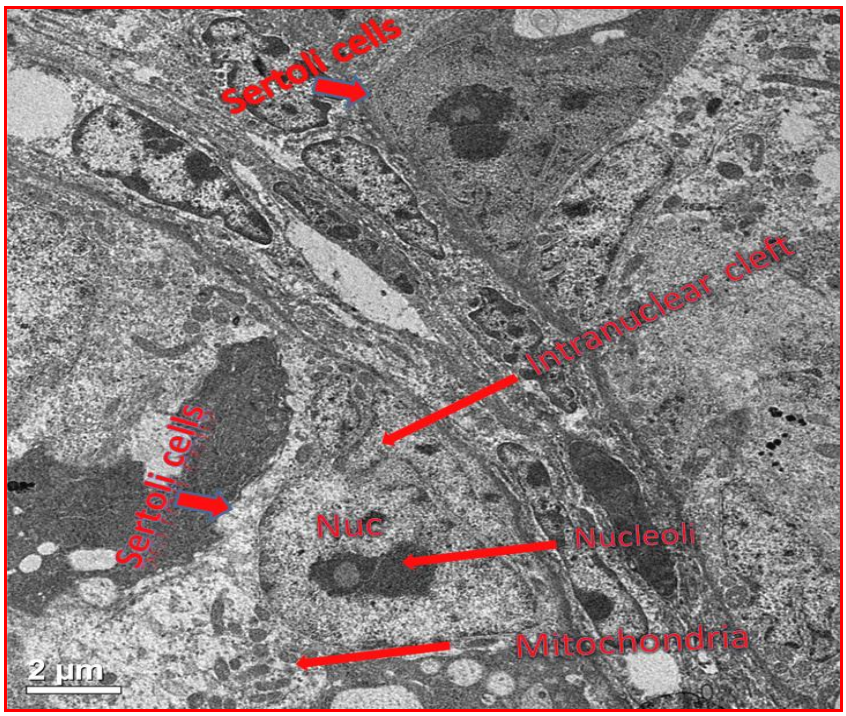

Fig. 4.193. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE SEROLI CELLS HAVING IRREGULARLY SHAPED NUCLEUS WITH INTRANUCLEAR CLEFT

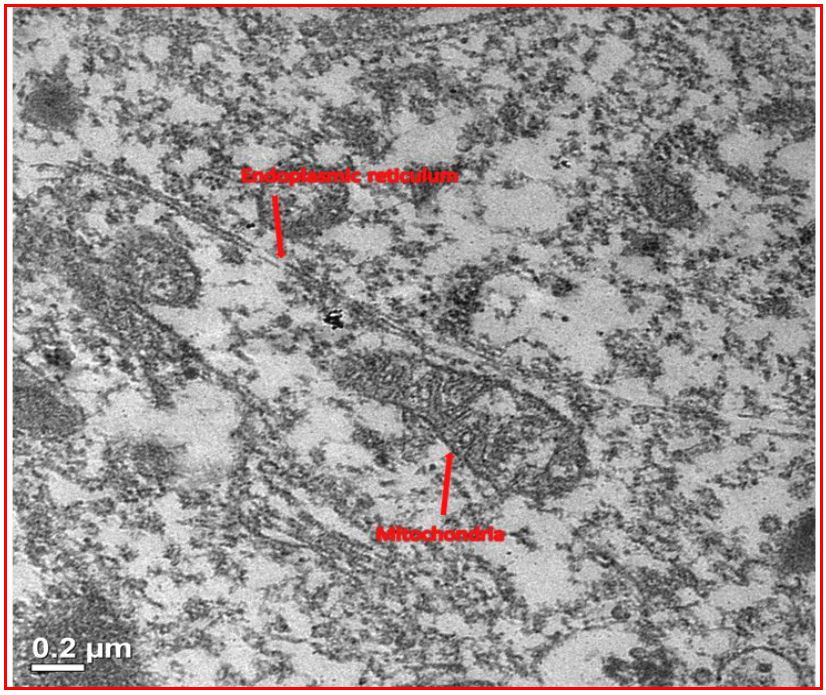

Fig. 4.194. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE MITOCHONDRIA AND ENDOPLASMIC RETICULUM PRESENT IN THE CYTOPLASM OF THE SEROLI CELLS

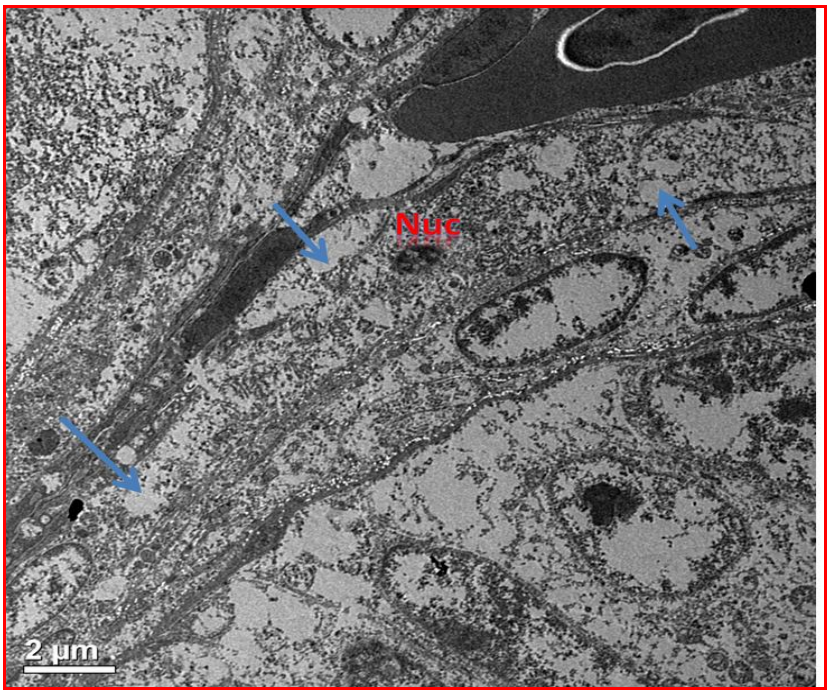

Fig. 4.195. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE ELONGATED LEYDIG CELLS WITH NUCLEUS (NUC) AND LIPID DROPLETS (→)

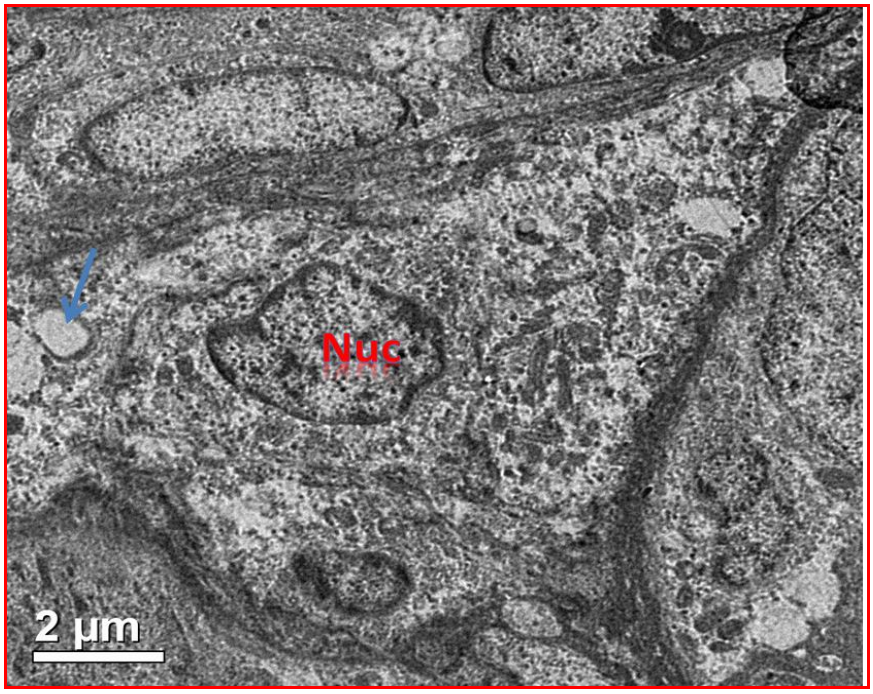

Fig. 4.196. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE POLYGONAL LEYDIG CELLS WITH NUCLEUS (NUC) AND LIPID DROPLETS (→)

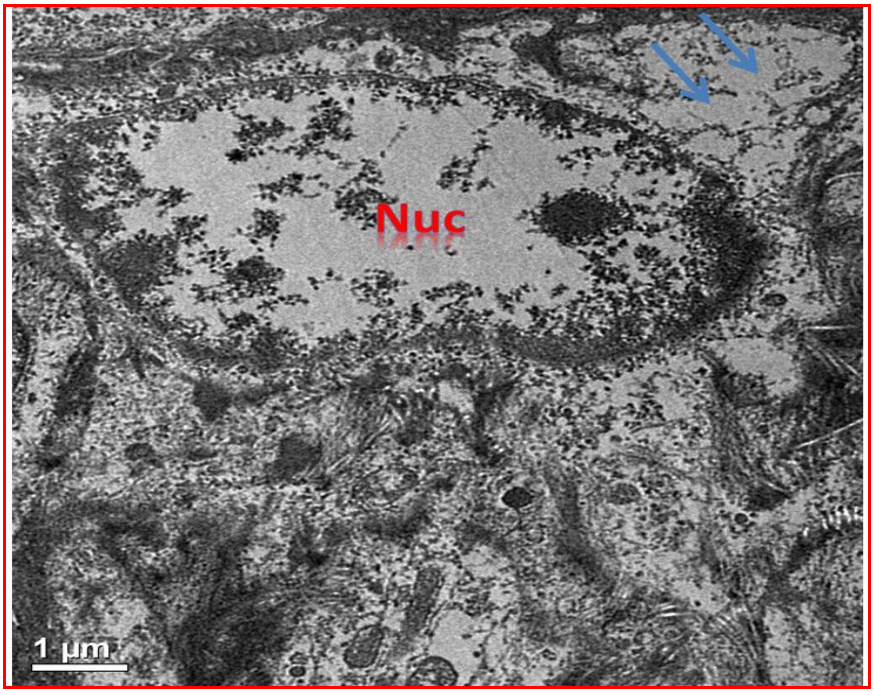

Fig. 4.197. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE POLYGONAL LEYDIG CELLS WITH NUCLEUS (NUC) AND LIPID DROPLETS (→)

