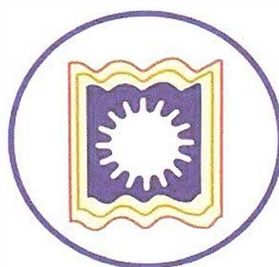


**GROSS AND HISTOMORPHOLOGICAL
OBSERVATIONS OF THE TESTIS AND ACCESSORY
GENITAL GLANDS OF INDIGENOUS BULL FROM
PREPUBERTAL STAGE TO ADULT**



PhD Thesis

By

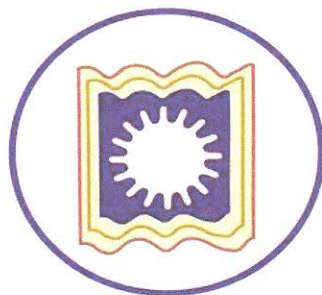
Gitaindro Nath Adhikary

**Department of Animal Husbandry and Veterinary
Science**

**Faculty of Agriculture, University of Rajshahi,
Rajshahi-6205, Bangladesh**

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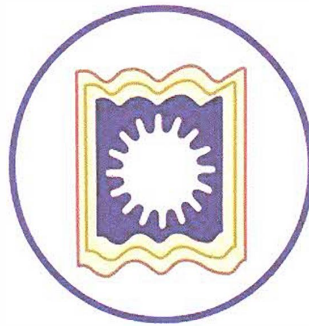
Session: 2012-2013

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Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY (PhD)

From the Faculty of Agriculture
In the Department of Animal Husbandry and Veterinary
Science

University of Rajshahi
Rajshahi-6205, Bangladesh

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Session: 2012-2013

February, 2016

DEDICATED TO

MY

**BELOVED ETERNAL FATHER
HIS HOLINESS JAYAPATAKA
SWAMI**

DECLARATION

I, hereby declare that the Thesis entitled **“Gross and histomorphological observations of the testis and accessory genital glands of indigenous bull from prepubertal stage to adult”** is authentic record of research work carried out by me under the guidance and Supervision of **Dr. Mst. Ismat Ara Begum**, Associate Professor, Department of Animal Husbandry and Veterinary Science, Faculty of Agriculture, University of Rajshahi, Rajshahi-6205. The Thesis contains no materials which has been accepted for the award of any other degree or diploma elsewhere, and to the best of my knowledge, the Thesis contains no material previously published or written by another person, except where due reference is made in the text.



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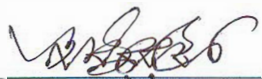
This is to certify that the PhD dissertation entitled "**Gross and histomorphological observations of the testis and accessory genital glands of indigenous bull from prepubertal stage to adult**" submitted to the University of Rajshahi, Faculty of Agriculture, for the award of the degree of Doctor of Philosophy (PhD) in the Department of Animal Husbandry and Veterinary Science, is the original research work of **Gitaindro Nath Adhikary**, a bona-fide PhD research fellow, carried out under our joint supervision. The dissertation has not been submitted to this University or to some other University so far and is submitted for the first time. It is further certified that this dissertation is fit for submission for the degree of Doctor of Philosophy (PhD) in the Department of Animal Husbandry and Veterinary Science and the candidate has fulfilled all the statutory requirements for the completion of the PhD program.



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LIST OF ABBREVIATIONS AND SYMBOLS

%	=	Percentage
&	=	and
μm	=	Micrometer
°C	=	Celsius
±	=	Plus minus
β	=	beta
<	=	Less than
>	=	Higher than
×	=	Magnification
*	=	Significant sign
/	=	oblique
=	=	Equal to
A	=	Alveoli (secretary)
AI	=	Artificial insemination
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
BM	=	Basement membrane
BV	=	Blood vessels
bwt	=	Body weight
Ca	=	Capsule
CS	=	Collecting Sinus
CT	=	Connective tissue
DLS	=	Department of Livestock Services
DNA	=	Deoxyribonucleic Acid
DPX	=	Dextrin Plasticized Xylene
cm	=	Centimeter
ED	=	Excretory Duct
Edn.	=	Edition
<i>et al.</i>	=	and Others
FAO	=	Food and Agricultural Organization
Fig.	=	Figure
Fm	=	Fibro muscular
FSH	=	Follicular Stimulating Hormone
G	=	Chest girth
GDP	=	Gross Domestic Product