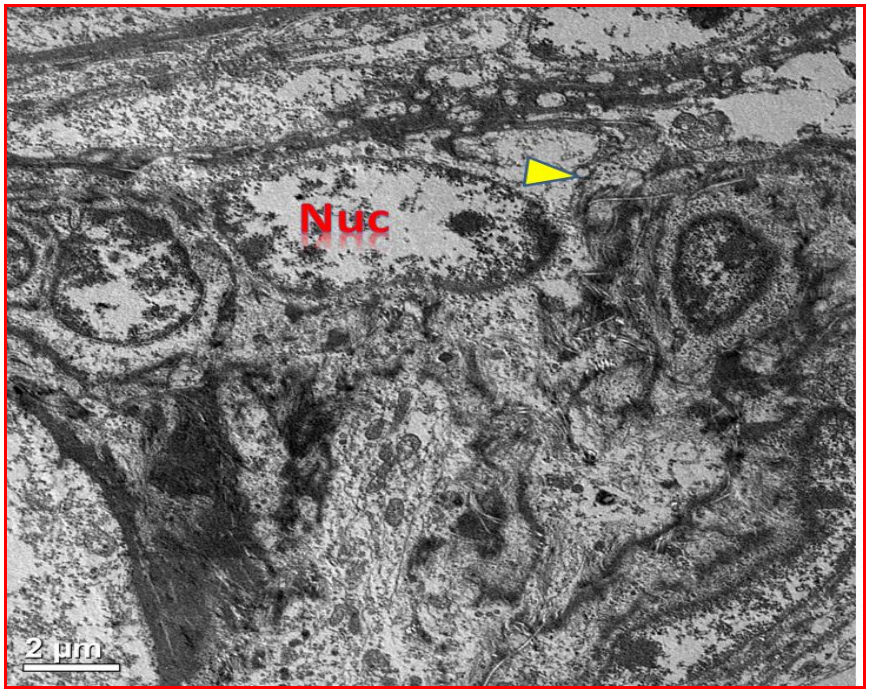

Fig. 4.198. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE POLYGONAL LEYDIG CELLS WITH NUCLEUS (NUC) AND REMNANTS OF LIPID DROPLETS (►)

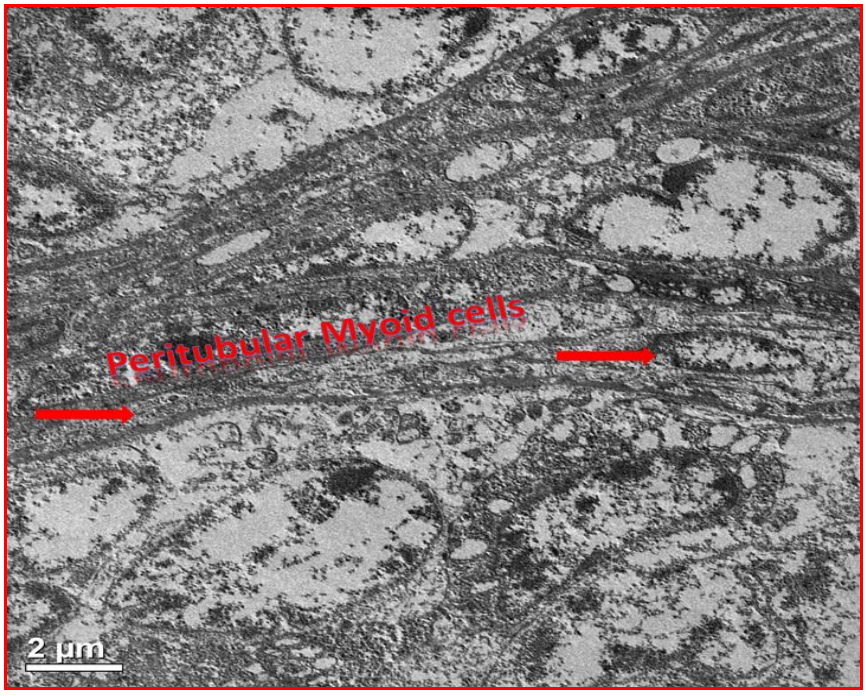

Fig. 4.199. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE OVERLAPPING PERITUBULAR MYOID CELLS WITH ELONGATED NUCLEUS

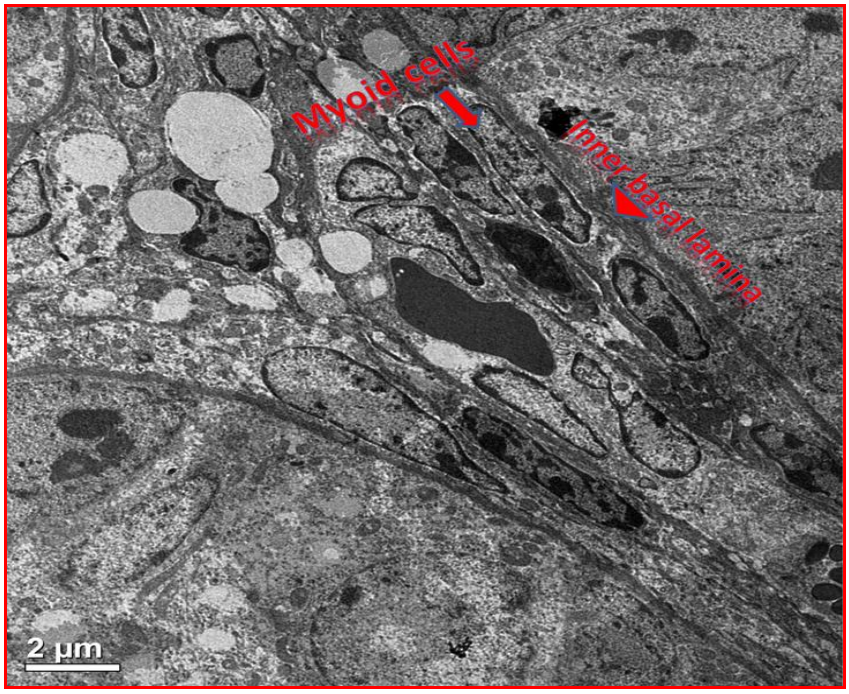

Fig. 4.200. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE OVERLAPPING PERITUBULAR MYOID CELLS WITH ELONGATED NUCLEUS OUTSIDE THE BASAL LAMINA OF SEMINIFEROUS TUBULES

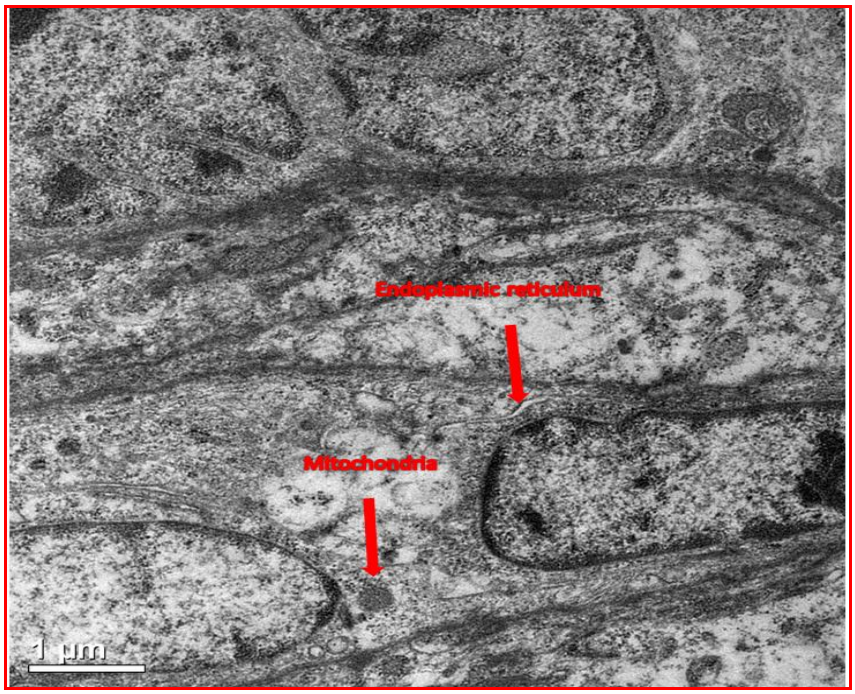

Fig. 4.201. TRANSMISSION ELECTRON MICROGRAPH SHOWING THE PERITUBULAR MYOID CELL CYTOPLASM WITH MITOCHONDIA AND ENDOPLASMIC RETICULUM

4.5. HAEMATOLOGY

Haematological parameters were detected using digital analyzer. The result obtained at different age groups were discussed in the following subheads.

4.5.1. Red Blood Cell count (RBC)

The RBC counts from 1 month to 40 weeks of male Pati duck were presented in Table 4.5. The mean values of RBC in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 2.51 ± 0.12×10^3/mm^3, 2.38 ± 0.06×10^3/mm^3, 2.83 ± 0.11×10^3/mm^3, 2.85 ± 0.18×10^3/mm^3 and 2.72 ± 0.19×10^3/mm^3 respectively. There was no significant difference among the different age groups, thus no age related change in the RBC count in male Pati duck of Assam (Fig. 4.202). The finding was comparable to the records by Mostaghni *et al.* (2005) in Flamingo (2.27 ± 0.29 ×10^{12}/l) and Black-headed gull (2.89 ± 0.45 ×10^{12}/l). Olayemi *et al.,* (2006) also recorded the RBC count in male Nigerian laughing dove (2.78 ± 0.44 × 10^{12}/L)) and Nigerian duck (2.43 ± 0.58 ×10^{12}/L). The value observed in the present study was lower than those determined by Kecici and Col (2011) in male pheasants chick (1.82±0.4 ×10^6/mm3) while it was similar with adult (2.55±0.5 ×10^6/mm3). The finding was also lower than the value recorded by Okeudo *et al.* (2003) in Southern Nigerian duck (3.31 ± 0.10 × 10^6/mm^3). The difference in findings can be attributed to difference in species and breed. Difference in environment may also have some effect on the difference. The male sex hormone, testosterone, was implicated to be responsible for the higher erythrocyte values in the male (Fried *et al.,* 1964). Olayemi *et al.* (2006) suspected that testosterone plays an insignificant role in the erythropoesis of the Nigerian laughing dove.

4.5.2. White Blood Cell count (WBC)

The WBC counts from 1 month to 40 weeks of male Pati duck were presented in Table 4.5. The mean values of WBC in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 58.20±1.55 M/mm^3, 52.81±4.30 M/mm^3, 60.37±1.26 M/mm^3, 47.00±7.15 M/mm^3 and 30.82±3.88 M/mm^3 respectively (Fig. 4.203). WBC count in 20 weeks was significantly higher ($P< 0.05$) than the other age groups while the count in 40 weeks was

significantly lower (P< 0.05). The finding was much higher than the recorded count by Menon *et al.* (2013) in Emu (12.2 ± 0.6 × 10^9/L); Mostaghni *et al.* (2005) in Flamingo (5.93 ± 1.25 ×10^9/l) and Black-headed gull 2.25 ± 0.42 ×10^9/l). Olayemi *et al.* (2006) also recorded the WBC count in male Nigerian laughing dove (0.75 ± 0.28 × 10^9/L) and Nigerian duck (16.96 ± 2.23 × 10^9/L). Okeudo *et al*. (2003) recorded that in Southern Nigerian Duck WBC count was (23.81 ± 0.88 × 10^3/mm^3). The difference in finding may be attributed to species and breed difference.

4.5.3. Lymphocyte (%)

The lymphocyte percentage in the blood of male Pati duck from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of lymphocytes in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 58.59±2.55%, 53.48±1.29%, 54.82±4.24%, 54.39±3.71% and 55.53±2.88% respectively (Fig. 4.204). There was no significant difference in the percentage of lymphocyte at different age group of male Pati duck. Lymphocyte was observed to be highest in 1 month old whereas it was lowest in 6-8 weeks old. The finding was in conflict with the observations by Kecici and Col (2011) in pheasants where it was lowest in chick (62.0 ± 9.2%) and highest in adult (73.3 ± 11.1%) which were comparatively higher that any age groups of Pati duck. The observed values in the present study was also higher than the findings by El-Katcha *et al.,* (2017) in Pekin ducking (31.36%), Menon *et al.* (2013) in Emu (31.8 ± 1.4%); by Mostagni *et al.* (2005) in Flamingo (35.14±2.1%) and Black headed gull (42.66±4.7%). However the finding in Pati duck was much lower than the observation by Okeudo *et al. (*2003) in Southeastern Nigerian Duck 80.09% and Sturkie (1986) in male Indian native duck 68.0%. The difference in findings may be ascribed to differences in avian species, the management procedure, and the physical and environmental conditions.

4.5.4. Monocyte (%)

The monocyte percentages in the blood of male Pati duck from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of monocytes in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 4.80±0.07%, 5.17±0.12%, 5.00±0.16%, 5.80±0.45% and 12.31±1.24% respectively. The percentage of monocyte

was significant higher (P< 0.05) at 40 weeks of age (Fig. 4.204). The finding at 40 weeks was similar to the findings by El-katcha *et al.* (2017) in Pekin duckling (12.40%) while the finding in other age group was much lower. The observed value at 1 month old was in accordance with the findings by Menon *et al.* (2013) in male Emu (4.0 ± 0.5%). The observation in Pati duck of Assam was in conflict with Kecici and Col (2011) in pheasants as a decreasing trends was observed from chick (4.4 ± 2.0%) to adult (2.5 ± 1.1%). The difference in findings may be attributed to differences in avian species, the management procedure, and the physical and environmental conditions.

4.5.5. Neutrophil or Heterophils (%)

The neutrophil percentages in the blood of male Pati duck of Assam from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of neutrophils in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 34.63 ± 2.91%, 30.77 ± 1.75%, 37.78 ± 4.20%, 43.52 ± 2.25% and 25.44 ± 2.76% respectively. The heterophils percentage in 30 weeks age group was significantly higher (P< 0.05) than the other age groups, while 40 weeks was significantly lower (P< 0.05) than the others (Fig. 4.204). The mean percentage neutrophil was recorded by El-katcha *et al.* (2017) in Pekin duck (39.60%) which was in the same range as Pati duck of Assam. The findings by Mostaghni *et al.* (2005) in Flamingo (64.71 ± 4.47%) and Black-headed gull (57.33 ± 12.2%); Menon *et al.* (2013) in male Emu (63.2 ± 1.6%) were much higher than the findings in Pati duck of Assam. Sturkie (1986) recorded that the heterophils in Pekin duck was 52%. However a mean heterophils of 15.33 ± 4.16% was recorded by Okeudo *et al.* (2003) in male Southeastern Nigerian duck which was lower than the findings in Pati duck. Kecici and Col (2011) observed a decreasing trend from chick (28.3 ± 8.6) to adult (21.1 ± 11.3) pheasant which was in conflict with the findings in Pati duck of Assam. The difference in findings may be attributed to differences in avian species, breed, the management procedure, and the physical and environmental conditions of the birds studied.

4.5.6. Eosinophil (%)

The eosinophil percentages in the blood of male Pati duck from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of eosinophil in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 3.30±1.80%, 5.82±1.33%, 2.27±0.52%, 3.65±0.21% and 5.48±0.83% respectively. There was no significant difference among the different age groups. The study showed that eosinophil was highest in 6-8 weeks age while it was lowest in 20 weeks old (Fig. 4.204). The finding was in the same range with the findings by Okeudo *et al.* (2003) in Southeastern Nigerian male duck with mean eosinophil 5.67 ± 2.08%. However, the observation was in conflict with the findings by Kecici and Col (2011) in pheasants where eosinophil concentrations decreased from chick (3.2 ± 2.0%) to adult (2.4 ± 1.1%). The mean percentage eosinophil was recorded by El-katcha *et al.* (2017) in Pekin duck (11.70%) which was much higher while the findings by Menon *et al.* (2013) in male Emu was much lower (0.9 ± 0.2%) than the findings in Pati duck of Assam. The differences in findings may relate to season, species and/or technique.

4.5.7. Basophil (%)

The basophil concentrations in the blood of male Pati duck from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of basophil in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 0.33 ± 0.12%, 0.47±0.11%, 0.27 ± 0.07%, 0.27 ± 0.03% and 0.45±0.04% respectively. There was no significant difference between the different age groups. It was observed that basophil was highest in 6-8 weeks and lowest in 30 weeks of age (Fig. 4.204). The finding was in accordance with Menon *et al.* (2013) record in male Emu (0.5 ± 0.1%). The finding was in conflict with the observations by Kecici and Col (2011) in pheasants where basophil percentage decreased from chick (2.13 ± 1.1%) to adult (0.73 ± 0.7%). The mean percentage basophil was recorded by El-katcha *et al.* (2017) in Pekin duck (4.93%) which was much higher than the observed value in Pati duck of Assam. The differences in findings may relate to season, species and/or technique.

4.5.8. Hemoglobin (Hb)

The hemoglobin value from 1 month to 40 weeks of male Pati duck were presented in Table 4.5. The mean values of Hb in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 13.92±0.64 g/dl, 12.92±0.99 g/dl, 16.08±0.55 g/dl, 14.92±1.04 g/dl and 14.13±1.03 g/dl respectively. There was no significant difference in the Hb value among the different age group with highest Hb observed in 20 weeks age group while lowest was observed in 6-8 weeks age group (Fig. 4.205). The Hb value observed in the present study was in a similar range with the observation by Mulley (1979) in male Black duck (12.88 ± 1.25 g/100 ml), Okeudo *et al.* (2003) in Southeastern Nigerian male duck (15.67 ± 0.29g/100 ml) and Oladele *et al.* (2007) in Mallard duck (13.37 ± 0.20g %). Mostaghni *et al*. (2005) recorded the Hb value in Flamingo (117.8±59 g/l) and Black headed gull (123 ± 13.3 g/l). Olayemi *et al.* (2006) also recorded the Hb value in male Nigerian laughing dove (148.60 ± 22.10 g/L) and Nigerian duck (136.10 ± 20.40 g/L). Birds that don't fly have higher Hb than bird which frequently flies (Bond and Gilbert, 1958 and Olayemi *et al.* (2006) which may be the reason for lower Hemoglobin observed in Pati duck. The change in Hb may be attributed to change demands of oxygen for activity.

4.5.9. Packed Cell Volume (PCV) %

The PCV percentages in the blood of male Pati duck from 1 month to 40 weeks were presented in Table 4.5. The mean concentrations of PCV in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 36.15±1.92%, 35.68±2.02%, 42.15±1.13%, 42.87±2.76% and 45.12±2.43% respectively. The value of PCV for 1 month and 6-8 weeks was significantly lower than the other age groups (Fig. 4.206). The findings in Pati duck of Assam was in the similar range with the observation by Mulley RC (1979) in Black duck (40.00 ± 3.82%), Oyewale and Ajibade (1990) in Pekin duck (41.38 ± 4.08%), Okeudo *et al.* (2003) in Southeastern Nigerian male duck (46.00 ± 1.73%) and Oladele *et al.* (2007) in Mallard duck (40.12 ± 0.58)%. Mostaghni *et al*. (2005) recorded the PCV percentage in Flamingo (35.21 ± 1.6%) and Black-headed gull (39 ± 2.52%). Olayemi *et al*. (2006) recorded the PCV in male Nigerian laughing dove (43.58 ± 7.33%) and Nigerian duck (42.58 ± 5.67%). A much higher percentage of PCV was recorded by

Menon *et al*. (2013) in Emu (50.3 ± 1.4%). Higher PCV and hemoglobin concentrations in tropical poultry breeds over exotic breed might be due to inherent physiological traits in these local breeds involving their hemopoetic system (Oluyemi and Ologhobo, 1998; Nwosu, 1979) which probably enhances the dissipation of useless energy Okeudo *et al*. (2003). The difference in the PCV concentrations observed may also be attributed to breed and species difference as Orji *et al.* (1986), reported strong species and sex effects on avian hematological parameters.