GE	=	Germinal Epithelium
gm	=	Gram
H & E/HE	=	Hematoxyline and Eosin
Id	=	Interlobular duct
Ic	=	Interlobular connective tissue
Kg	=	Kilogram
lb	=	Pound
LC	=	Leydig Cells
L	=	Length
LH	=	Lutenizing hormone
m	=	Meter
ml	=	mililiter
mm	=	Millimeter
n	=	Number of observation
No.	=	Number
PAS	=	Periodic Acid Schiff
PGC	=	Premordial Germ Cell
PI	=	Permanent Incisor
SAS	=	Statistical Analysis System
SAU	=	Sylhet Agricultural University
SE	=	Standard error
SC	=	Sex Cord
SCRF	=	Scrotal circumference
±SE		Plus minus Standard Error
ST	=	Seminiferous Tubule
Sz	=	Spermatozoa
TA	=	Tunica Albuginea
TM	=	Tunica Muscularis
USA	=	Unitated States of America
W	=	Live body weight

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ACKNOWLEDGEMENTS

All praises are due to the Supreme Personality of Godhead, **Lord KRISHNA**, Supreme authority of this universe, who enabled the author to pursue higher education in Doctor of Philosophy complete the research work and the thesis for the degree of Doctor of Philosophy.

With great pleasure, the author would like to express his deepest sense of gratitude, sincere appreciation, profound regards and immense indebtedness to his reverend teacher and principal research supervisor Dr. Mst. Ismat Ara Begum, Associate Professor, Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi, for his research planning, scholastic guidance, supervision, sympathetic supervision, valuable suggestions and comments throughout the entire period of the study, research work and in preparation of this manuscript.

With enormous gratification, the author would like to express his sincere sense of appreciation, profound regards and immense indebtedness to his reverend teacher and former principal research supervisor and co-supervisor Dr. Shah Md. Abdur Rauf, Associate Professor, Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi, for his research setting up, scholastic direction, supervision, sympathetic care, valuable suggestions and comments throughout the entire period of the study, research work and in preparation of this manuscript.

The author feels proud to express the highest gratefulness and deepest sense of gratitude to his honorable teacher which keenly related with research period and research co-supervisor, Professor Dr. Muhammad Nazrul Islam, Department of Anatomy and Histology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet for his kind co-operation, generous technical help and constructive and constant inspiration, throughout

the research work that made the author able for successful completion of the thesis.

With huge execution, the author would like to express his sincere sense of appreciation, profound regards and immense indebtedness to Chairman Dr. Kh. Md. Mozaffor, Associate Professor, Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi, for his special follow up about research progress, academic direction, valuable suggestions and comments throughout the entire period of the study, research work and in preparation of this manuscript.

The author wishes to express the warm sense of gratitude and heart-felt appreciation to his respected teachers Professor Dr. Md. Jalal Uddin Sarder, Dr. Syed Sarwar Jahan, Associate Professor, Associate Professor Dr. Moizur Rahman, Associate Professor Golbar Hossain, Assistant Professor Rashida Khatun with all respected teacher of the Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi for their constant encouragement, valuable suggestions and cordial co-operation during whole study period.

The author wishes to express the warm sense of gratitude and heart-felt appreciation to his respected teachers, Professor Dr. Md. Nazrul Islam, Assistant Professor DR. Bashudeb Paul and DR. Sonjoy Sarkar in the Department of Anatomy and Histology with his all colleagues of the Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet for their constant encouragement, valuable suggestions and cordial co-operation during whole study period.

The author wants to express cordial thanks and appreciation to the Laboratory technicians and Laboratory attendants of the Department of Anatomy and Histology, Faculty of Veterinary and Animal Science, Sylhet Agricultural University, Sylhet, Department of Anatomy and Histology, Faculty of

Veterinary Science, Chittagong Veterinary and Animal Sciences University, Chittagong and the Department of Animal Husbandry and Veterinary Science, University of Rajshahi, Rajshahi for their support and co-operation during study period.

The author expresses special thanks to all the staffs of the Central Library of Sylhet Agricultural University, Sylhet, for their assistance during the research period.

The author expresses also special thanks to Kh. Habibul Alam, Senior Scientific Officer, Fruit Research Station, BARI, Binodpur, Rajshahi, for their assistance in digital microphotography during the research period.

Last but no way to the least, the author wishes to express ever gratefulness and immensely indebtedness to his respected parents (Bishwa Nath Adhikary and Kusum Bala Adhikary) for their heartiest blessings, wife and son (Swapna Roy and Tushar Kanti Adhikary), brother (Nillkanta), for their affectionate feelings, encouragement and all sorts of sacrifices for the author's higher education and successful completion of his academic life.

February, 2016

The Author

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ABSTRACT

A study was conducted in the post graduate laboratory of the Dept. of Anatomy and Histology, Sylhet Agricultural University and Dept. of Animal Husbandry and Veterinary Science, University of Rajshahi with aimed to evaluate the gross and histological features of testis and accessory genital glands in details and their chronological development of testes and accessory genital glands from prepubertal stage to adult during July 2012 to June 2014. Twenty eight bulls of three age groups were selected from the local market: the prepubertal group (<1 year n=4), pubertal group ((1.5- 2.5 years, n=16) and post pubertal or adult group (above 3 years, n=8). Body weight and scrotal circumference were recorded before slaughter and various testicular and accessory glandular parameters were recorded after slaughter. The left testicular size of prepubertal bulls was slightly larger than the right. The left and right testicular weight was 18.57 ± 1.40 gm and 16.10 ± 1.64 gm respectively in prepubertal bull, 129.22 ± 19.89 gm and 123.86 ± 19.53 gm respectively in pubertal bull, 204.36 ± 14.10 gm and 188.41 ± 13.59 gm respectively in adult bulls. Among three layered testicular covering, the thickness of tunica albuginea varied among different age groups and the thickness increases with the advancement of age. The parenchyma of testis was divided into lobes and lobules by connective tissue septa originating from tunica albugenia. The testicular parenchyma of prepubertal bulls consisted of solid non luminated, coiled and straight tubular sex cord having basement membrane and filled up by the centrally placed large primordial germ cells with varying numbers and peripherally located small indifferent basal cells and interstitial tissue. The parenchyma of pubertal and adult bull consisted of interstitial tissue and seminiferous tubules containing complex stratified epithelium, the spermatogenic cells and Sertoli cells. The cells were four categories the spermatogonia, primary spermatocytes, secondary spermatocytes and spermatid arranged in chronological layers from outward to inward of the tubules. Spermatozoa are the mature form of spermatids contains oval shaped

head and flagellated tail. All four types of cells were found within the fully luminated seminiferous tubules at or after 18 months of pubertal bull. The spermatogenic lineages were arranged in 5-8 layers in pubertal and 3-5 layers in adult bull. Whereas spermatozoa were less in early pubertal stage and increased along with the advancement of age. This observation revealed that spermatogenic cells and seminiferous tubules became functional at early pubertal stage and fully functions at late pubertal and adult bulls. The seminiferous tubules become more convoluted and intertubular space decreases with the advancement of age of the animals. The cross sectional lengths, breadths of the seminiferous tubules were higher in adult than the pubertal bulls. The interstitial tissues consisted of loose connective tissue, blood and lymph vessels, fibroblasts or fibrocytes, free mononuclear cells and the interstitial endocrine cells of Leydig lodged in the collagen fibrous network in between the seminiferous tubules. The interstitial cells of Leydig were large polymorphs, ovoid to polygonal in shape with spherical nuclei and granular cytoplasm remained singly or in groups. The weight, length, width and the thickness of the both left and right vesicular glands were recorded separately after slaughter of each animal. The left and right vesicular gland shows significantly different ($p < 0.01$), in weight and length in every group. Left vesicular glands were slightly higher than the right in all parameters. The glandular unit of the vesicular gland showed folded mucosa, lined with pseudostratified columnar epithelium. Three types of cells were identified in the epithelium A, B and C cell. Type A cells were tall columnar cells having distinct cell boundaries with the oval, round or elongated nucleus. Type B cells were located in the basal lamina having round or oval nucleus with indistinct cell boundaries. Type C cells were narrow columnar cells interspersed between A cells with darkly stained cytoplasm. Lamina propria consisted of loose connective tissue surrounded the alveoli, tubules and some solid end pieces. The numbers of secretory end pieces were variable. The diameters of luminated or non-luminated acini of the glandular end pieces and ducts were increased