TABLE 4.5. AVERAGE HAEMATOLOGICAL PARAMETERS OF PATI DUCK DURING POST NOETAL DEVELOPMENT

HAEMATOLOGY	AGE				
	1 Month	**6-8 Week**	**20 Week**	**30Week**	**40Week**
RBC (10^3/mm3)	2.51±0.12	2.38±0.06	2.83±0.11	2.85±0.18	2.72±0.19
WBC (M/mm3)	$58.20±1.55^{AB}$	$52.81±4.30^{AB}$	$60.37±1.26^{A}$	$47.00±7.15^{B}$	$30.82±3.88^{C}$
Lymphocyte (%)	58.59±2.55	53.48±1.29	54.82±4.24	54.39±3.71	55.53±2.88
Monocyte (%)	$4.80±0.07^{B}$	$5.17±0.12^{B}$	$5.00±0.16^{B}$	$5.80±0.45^{B}$	$12.31±1.24^{A}$
Heterophil (%)	$34.63±2.91^{B}$	$30.77±1.75^{BC}$	$37.78±4.20^{AB}$	$43.52±2.25^{A}$	$25.44±2.76^{C}$
Eosinophil (%)	3.30±1.80	5.82±1.33	2.27±0.52	3.65±0.21	5.48±0.83
Basophil (%)	0.33±0.12	0.47±0.11	0.27±0.07	0.27±0.03	0.45±0.04
Hb (g/dl)	13.92±0.64	12.92±0.99	16.08±0.55	14.92±1.04	14.13±1.03
PCV (%)	$36.15±1.92^{B}$	$35.68±2.02^{B}$	$42.15±1.13^{AB}$	$42.87±2.76^{A}$	$45.12±2.43^{A}$

* Means with different superscript are significantly different.

Dependent Variable: WBC (m/mm3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3356.507713	839.126928	7.89	0.0003
Error	25	2659.577833	106.383113		
Corrected Total	29	6016.085547			

Dependent Variable: Monocyte (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	246.3564800	61.5891200	28.96	<.0001
Error	25	53.1666667	2.1266667		
Corrected Total	29	299.5231467			

Dependent Variable: Neutrophil (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1129.110280	282.277570	5.62	0.0023
Error	25	1255.682017	50.227281		
Corrected Total	29	2384.792297			

Dependent Variable: PCV (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	430.367187	107.591797	3.97	0.0125
Error	25	676.803350	27.072134		
Corrected Total	29	1107.170537			

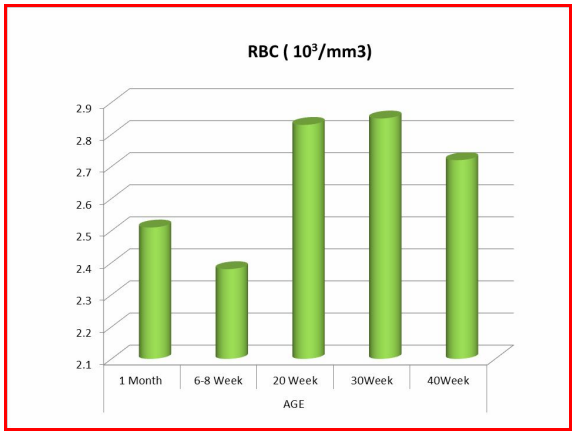

Fig. 4.202. GRAPHICAL REPRESENTATION OF THE AVERAGE RED BLOOD CELLS (RBC) COUNT AT DIFFERENT AGE GROUP OF *PATI* DUCK

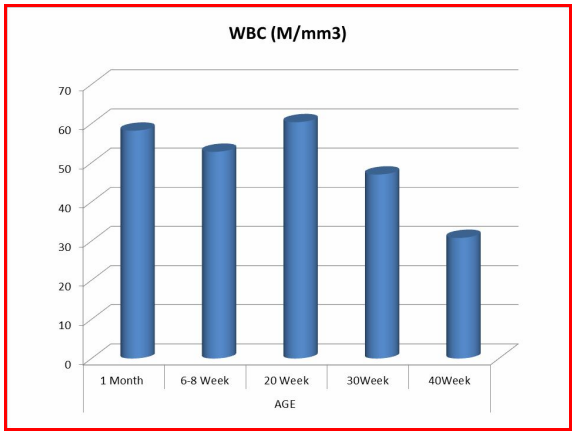

Fig. 4.203. GRAPHICAL REPRESENTATION OF THE AVERAGE WHITE BLOOD CELLS (WBC) COUNT AT DIFFERENT AGE GROUP OF *PATI* DUCK

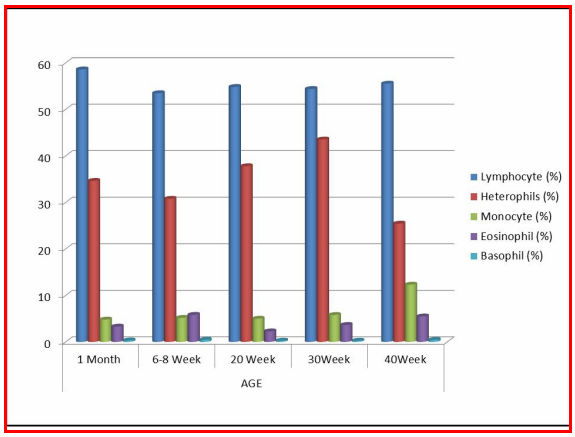

Fig. 4.204. GRAPHICAL REPRESENTATION OF THE AVERAGE PERCENTAGE OF LEUKOCYTES AT DIFFERENT AGE GROUP OF *PATI* DUCK

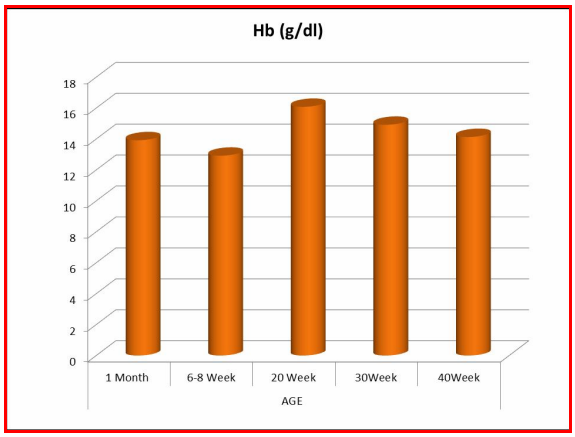

Fig. 4.205. GRAPHICAL REPRESENTATION OF THE AVERAGE HAEMOGLOBIN AT DIFFERENT AGE GROUP OF *PATI* DUCK

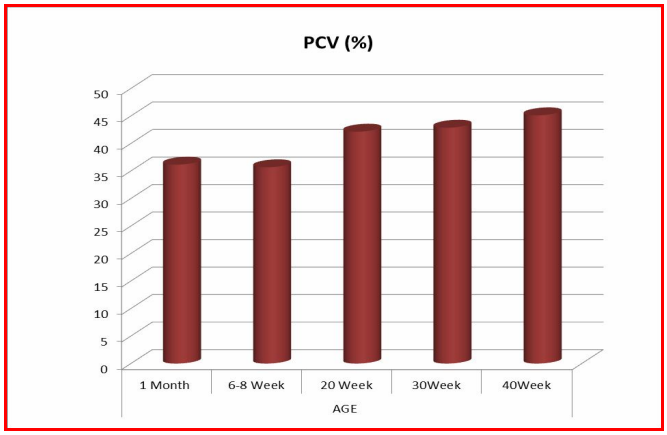

Fig. 4.206. GRAPHICAL REPRESENTATION OF THE AVERAGE PACKED CELL VOLUME (PCV) AT DIFFERENT AGE GROUP OF *PATI* DUCK

4.6. HORMONE

4.6.1. Triiodothyronine (T3)

The mean values of triiodothyronine in male Pati duck from 1 month to 40 weeks were presented in Table 4.6. The mean level of T3 in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 3.37 ± 0.2 nmol/L, 3.23 ± 0.30 nmol/L, 4.18 ± 0.26 nmol/L, 3.11 ± 0.42 nmol/L and 2.92 ± 0.28 nmol/L. There was no significant difference among the different age group. The concentration of T3 decreases with age with the exception of 20 weeks age group (Fig. 4.207). Biswas *et al.* (2010) also observed a decrease in T3 concentrations from 6 weeks (4.83± 0.16 ng/ml) to 30 (0.86 ± 0.10 ng/ml) weeks in White Leghorn, Kadakhnath and Aseel. Sinurat *et al.* (1987) observed a daily decrease in the T3 level in broiler with the initial mean value of 0.85-0.95µg/l to 0.85-0.91µg/l after 20 days. A decrease in T3 with age indicated that the hormone played an important role in growth and development of birds (Biswas *et al.,* 2010) since T3 is metabolically more active than T4 in avian (Klandorf *et al.,* 1981).