gradually and significantly ($p<0.01$) with the advancement of age. The epithelial height of the duct and alveoli were increased with the advancement of age. Cytoplasmic secretory granules and secretion increased along with the advancement of age indicating the gland became more functional along with the advancement of age. The prostate glands of bull consisted of corpus prostate and pars disseminata portion. All three groups of bull showed the corpus prostate as band like structure. The both portions enclosed by the connective tissue capsule composed of mainly collagen and smooth muscle cells. Interlobular connective tissue decreased significantly ($p<0.01$) with the advancement of age. The secretory ends pieces were lined by simple cuboidal epithelium, simple low columnar epithelium and simple high columnar epithelium in prepubertal, pubertal and adult bulls respectively. The height of the lining epithelium increased with the advancement of age significantly ($p<0.01$). Cytoplasmic secretory granules and secretion increased along with the advancement of age as a result nuclei become flattened and pushed toward the basement membrane. The average diameters of the ducts, enfolding and the height of the lining epithelium increased significantly ($p<0.01$) with the advancement of age. These findings revealed that function of the prostate gland increased along with the advancement of age and reached maximum at adult stage. The left bulbourethral gland is larger than the right in all parameters in all three groups. The weight, length, width and the thickness of the both left and right glands were increased significantly ($p>0.01$) along with the age and reached maximum at adult stage. The thickness of the capsule increased significantly ($p>0.01$) with age whereas interlobular and intralobular connective tissue thickness decreased significantly ($p>0.01$) with the advancement of age. The gland was tubulo-alveolar type lined by pyramidal or columnar epithelium. In prepubertal bull epithelial height is least, cytoplasm having less cytoplasmic granules with rounded nuclei. In pubertal bull epithelial height and cytoplasmic granules increased, nuclei became oval and pressed toward the basement membrane. In pubertal bull the epithelial height

and cytoplasmic granules further increased and nuclei became flatted and located near the basement membrane indicating the function of the gland increased along with the advancement of age and reached maximum stage at adult age. Above gross and histomorphological data will provide the database for the first time of testes and accessory glands of indigenous bull at different ages for making the ancestry of real scenario which may lead to the gateway for perspective future research. This information may help students, scientists and breeders for advance study, to select indigenous breeding bulls for quality tasty meat and to conserve our indigenous cattle.

CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

Cattle are the most versatile component among other livestock species available in relation to existing integrated agricultural farming system in Bangladesh. It is an inseparable and integral part of the small holder subsistence farming. There are about 24.5 million cattle population in Bangladesh, which is about 1.79% of the world and 5.47% of the Asian cattle population (FAO, 2004). Bangladesh is currently estimated 26.82 million cattle (BBS, 2010). Number of cattle per livestock household is 3.5 and that of 0.94 for all household (BBS, 2002). These populations are important for milk, meat and hide. Export quality of hide increases our foreign currency as well as national economy. But the production of milk and meat as per requirement of people per capita are not satisfactory label yet. The annual meat production in Bangladesh is about 446000 metric ton, where the beef contributes 182000 metric ton of the total meat production (FAO, 2005). Nowadays a large number of farmers involved in bull fattening just before 3-4 months of Eid-ul Azaha, when they can sell the animals with profitable prices. Even some landless people carry out fattening programme year round as a way of their livelihood. Cattle fattening for beef production has become an important business of the small farmers in Bangladesh (Sabur *et al.*, 2000).

By developing and adopting modern technologies generated through research on breeding, feeding, management and disease control can raise the production to a much higher extent. Indigenous bull found all over the country is the heritage and important asset of Bangladesh. They have tremendous demand in every part of the economy of our country for their strength and resistance against different diseases. Indigenous cattle populations especially bulls are collectively insufficient to make up our demand for tasty and tender meat. On the other hand, every year large number of indigenous bulls is sacrificed for meat and in religious festival all over the country. So, Government taking various sustainable development programs such as beef fattening for meat, cross breeding policy for milk and sire selection for genetically potential new progeny etc. Recent development in artificial insemination program is the most popular program for developing our native indigenous cattle for milk production. As a result, widespread

uses of artificial insemination by different cross breed bull, number of indigenous breeding bull decreases day by day, whereas the indigenous bull meat has more demand than that of crossbreed bull. The development of a bull is the progression of the pre-pubertal animal towards maturation involving differentiation and morphogenesis of the reproductive tract. Development is influenced and driven by the inherent genetic makeup of the animal and initiate by increased levels of reproductive hormones. The process is greatly influenced by nutrition, environment and other exogenous factors (Lawrence, 1997). Reproductive efficiency is a major determinant of half cow-half profitability. Bulls are considered as half of the herd in any breeding program. Not only they are responsible for determining conception rate of the cow herd but also for establishing genetic quality of the offspring. A positive outcome in any breeding program begins with the ability of a breeder to recognize the importance of using a fertile sire.

Early puberty shows early maturity of indigenous bull causes muscular development of bull minimizes the farmers cost by early selling. Puberty in bull is defined as the age at which an ejaculate contains 50 million spermatozoa with a minimum motility of 10% (Evans *et al.*, 1995) and the age or time at which the generative organs become functional and reproductive capacity may occur (Roberts, 1956). However along with the bull maturation, the number of sperm cells per ejaculate and the cell motility will continue to increase. Puberty occurs at approximately 11 months of age, though it can vary in individuals depending on biological type, nutrition and health status (Taylor, 1994).

In bulls, puberty may be defined as the age at which spermatozoa are first evident in the ejaculate (Stewart *et al.*, 1980). The onset of puberty is a gradual process and is variable in times since it may be influenced within a species by the plane of nutrition, breed of animals, cross-breeding, methods of management, presence of chronic diseases and individual differences (Bellows *et al.*, 1964; Amann, 1981). Secondary sex signs, sexual desire, ability to copulate and presence of viable spermatozoa in the ejaculate characterize the onset of puberty in male. Nutrition is one of the important factors that let animals reach at puberty in due time. A growing bull must receive adequate nutrition from birth through at least 8-10 months to reach puberty at the

youngest possible age (Flipre and Almquist, 1961). Delayed puberty results from low plane of nutrition in young male animals (Bratton *et al.*, 1956). Usually, dairy cattle reach puberty when the body weight is 30-40% of the adult weight, whereas in beef cattle this ranges between 45-50% (Roy *et al.*, 1975).

Determination of scrotal circumference (SC) is an essential aspect of breeding soundness examination of bulls because of its significance as indicator of puberty, total sperm production, semen quality and pathological conditions of the testes (Ott, 1991). Young bulls with smaller scrotal circumferences have significantly lower fertility than the bull with larger scrotal circumferences. Bull with small testicles was shown to have semen with higher percentage of sperm defects (Achnelt *et al.*, 1964). The SC was positively correlated with fertility.

The young bulls (18 months) with scrotal circumference more than 27 cm have higher concentration of testosterone and estradiol 17 β and denser semen than the counterpart bulls with scrotal circumference less than 23 cm. Bulls with risk of subnormal testicular development have hereditary factors associated with abnormal spermatogenesis and low fertility (Aehnelt and Hahn, 1965). As the bull matures the scrotal circumference increases leading to an increase in the percentage of normal and live spermatozoa, sperm motility and the number of spermatozoa per ejaculate. The maturation of bulls and subsequent enlargement of testes result in the decreased proportion of proximal cytoplasmic droplets as well as knobbed and ruffled acrosomes (Evans *et al.*, 1995). According to the society of Theriogenology (Ball *et al.*, 1983) minimum scrotal circumference for bulls irrespective of breeds should be 30cm at 15 months or less, 31 cm at more than 15 and less than 18 months, 32cm at more than 18 months and less than 21 months, 33cm at more than 21 months and less than 24 months of age. There are great differences between breeds of bull in average scrotal circumference at any given age (Barth, 1997). Additionally, scrotal circumference is a reasonable predictor of puberty. Most bulls have undergone puberty when scrotal circumference reaches 28 to 29 cm (Taylor, 1994).

Testicular size of the sire lines is an important trait in the selection of males practically those used in AI program (Ying and Johnson, 1996). Scrotal circumference has also been found to be correlated with fertility of bulls (Mateose *et al.*, 1978).

The testes of bull are relatively larger and have an elongated, oval outline. The long axis is vertical and the attached border being caudal. The medial surface somewhat flattened. A testes of an adult bull, in contrast, measures on the average about 10-12 cm in length excluding the epididymis. The width is about 6-8 cm and the craniocaudal diameter is about the same. The weight is about 300 gm. Most often the left testicle is slightly larger and lower in position, giving the scrotum an asymmetrical appearance. The surfaces are convex and smooth. The extremities, like dorsal and ventral are blunt and rounded (Macleod *et al.*, 1964). The testes represented two surfaces, two borders and two extremities. The medial and lateral surfaces are convex. But the convexity of the lateral surface is much more than that of the medial surface. The free border is ventral and somewhat convex. The attached border is dorsal. The head extremity and tail extremity are rounded (Getty, 1975).

Testicular size could be correlated for sperm output (Amann and Almquist, 1976). The curve of testis growth is highly correlated to sperm output potential of bulls ejaculate and this is particularly true in young bulls, where the correlation between estimated testis size and daily sperm output (Hahn *et al.*, 1969; McMillan and Hafs, 1968).