4.6.2. Thyroxine (T4)

The mean values of thyroxine in male Pati duck from 1 month to 40 weeks were presented in Table 4.6. The mean level of T4 in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 15.06 ± 3.9nmol/L, 12.01 ± 3.88nmol/L, 3.72 ± 1.05nmol/L, 9.94 ± 4.19 nmol/L and 6.12 ± 1.49 nmol/L. There was no significant difference among the different age group. Maximum level of T4 was observed in 1 month age group while minimum level was observed in 20 weeks age group. There was a gradual decrease in T4 level with age from 1 month to 20 weeks of age which increased at 30 weeks and later decrease at 40 weeks (Fig. 4. 208). The finding was comparable with observations by Biswas *et al.* (2010) in White Leghorn where T4 concentration decreased from 6 weeks (16.25 ± 1.92ng/ml) to 30 weeks (13.79 ± 1.21ng/ml) of age. Harvey *et al.* (1980) recorded T4 concentration of 6.12±0.22ng/ml during dark period and 5.00 ± 0.22ng/ml during light period in domestic duck. Sinurat *et al.* (1987) observed a daily decrease in the T4 level in broiler with initial mean value of 1.13-1.26µg/l to 0.72-0.81µg/l after 20 days. The higher concentration of hormone during early age may be attributed to an

increased metabolic rate, especially to energy production as well as to their involvement in the growth and development of the bird at early age of life. (Biswas *et al.,* 2010)

4.6.3. Testosterone

The mean values of testosterone hormone in male Pati duck from 1 month to 40 weeks were presented in Table 4.6. The mean level of testosterone in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 0.04 ± 0.01nmol/L, 0.06 ± 0.01nmol/L, 0.15 ± 0.03nmol/L, 0.3 ± 0.11nmol/L and 0.42 ± 0.08 nmol/L. The hormone level was lowest at 1 month age group which increased with age and maximum level was observed in 40 weeks age group (Fig. 4.209). There was significant difference ($P< 0.05$) in the hormone level among the age groups. Abdul Rahman (2018) recorded the testosterone concentration at 4 weeks (0.09 ± 0.04ng/ml), 8 weeks (0.12 ± 0.04ng/ml), 20 weeks (0.28 ± 0.04ng/ml) and 32 weeks (0.18 ± 0.037ng/ml) which were approximately similar to the findings in Pati duck. Hau *et al.* (2010) also recorded a testosterone concentration of 0.2 ng/ml in Tropical birds. Whereas Tanabe *et al.* (1983) recorded a testosterone level of 111.8 ± 16.0 ng/ml in 14 days old duck which was comparatively higher than the findings in the present study. Simoes *et al.* (2017) observed also highest testosterone concentrations of 76.91 ± 0.20 ng/dl during peak production. The difference in the findings may be attributed to sample collection time as some avian have gonadally active period (breeding season) and in-active period which may affect the hormone level. Testosterone levels showed seasonality, with the highest levels observed during peak reproduction and at the beginning of quiescence in domestic duck (Simoes *et al*., 2017).

4.6.4. Cortisol

The mean values of cortisol hormone in male Pati duck from 1 month to 40 weeks were presented in Table 4.6. The mean level of cortisol in 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 9.37 ± 4.33 nmol/L, 7.99 ± 2.05 nmol/L, 4.71 ± 1.4 nmol/L, 12.84 ± 6.28 nmol/L and 30.78 ± 4.39 nmol/L. The cortisol hormone reached maximum level at 40 weeks which was significantly higher than the other age groups (Fig. 4.210). Flament *et al.* (2012) reported cortisol level of 4.25 ± 1.36 ng/ml in 8weeks old hybrid duck which was lower than the findings in 6-8 weeks old Pati duck. A cortisol

level of 8.75 ± 0.62 ng/ml in 2 days old chick was reported by Kalliecharan and Hall (1974), 0.56 ± 0.17 ng/ml in 14 days old chick by Tanabe *et al*. (1983). Schmidt and Soma (2008) observed a decreasing trend of cortisol concentration with age in songbird. Cortisol is the preferred Glucocorticoids for immune regulation during development in birds (Schmidt and Soma, 2008; Breuner, 2008).

TABLE 4.6. AVERAGE HORMONAL PARAMETERS OF PATI **DUCK DURING POST NATAL DEVELOPMENT**

HORMONE (nmol/L)	**AGE**				
	1 Month	**6-8 Weeks**	**20 Weeks**	**30 Weeks**	**40 Weeks**
Triiodothyronine (T3)	3.37 ± 0.2	3.23 ± 0.30	4.18 ± 0.26	3.11 ± 0.42	2.92 ± 0.28
Thyroxine (T4)	15.06 ± 3.9	12.01 ± 3.88	3.72 ± 1.05	9.94 ± 4.19	6.12 ± 1.49
Testosterone	0.04 ± 0.01^{C}	0.06 ± 0.01^{C}	0.15 ± 0.03^{BC}	0.3 ± 0.11^{AB}	0.42 ± 0.08^{A}
Cortisol	9.37 ± 4.33^{B}	7.99 ± 2.05^{B}	4.71 ± 1.4^{B}	12.84 ± 6.28^{B}	30.78 ± 4.39^{A}

Dependent Variable: TESTOSTERONE

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Week	4	0.65205013	0.16301253	7.07	0.0006
Error	25	0.57657683	0.02306307		
Total	29	1.22862697			

Dependent Variable: CORTISOL

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
week	4	2537.659900	634.414975	6.32	0.0012
Error	25	2511.535855	100.461434		
Total	29	5049.195755			

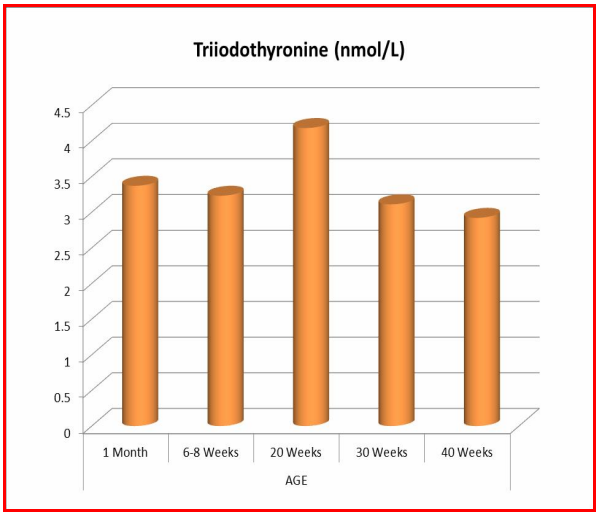

Fig. 4.207. GRAPHICAL REPRESENTATION OF THE AVERAGE TRIIODOTHYRONINE (T3) VALUE AT DIFFERENT AGE GROUP OF PATI DUCK

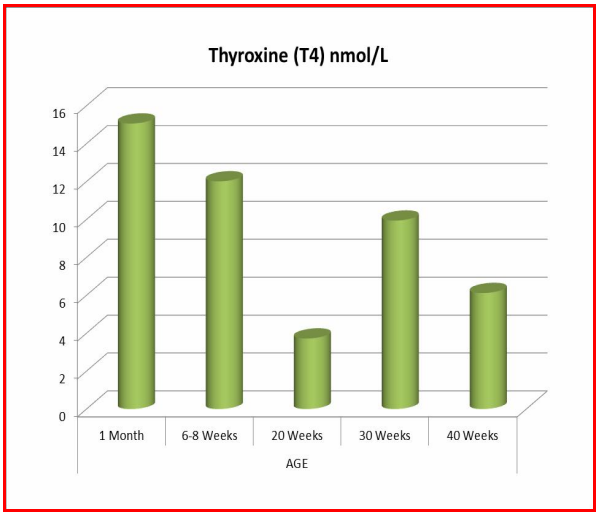

Fig. 4.208. GRAPHICAL REPRESENTATION OF THE AVERAGE THYROXINE (T4) VALUE AT DIFFERENT AGE GROUP OF PATI DUCK

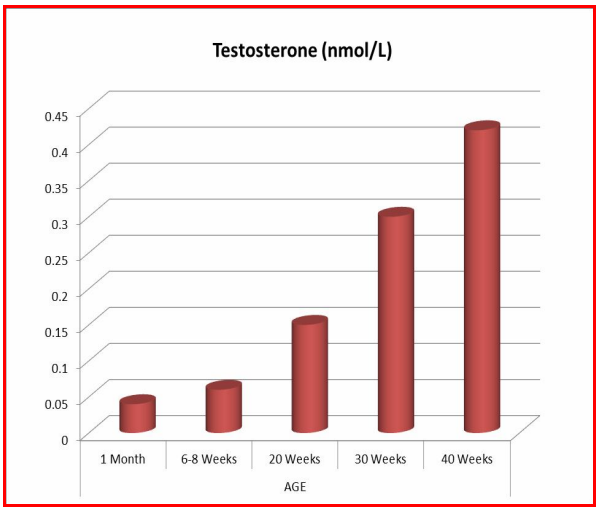

Fig. 4.209. GRAPHICAL REPRESENTATION OF THE AVERAGE TESTOSTERONE VALUE AT DIFFERENT AGE GROUP OF PATI DUCK

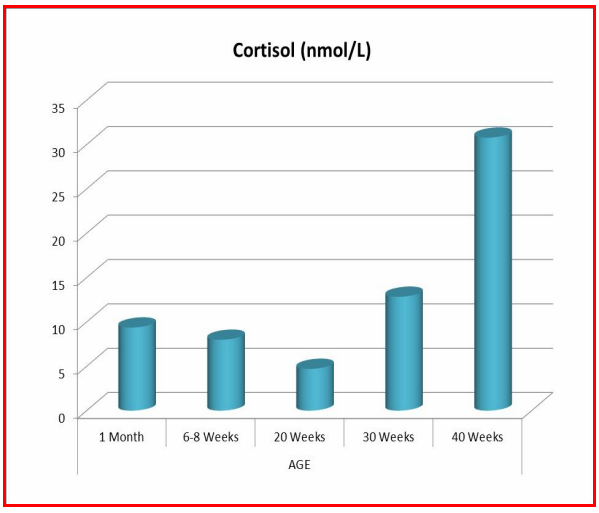

Fig. 4.210. GRAPHICAL REPRESENTATION OF THE AVERAGE CORTISOL VALUE AT DIFFERENT AGE GROUP OF PATI DUCK

4.7. BIOCHEMISTRY

Biochemical parameters were estimated on the serum of Pati duck of Assam collected at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks. The post-natal changes in the serum metabolites and enzymes were discussed in the following sub-heads.

4.7.1. ALP (alkaline phosphatase)

The alkaline phosphatase levels from 1 month to 40 weeks of male Pati duck of Assam were presented in Table 4.7. The mean values at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 326.49 ± 19.83 U/L, 320.38 ± 48.93 U/L, 230.54 ± 23.95 U/L, 358.57 ± 37.24 U/L and 130.62 ± 33.85 U/L respectively. There was significant difference ($P<0.05$) among the different age groups. The levels of ALP in 1 month, 6-8 weeks and 30 weeks were significantly higher than that of 20 and 40 weeks, where the maximum serum alkaline phosphatase level was observed at 30 weeks and minimum level at 40 weeks (Fig. 4.211). The lower value of ALP observed when the birds were at 20 weeks and 40 weeks may be due to decreased metabolism of liver as reported by Sinha *et al.* (2017). The findings differed from observation by Sinha *et al.* (2017) in Pati duck where the serum ALP was in a decreasing trend from 2 weeks (185.062 ± 1.365 U/L) to 40 weeks (12.912 ± 0.209 U/L). Similarly, Deka (2018) also observed a decreasing trend of ALP in Pati duck of Assam from 1 weeks old (337.01 ± 8.77 UL) to 42 weeks old (72.33 ± 3.07 U/L). The level of ALP observed in the study were comparatively higher than that of the findings reported by Deka (2018) when compared between the same age groups. The ALP level at 40 weeks (130.62 ± 33.85 U/L) recorded in the study was comparatively higher than the findings by Deka *et al.* (2017) in 42 weeks Pati duck (28.10 ± 1.87 UL) and Chara-Chemabali duck (51.03 ± 1.52UL). Mahanta *et al.* (1994) observed the level of ALP in Pati duck to be 82.46 ± 5.29 µ moles p-nitrophenol/min/liter. An ALP range of 51 – 202 U/L was observed by Franco *et al.* (2010) in Indian runner ducks with an average of 117 ± 51.9 U/L which was lower that the findings observed. The difference in the findings observed in the study maybe due to difference in sex of birds used, as the study was conducted only in male Pati duck of Assam. The difference may also be due to difference in season of collection as

Hochleithner *et al*. (1994) had reported the seasonal variation in ALP. The increased in ALP activity result from increased cellular synthesis. Juvenile birds have higher Alkaline phosphatase activity in comparison to adult due to bone growth and development.

4.7.2. AST/SGOT (Aspartate Transaminase)

In the present study the aspartate transaminase levels from 1 month to 40 weeks of male Pati duck were presented in table 4.7. The mean values at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 14.97 ± 6.31 U/L, 20.8 ± 6.8 U/L, 35.02 ± 11.33 U/L, 16.21 ± 3.12 U/L and 18.85 ± 4.58 U/L respectively. There was no significant difference among the different age group of male Pati duck. It was observed that AST level increased with age from 1 month to 20 weeks of age, but decrease at 30 weeks and 40 weeks of age (Fig. 4.212). The AST value observed was in the same range with the findings by Franco *et al.* (2010) in adult Indian Runner duck (26.1 ± 8.8 U/L) and El-katch *et al.* (2017) in growing Pekin duckling (20 µ/l). However the value observed was in conflict with the findings of Sinha *et al.* (2017) where the AST level increased from 2 weeks (46.39 8± 1.305) till 40 weeks (419.553 ± 1.169) of age. The levels of AST observed in the study were very low in comparison to the findings by Sinha et al., (2017) in Pati duck of Assam; Franco *et al*. (2010) in American Flamingoes (154.5 ± 33.6 U/L); Mulley (1979) in black duck (61.00 ± 24.00 U/L); Bowes *et al.* (1988) in 1 month old Broiler (184 ± 38 U/L) and White Leghorn (188 ± 16 U/L); and Mostagni *et al*. (2005) in Flamingo (70.83 ± 19.77 IU/l) and Black headed gull (92.66 ± 17.14 IU/l). The increase in AST value from 1 month to 20 weeks may due increased in body weight with age as Satish (2013) reported an increase in AST enzyme activity with body weight.

4.7.3. ALT/SGPT (Alanine Transaminase)

The level of alanine transaminase in the present study from 1 month to 40 weeks of male Pati duck were presented in Table 4.7. The mean values at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 39.22 ± 15.29 U/L, 27.25 ± 7.29 U/L, 30.35 ± 4.69 U/L, 15.83 ± 5.51 U/L and 28.83 ± 9.17U/L respectively. There was no significant difference between the age groups. The highest level of serum ALT was observed at 1 month age group where it decreased with increase in age till it reached minimum level at

30 weeks age group (Fig. 4.213). The level of ALT observed was in the same range to the findings by Franco *et al.* (2010) in Indian Runner duck (24.8 ± 5.2 U/L) and American Flamingos (26.0 ± 5.9 U/L); by Mulley (1979) in male black duck (12.90 ± 7.70 U/L); and by El-katcha *et al*. (2017) in growing Pekin duckling (18.8µ/l). However the finding was in contrast to the findings by Sinha *et al.* (2017) in Pati duck of Assam where ALT levels were observed to increase from 2 weeks (250.971± 0.597 U/L) to 40 weeks (1021.240± 0.590 U/L). The finding in Pati duck was higher than the findings by Mostagni *et al.* (2005) in Flamingo (4.2 ± 0.2 IU/l) and Black headed gull (9.21 ± 1.2IU/l). ALT levels tends to below the level of detection for an analyzer in some avian species Hochleithner *et al.* (1994) which maybe the reason for the low ALT level observed in Pati duck of Assam

4.7.4. Total protein

The total protein levels from 1 month to 40 weeks of male Pati duck were presented in Table 4.7. The mean values at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 4.65 ± 0.033 g/dl, 5.27 ± 0.76 g/dl, 5.69 ± 0.86 g/dl, 6.73 ± 0.93g/dl and 4.24 ± 0.18 g/dl respectively. Total protein level increased with age from 1 month till 30 weeks age. But a drastic decrease was observed at 40 weeks (Fig. 4.214). The finding was similar to the findings of Sinha *et al.* (2017) in Pati duck where total protein levels increased from 2 weeks (3.085 ± 0.061 g/dl) till 40 weeks (4.783 ± 0.014 g/dl). The total protein observed in the study was also similar to the findings by Mulley (1979) in male Black duck (4.57 ± 0.22 g/100 ml) and El-katcha *et al.* (2017) in growing Pekin duckling (5.7 g/dl). However, the total protein observed in 1 month age group Pati duck was lower than the findings by Rezende *et al.* (2017) in male (24.60 ± 4.20 g/L) broiler of 1 month old; by Bowes *et al.* (1989) in 1 month old broiler (34.4 ± 2.70 mg/dl) and white leghorn (33.90 ± 2.20 mg/dl). The total protein observed in adult Pati duck was also much lower than the findings of Mostaghni *et al.* (2005) in adult Flamingo (55±4.7 g/l) and Black-headed gull (51±8.1 g/l). The lower total protein in male than female was attributable to higher albumin level in female (Rezende *et al.,* 2017). According to Bell (1971), total serum protein is influenced by breed, age, physiological state, environment and antigen exposure and levels can be extremely variable. The total protein observed in Pati duck of

Assam was within or close to the normal total protein level in bird *i.e.* 3-5 g/dl (Gee *et al.* 1981; Lewandowski *et al.*, 1986; Palomeque *et al.*, 1991 and Mostagni *et al.*, 2005).

4.7.5. Albumin

The albumin levels from 1 month to 40 weeks of male Pati duck were presented in Table 4.7. The mean value at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 2.49 ± 0.64 g/dl, 2.76 ± 0.64 g/dl, 3.17 ± 0.37 g/dl, 3.88 ± 0.58 g/dl and 1.52 ± 0.20 g/dl respectively. There was significant difference ($P < 0.05$) among the different age groups. Albumin level of 40 weeks was significantly lower from the other age group. 1 month and 6-8 weeks albumin level was also significantly different from 20 and 30 weeks (Fig. 4.215). The findings were slightly lower than the observations by El-katcha *et al.*, (2017) in growing duckling (4.4g/dl). However the albumin level observed in the study were much lower than the findings by Olayemi *et al.* (2006) in adult Nigerian duck (12.20 ± 2.90) and Nigerian laughing (10.20 ± 4.20); by Rezende *et al.* (2017) in broiler of 1 month old male (10.60a ± 1.90 g/L) and female (13.70 ± 1.90 g/L); Jerabek *et al.* (2018) in Hybrid mallard duck (32.6 g/l); Bowes *et al.* (1988) in 1 month old Broiler (12.9 ± 1.2 mg/L) and white (Leghorn 13.3 ± 1.6 mg/L); and Mostagni *et al.* (2005) in Flamingo (17.1 ± 2.7 g/l) and Black-headed gull (18.3 ± 2 g/l). According to Bowes *et al.* (1988) serum albumin increased when protein intake exceeds the amount required for growth and maintenance

4.7.6. Creatinine

The results of creatinine levels from 1 month to 40 weeks of male Pati duck were presented in Table 4.7. The mean value at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 6.14 ± 2.58 mg/dl, 0.81 ± 0.25 mg/dl, 1.48 ± 0.59 mg/dl, 0.9 ± 0.2 mg/dl and 2.62 ± 1.95 mg/dl respectively. There was significant difference ($P < 0.05$) among the different age groups. The level of creatinine for 1 month and 40 weeks were significantly higher than 6-8, 20 and 30 weeks age groups. Highest level of creatinine was observed in 1 month age group and minimum level at 30 weeks age group (Fig. 4.216). The findings were similar to the finding by El-katcha *et al.* (2017) in growing Pekin duckling (1.0 mg/dl). The finding was in contrast to the findings by Sinha *et al.*

(2017) in Pati duck of Assam where minimum level was observed in 2 weeks (3.135± 0.058mg/dl) and maximum level at 40 weeks (6.616± 0.041mg/dl). This might be due to the fact that creatinine is mainly produced by the metabolism of creatine or creatine phosphate in skeletal muscle (Sinha *et al.,* 2017). The creatinine level observed in the study was much lesser than the findings by Bowes *et al.* (1988) in 1 month old broiler (44.8 ± 5.6 mg/dl) and white leghorn (48.8 ± 4.5 mg/dl). Creatine dehydrating mechanism were absent in fowl (Bowes *et al*., 1988) which can be the reason for the low level of creatinine observed in Pati duck of Assam.