Testes of an adult bull, in contrast, measures on the average about 10-12 cm in length excluding the epididymis. The width is about 6-8cm and the cranio-caudal diameter is about the same. The weight is about 300 gm (Getty, 1975).

After removing scrotum the testis has the parietal layer of the tunica vaginalis remained attached to the scrotum, whereas the visceral layer, the peritoneal covering of the testis (and epididymis), remained intimately associated with the underlying capsule of the testis, the tunica albuginea. The visceral layer of the tunica vaginalis consisted of a mesothelium and a connective-tissue layer that blends with the tunica albuginea (Dellmann, 1992).

The tunica albuginea is a solid capsule of dense irregular connective tissue. It consists predominantly of collagen fibers, a few elastic fibers, and myofibroblasts. Intratunical interstitial endocrine cells occur in the cat. Meandering branches of the testicular artery and a network of anastomosing veins constitute the vascular layer of the tunica albuginea (Dellmann, 1992). In bull, the tunica albuginea is thin; it contains many

elastic fibers but no smooth muscle. The parenchyma is yellowish in color (Getty, 1975).

Leydig cells in mammals are in general relatively large, polyhedral, epitheloid cells surrounded by a typical plasma membrane which is usually covered with microvilli; each cell has a single, spherical or ovoid, eccentrically located nucleus with one to three large nucleoli. The cytoplasm contains moderate numbers of average sized mitochondria with mostly lamellar cristae and a well developed Golgi complex (Fawcett *et al.*, 1973).

The convoluted seminiferous tubules in most mammals are tortuous two ended loops with a diameter between 150 and 300 μm . They are lined by the stratified germinal epithelium, surrounded by a lamina propria. Histologically, the seminiferous tubules have 3 components: lamina propria, somatic cells and spermatogenic cells (Dellmann, 1992).

The diameters of seminiferous tubules are between 200 to 250 μm and an average ram testis contains rarely 3000million of tubules. The tubules are surrounded by a specialized peritubular tissue which consists of several layers of non cellular material and myeloid cells, which are contractile and probably cause peristaltic contraction of the tubules to assist in the transport of spermatozoa (Setchell and Brooks, 1988).

During puberty, sustentacular cell differentiation is accompanied by a morphologic transformation and loss of mitotic capability. The adult sustentacular cells are irregularly outlined, elongated cells. Their broad bases rest on the basal lamina and the remaining cytoplasm extend upward to the tubular lumen. They are rather evenly shaped; about 25 to 30 sustentacular cells are seen in a cross section of a seminiferous tubule (Dellmann, 1992). Their sides are markedly uneven, showing pits and depressions into which fit the adjoining germ cells. The nucleus is ovoid and pale staining with finely dispersed chromatin, and it usually contains one or more prominent nucleoli. The nuclear membrane often shows a characteristic longitudinal groove (Copenhaver *et al.*, 1978).

The spermatogenic cells lie between the cells of Sertoli in an orderly manner, with four to eight layers occupying the space between the basal lamina and the lumen. In the

immature testis, only the primitive germ cells or spermatogonia are present. With the onset of sexual maturity, the spermatogenic cells are represented in all stages of differentiation and are arranged in several distinct layers (Copenhaver *et al.*, 1978).

The production, differentiation and presence of male gametes in the testis represent inimitable challenges to the immune system as they are unique to the body and appear long after the maturation of immune system and formation of systemic self tolerance. Immune cells are found within normal testes in the interstitial compartments, these are implicated in process that makes the testes and immunologically privileged site where germ cells are protected from autoimmune attack (Anton *et al.*, 1998).

The accessory glands of the male reproductive system composed of vesicular glands, bulbourethral or Cowper's glands and prostate glands. The secretions of accessory genital glands regulate the ejaculatory process. The secretion of the accessory glands contribute for the most ejaculated volume (Ashdown and Hafez 1995; Campos, 2003). The contribution of each gland to sperm volume varies among species, due to differences in gland size, and even absence of one, or more of them, in some species (Gonzalez, 2002). The seminal vesicles having major role to produce viscid, yellowish secretion contains spermatozoa activating substances such as fructose, citrate, inositol, prostaglandins and several proteins act as a source of energy for the sperm motility. Secretary product of the vesicular gland is rich in fructose which serves as the energy source for ejaculated spermatozoa (Dellmann and Eurell, 1998).

Prostate gland is the most important accessory genital gland of male genital system which has major contribution in seminal plasma which plays important role in male reproduction. Its functional significance lies in neutralizing the seminal plasma and to initiate active movement of the ejaculated spermatozoa (Eurell and Frappier, 2007; Hafez 1987). Before eighteenth century (Hunter, 1972) it was believed that the sound animal economy depends upon a perfect relationship between the testes and the accessory reproductive glands. Later, in the twentieth century techniques were used to isolate the prostatic acini, characterize and assay the androgens (Moore *et al.*, 1930). Histological and histochemical studies have been made on the prostate gland of cattle (Stallcup, 1969), buffalo (Sudhakar *et al.*, 1984, 1985), ram (Roy *et al.*, 1985), man (Copenhaver 1964; Bloom and Fawcett 1968) in the light microscope and in rat

(Brandz and portella, 1960; Kanai and Tadao, 1961) by the electron microscope. The staining characteristics of prostate glands and the secretory granules have been studied in bovine male (Trotter 1959). Hypertrophy and cancer of the prostate are the two major problems facing both veterinary and human medicine. Abundant literatures are available regarding the anatomical features of the prostate gland of the most mammals, but the literature on the indigenous bulls of Bangladesh is lacking. Hence, this work was taken to record the sequential changes in anatomical and histological features from the prepubertal stage to adult.

The bulbourethral glands are the accessory sex gland located dorso-lateral to the penile portions of the urethra, at the root of the penis (Eurell and Frappier, 2007), either sides of the pelvic urethra cranial to the ischiatic arch. They are covered by a thick fibrous capsule and the bulbocavernosus muscles (Dellmann and Wrobel, 1981). The secretion of accessory genital glands of bull is very important during ejaculation. At the time of ejaculation, seminal plasma constitutes 64-70% of the ejaculate and 31-32% comes from the prostate and bulbourethral gland. Bulbourethral gland takes part important roles for lubrication and making the semen less watery to provide a suitable living environment for the sperm. The proteinaceous mucous secretion produced by the bulbourethral gland clears the urethra during the ejaculatory stage (Banks, 1981; Dellmann and Wrobel, 1981) and it is the most important component of the first fraction of ejaculate. Abnormalities of the bulbourethral gland may therefore affect bull fertility. Grossly, bulbourethral adenitis was an uncommon finding in one abattoir survey (Bagshaw and Ladds 1974). Certain anatomical, histological and histochemical studies have been conducted on the bulbourethral gland of goat (Archana *et al.*, 2011; Kundu, 1980; Gupta and Singh, 1982; Pawar *et al.*, 1986 and Pyne *et al.*, 1991). But in case of bulls, there is no such available literature in this regard. The present work has been conducted on bulbourethral glands of indigenous bull of Bangladesh from prepubertal stage to adult to record a sequential feature of the histometric changes of bulls.

The testes are a pair of primary reproductive organs that maintain the health of the male reproductive system. The primary functions of the testes are to produce sperm and androgens, especially testosterone a hormone that is vital to the normal development of

male physical characteristics. Sperm production is directly correlated to testicular size. In adult bull, testosterone maintains libido, muscle strength, and bone density. Disorders of the testes may cause fall in the testosterone production. A major constrain in bull reproduction is the hypogonadism, a testicular disorder associated with low testosterone. Having very low testosterone level causes a variety of problems in bull including: decreased sex drive, diminished muscle mass, low sperm count (reduced fertility), loss of body hair. The knowledge of the anatomy and histology of the testis and accessory genital glands are essential as this knowledge is important in many cases those determine the breeding value of a bull in reproduction including the healthy development of male sex organs, quality semen production, maintaining libido and muscular development.

Although, sporadic information on the gross and histomorphology of the testes of bull is available in the literatures, details gross histomorphological reports on testes and accessory genital glands of indigenous bulls are not available. In context the present situation, scientific breeding practice of considerable standard including the histomorphological knowledge on testes and accessory genital glands of indigenous bull from prepubertal to pubertal stages provide valuable information to the instructors, veterinarians, students and other research personnel working in the field of reproduction for the ancestry of the indigenous bulls of Bangladesh which will helps in the improvement of milk and meat production in indigenous cattle that ultimately will improve our economic condition, health status, alleviate poverty and create opportunity of self employment.

Therefore, the present research has been selected for making a real scenario in the part of ancestry of indigenous bulls by pursuing the following objectives with the hope that it will open a new gateway to study the-

- i) Gross and histoarchitectural features of testis and accessory genital glands in details.
- ii) Gradual changes or development of testes and accessory genital glands from prepubertal stage to adult.