4.7.7. BUN (Blood Urea Nitrogen)

The Blood Urea Nitrogen levels for Pati duck from 1 month to 40 weeks were presented in Table 4.7. The mean value of BUN at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 99.59 ± 24.89 mg/dl, 126±26.63 mg/dl, 86.47 ± 19 .05 mg/dl, 87.83 ± 33.19 mg/dl and 55.91 ± 16.12 mg/dl, respectively. There was no significant difference ($P < 0.05$) among the different age groups of male Pati duck. The BUN level was highest at 6-8 weeks age group, followed by 1 month age group. Minimum BUN level was observed at 40 weeks age group (Fig. 4.217). The findings in 40 weeks Pati duck was in similar range with the findings by El-katcha *et al.* (2017) in growing Pekin duckling (57.2 mg/dl). However the observation was in conflict with the findings by Franco *et al*. (2010) in flamingos and Indian runner ducks where serum BUN were <2mg/dL. Urea levels can be highly increased in dehydration in birds (Hochleithner, 1994) and were reported to be useful in diagnosing renal failure in pigeons (Lumeij, 1997) which help in the diagnosis of many diseases.

4.7.8. LDH (LACTATE DEHYDROGENASE)

The lactate dehydrogenase levels from 1 month to 40 weeks of male Pati duck were presented in Table 4.7. The mean values at 1 month, 6-8 weeks, 20 weeks, 30 weeks and 40 weeks were 302.71 ± 73.95 U/L, 196.24 ± 52.14 U/L, 209.83 ± 76.25 U/L, 153.66 ± 44.23 U/L and 218.54 ± 63.48 U/L respectively. There was no significant difference among the age groups. It was observed that LDH enzyme was highest at 1 month old and lowest at 30 weeks of age (Fig. 4.218). The observation was similar to

Bell (1971) in domestic fowl where higher level of LDH was observed in young birds. The level of LDH observed in the present study was similar with the findings by Mulley (1979) in Black duck male (351.80 ± 80.50 U/L). However the finding in 1 month old was much lower than the findings by Bowes et al (1988) in 1 month old broiler (817 ± 259 U/L) and white leghorn (490 ±138 U/L). Skeletal and cardiac muscle can be significant sources of LDH in serum (Bowes et al. 1988) where an increased skeletal and cardiac workload in younger birds may result in higher level of LDH.

TABLE 4.7. AVERAGE SERUM METABOLITES AND ENZYMES OF PATI DUCK DURING POST NOETAL DEVELOPMENT

	AGE				
	1 Month	**6-8 Week**	**20 Week**	**30 Week**	**40 Week**
ALP (U/L)	326.49± 19.83^{AB}	320.38± 48.93^{AB}	230.54± 23.95^{BC}	358.57± 37.24^{A}	130.62± 33.85^{BC}
AST (U/L)	14.97 ± 6.31	20.80 ± 6.80	35.02 ±11.33	16.21 ±3.12	18.85 ±4.58
ALT (U/L)	39.22 ±15.29	27.25 ± 7.29	30.35 ± 4.69	15.83 ± 5.51	28.83 ± 9.17
Total Protein (g/dl)	4.65 ± 0.033	5.27 ± 0.76	5.69 ± 0.86	6.73 ±0.93	4.24 ± 0.18
Albumin (g/dl)	2.49± 0.64^{AB}	2.76 ± 0.64^{AB}	3.17 ± 0.37^{A}	3.88± 0.58^{A}	1.52 ±0.2^{B}
Creatinine (mg/dl)	6.14± 2.58^{A}	0.81 ± 0.25^{B}	1.48 ± 0.59^{B}	0.90± 0.20^{B}	2.62± 1.95^{AB}
BUN (mg/dl)	99.59± 24.89	126.00± 26.63	86.47 ± 19.05	87.83±33.19	55.91± 16.12
LDH (U/L)	302.71± 73.95	196.24± 52.14	209.83 ± 76.25	153.66± 44.23	218.54± 63.48

** Means with the different superscript are significantly different.

DEPENDENT VARIABLE: ALBUMIN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Age	4	18.10246249	4.52561562	2.85	0.0451
Error	25	39.75228125	1.59009125		
Total	29	57.85474374			

DEPENDENT VARIABLE: CREATININE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Age	4	118.0519282	29.5129820	2.26	0.0915
Error	25	326.9255167	13.0770207		
Total	29	444.9774449			

DEPENDENT VARIABLE: ALP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Age	4	207000.7500	51750.1875	7.32	0.0005
Error	25	176801.3067	7072.0523		
Total	29	383802.0567			

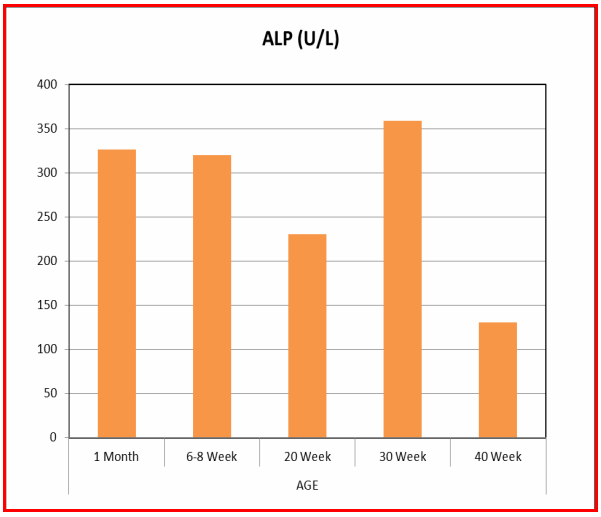

Fig. 4.211. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM ALKALINE PHOSPHATES (ALP) VALUE AT DIFFERENT AGE GROUP OF *PATI* DUCK

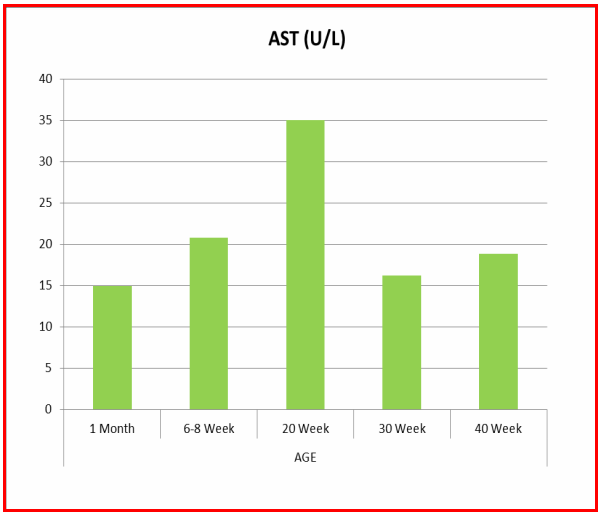

Fig. 4.212. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM ASPATATE TRANSAMINASE (AST) VALUE AT DIFFERENT AGE GROUP OF *PATI* DUCK

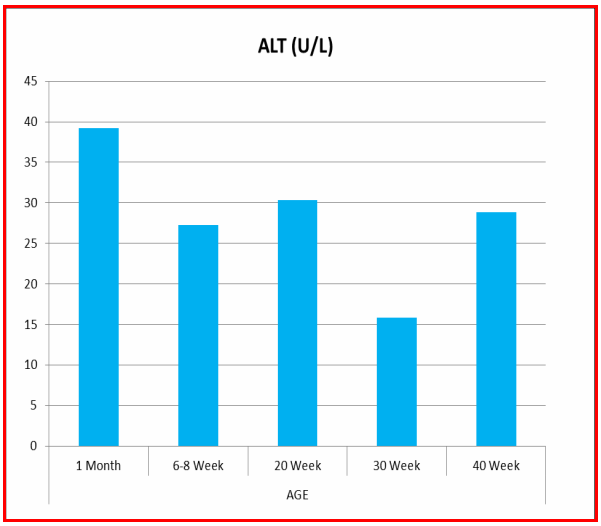

Fig. 4.213. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM ALANINE TRANSAMINASE (ALT) VALUE AT DIFFERENT AGE GROUP OF *PATI* DUCK

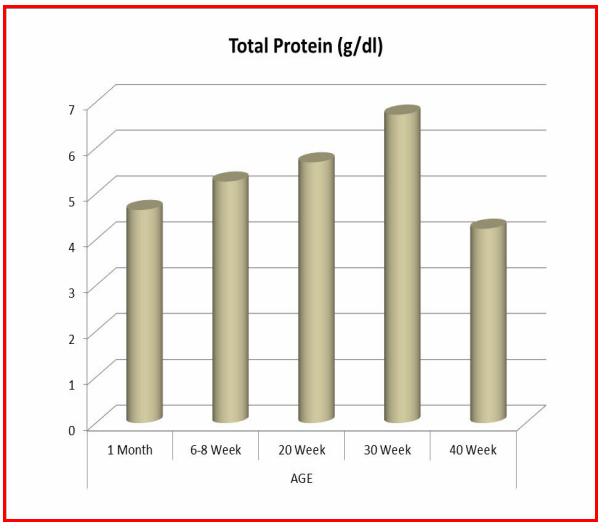

Fig. 4.214. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM TOTAL PROTEIN AT DIFFERENT AGE GROUP OF *PATI* DUCK

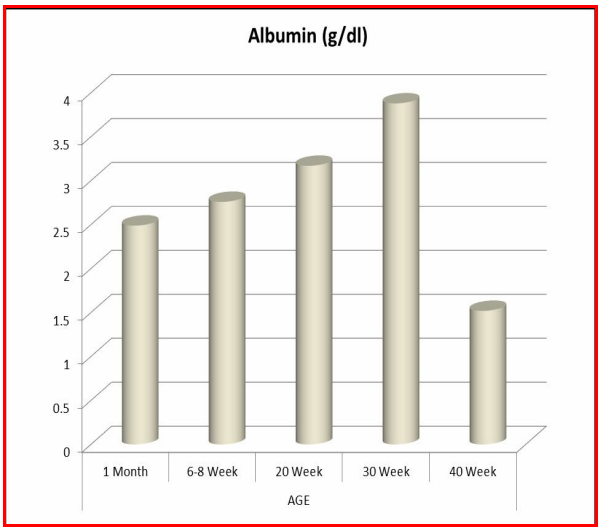

Fig. 4.215. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM ALBUMIN AT DIFFERENT AGE GROUP OF PATI DUCK

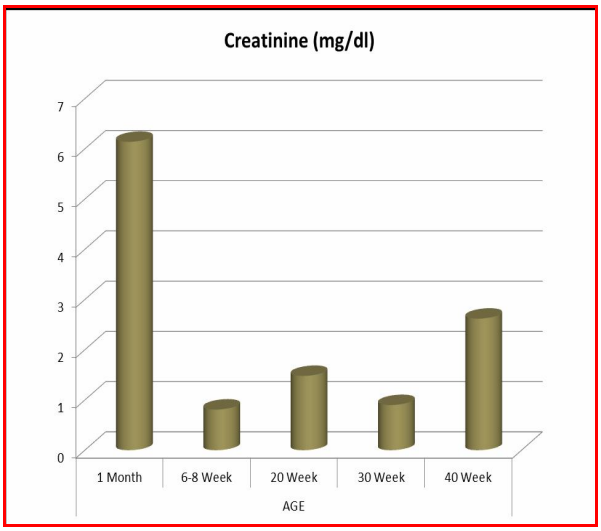

Fig. 4.216. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM CREATININE AT DIFFERENT AGE GROUP OF PATI DUCK

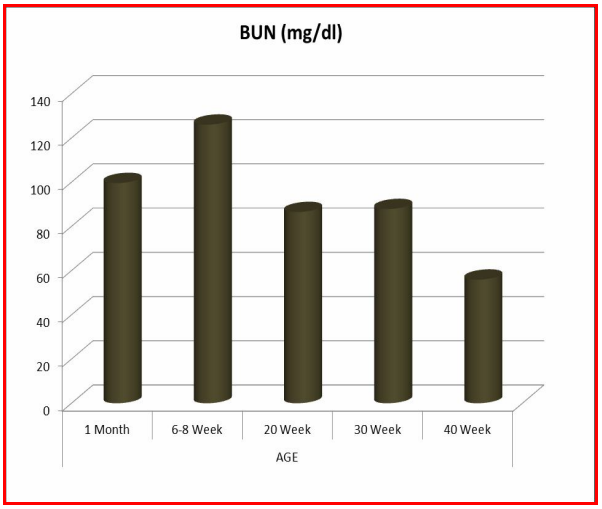

Fig. 4.217. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM BLOOD UREA NITROGEN (BUN) AT DIFFERENT AGE GROUP OF PATI DUCK

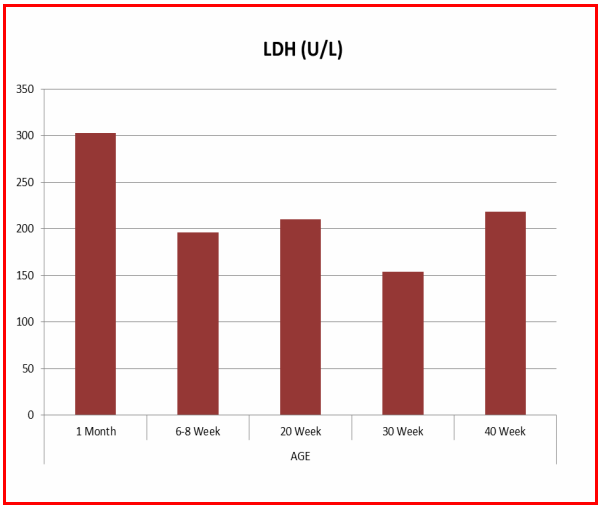

Fig. 4.218. GRAPHICAL REPRESENTATION OF THE AVERAGE SERUM LACTATE DEHYDOGENASE (LDH) AT DIFFERENT AGE GROUP OF PATI DUCK

CHAPTER - V

Summary and Conclusion

CHAPTER-V

SUMMARY AND CONCLUSION

The present study was undertaken to elaborate the post-natal changes in gross anatomical, histomorphological, histochemical and ultrastructural aspects of male genital system of Pati ducks (*Anas platyrhynchos)* of Assam and the changes in haematological, serum hormonal and biochemical during the postnatal development. Since there were no available literatures on the detailed anatomical study on the male genital system of Pati duck during the postnatal development hence the present study was designed to establish basic anatomical norms. The outcome of these research findings shall help Physiologist, Pathologist, Microbiologist and Poultry Scientists for carrying out further research work as well as to develop the disease control regime.

In the present investigation, total 30 numbers of apparently healthy Pati ducks were utilized for detailed anatomical study on gross, histomorphological, histochemical, ultrastructural, haematological, hormonal and biochemical.

The paired testis of Pati duck of Assam was located within the abdominal cavity ventral to the kidney till 20 weeks of age. With the advancement of age, the testis increased in size and were located between the cranioventral aspect of the kidney and caudoventral aspect of lung in adult birds. The paired testis of Pati duck were attached the dorsal body wall by connective tissue called mesorchium and located asymmetrically on either side of the midline with the left testis located slightly more cranial than the right testis. The testis of 1 month of age Pati duck was opaque white in colour with elongated rice grain like appearance. The colour turned slightly yellowish at 6-8 weeks of age. At 20 weeks it was creamy colour and oval shaped. The testis of 30 and 40 weeks were bean shaped with creamy to pinkish colour. The epididymis of the testis of Pati duck in 1 month, 6-8 weeks and 20 weeks of age was not distinct. But in 30 weeks and 40 weeks of age at the dorso medial aspect of testis was occupied by group of tubules strongly attached to the testis which was the epididymis. The epididymis extends upto the middle 2/3rd of the dorsomedial aspect of testis. The vas deferens convoluted in a zigzag pattern and emerged from the epididymis which ran parallel to the ureter at the

ventral aspect of kidney as to reach the cloaca. With the increase in age of the duck the diameter of vas deferens increased and the tightly coiled tube loosened.

The base of the phallus was attached to the ventral floor of the cloaca. From the base, the phallus coiled in anti-clockwise direction towards the apex. The ejaculatory groove and sulcus of the phallus run along the length of phallus and divide the shaft into two lateral bodies which ends at the tip of apex. The phallus of Pati duck was long, thick at the base with tapering coiled end. The length increased with age where maximum length was observed in 40 weeks age group.

The testis of Pati duck of Assam were covered by a thin capsule which have 3 layers *viz.* tunica serosa, tunica albuginea and tunica vasculosa. The thickness of the capsule increased with age. The tunica serosa was the outermost layer and consists of simple squamous epithelium. The tunica albuginea forms the bulk of the capsule, and is composed of dense connective tissue. The tunica vasculosa was formed by loose connective tissue, which consisted of fibroblasts and blood vessels.

The parenchyma of the testis consisted mainly of complex tubules which were highly convoluted which were separated by interstitial connective tissue**.** The testes were not divided into lobules and have no mediastinum testis. The diameter of the seminiferous tubules increased significantly with age. In 1 month old testis have no distinct layer of cells in the seminiferous tubules which was mainly composed of Sertoli cells along with few spermatogonium cells and vacuolated cells which have prominent nucleus. The numbers of sertoli, spermatogonium and vacuolated cells were gradually increased and were more distinct at 6-8 weeks of age. The testis of 20 weeks of age Pati duck of Assam showed that the seminiferous tubules have an increased number of cells which were arranged in 3 or 5 layers consisting of sertoli cells, spermatogonium and spermatocyte. In 30 and 40 weeks age group the seminiferous tubules was characterized by a multiple layered cell consisting of 8 to 17 layers with distinct layers formed by different stages of spermatogenesis. The interstitial cells consisted of leydig cells, fibroblast-like cells, collagenous fibres and reticular fibres, few elastic fibres, lymphatic vessels and blood vessels. The interstitial connective tissue occupy large portion of testis in 1 month and 6-8 weeks of age and were located in the space between the small

seminiferous tubules. At 20 weeks of age, as seminiferous tubules enlarged and the interstitium part of the testis decreased. The interstitial connective tissues were greatly reduced to a small space between the adjacent seminiferous tubules in 30 and 40 weeks.

The epididymal region of testis of Pati duck consisted of rete testis, efferent duct, collecting duct and epididymal duct which were separated by connective tissue. Rete testis was found within the capsule (intracapsular) and also outside the capsule (extracapsular) of the testis. The lining epitheliums was mostly cuboidal in 1 months, 6-8 weeks and 20 weeks which changes to simple squamous epithelium in 30 and 40 weeks of age. The epithelial lining of the efferent duct were folded villi-like lining which project into the lumen and were lined by ciliated pseudostratified columnar epithelium. The collecting ducts and epididymal duct were both lined by non-ciliated pseudostratified columnar epithelium.

The vas deferens was lined by pseudostratified columnar epithelium, the diameter increased with age. The epithelium at the cranial part of vas deferens was smooth while the caudal part has folded epithelium along with the basal cells.

The tubular phallus of Pati duck of Assam has a narrow lumen with its mucosal wall lined by non-keratinized stratified squamous epithelium. A layer of connective tissue which was consisted of numerous large lymphatic spaces was observed which was present in the entire circumference. Numerous blood vessels were present in the connective tissue surrounding the lymphatic space. The outer surface of the Phallus was lined by keratinized stratified squamous epithelium with numerous fibroblasts. Beneath the surface epithelium numerous glands and ducts were found along with connective tissue. The ducts were lined by cuboidal cells.

The activity of Alkaline Phosphatase enzyme decreased with age in the testis. Moderate activity in the Vas deferens and intense activity in the phallus. Acid phosphatase activity was strong in both testis and phallus while vas deferens showed weak activity. Weak to moderate activity of ATPase in the testis and Vas deferens. Intense activity in the Phallus. Enzymes activity was mainly observed in the capsule of

testis, peritubular area and lumen of seminiferous tubules and in the periductal area of epididymis. The enzymes activity was observed in all area of the phallus.

Two types of leydig cells were observed under TEM *viz.,* elongated and polygonal shaped with numerous lipid droplets along with mitochondria and endoplasmic reticulum. Sertoli cell nucleus had intranuclear cleft. In the peritubular space layers of overlapping myoid cells was found. Within seminiferous tubules cellular detailed of spermatogenic cells were observed.

Age related change observed with Testosterone hormone. T3 and T4 hormone were higher in younger age while Cortisol was higher in older groups.

Among the haematological parameters significant changes was found in PCV, WBC, monocyte and neutrophils.

ALP was the only serum enzyme which showed significant changes between age groups. Serum metabolites *viz.,* total protein, albumin and creatinine showed significant changes among the different age group.

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