- iii) Relation of age, body weight and scrotal circumference with the testicular parameters which may co-relate with the available data on testicular activity.
- iv) Provide sufficient informations on gross and histoarchitecture of testes and accessory genital glands of indigenous bull for breeder or scientist to co-relate the testicular parameters with libido and semen quality.

CHAPTER II
REVIEW OF LITERATURES

CHAPTER II

REVIEW OF LITERATURE

2.1 General aspect of review

This literature review intends to cover basic aspects of reproductive organs of indigenous bull (*Bos indicus*) such as occurrences of puberty of bull just from the prepubertal stage and the gross, histometric changes till their adult stage observed. Basic anatomy, histology and physiology of the bull's reproductive system as well as most important gross and histometric parameters were used to evaluate the changes in the sequential pattern from prepubertal to adult. Particular attention was given to the baseline study of the organizational changes which can help the determination of breeding indigenous bulls.

2.2 Basic anatomy, histology and physiology of the bull's reproductive system

The reproductive system of bull like all other mammalian farm animals consists of two testicles contained in the scrotum outside of the body cavity, the essential reproductive glands including ducts; with their coverings and appendages; the epididymis; the ductus deferentes, the vesicular glands; the prostate, a musculo-glandular organ; the two bulbourethral (or Cowper's) glands; the male urethra, a canal which transmits the generative and urinary organ (Getty, 1975).

The testes are covered by tunica albuginea and the parenchyma consists of the seminiferous tubules; which are surrounded and supported by intertubular connective tissue (Dellmann, 1992; Trutmann and Fiebiger, 1952; Copenhaver *et al.*, 1978). The essential constituents of the seminal fluid (the spermatozoa) are formed in the tubules of the testis and leave the body through the genital ducts (Copenhaver *et al.*, 1978).

The testis, as the male gonad, produces steroid hormones and male gametes. The parenchyma is the functional unit of the testis and is made up of seminiferous tubules and interstitial tissue. Leydig cells within the interstitial tissue are responsible for producing androgens to maintain male characteristics and spermatogenesis, whereas seminiferous tubules are the site for production of spermatozoa. The male germ line in

the seminiferous tubules of the adult testis includes spermatogonia, spermatocytes, and spermatids. Sertoli cells provide various necessary factors for the germ line cell types and support the proliferation and differentiation of immature undifferentiated germ cells to mature spermatozoa (Griswold, 1993).

2.2.1 Age

Ivanenko *et al.* (1976) studied the effects on bull sires (Swedish Red and White and Swedish Friesian breed bulls) and bulls age at collection and estimated for ejaculate volume, motility, total numbers of sperm cells per ejaculate and number of semen doses per collection. Motility and ejaculate of volume were significantly affected by bull sire ($P < 0.01$). The effects of bull and age at collection were highly significant ($P < 0.01$) for most of the traits.

Rodionovskii (1977) reported on semen characters in 2540 ejaculate of 39 bulls, aged 2 years and in ejaculates of surviving bulls up to 10 years of age. Ejaculate volume increased from 3.48 at 2 years to 5.70ml at 9 years. Sperm concentration decreased gradually with age.

Rao and Rao (1978) and Raju and Rao (1983) observed significant increased in the volume and motility percentage of spermatozoa and a significant decreased in the abnormalities percentage of sperm cells in the semen of Brown Swiss x Ongole bulls with a increased in their age over 15 months. The present report does not confirm their observation on an increased in concentration up to an age of 24 months.

Ramamohana Rao *et al.* (1979) reported on the semen characteristics of 3 cross- bred (F1) bulls comprising of two Holstein Friesian x Ongole and on Brown Swiss x Ongole bulls aged 15 months. The semen characteristics of 3 cross-bred (Fx) bulls from the age of 15-23 months consisting of 75 ejaculates were studied. The semen quality was poor at the beginning and improved gradually with age and was nearly normal at the age of 23 months in the cross bred (Fx) bulls studied.

Ziegler (1984) observed that in 990 ejaculates from Simmental bulls and 32298 first inseminations during 1980-82 from 10 older and 25 young bulls, the main difference in semen characters was for ejaculate volume 7.03VS 4.22ml). Bull age significantly

affected almost all semen characters. The 90 days non return rate after AI was significantly correlated with ejaculate volume (0.11) and with sperm concentration in the older bulls (0.22).

Tomar *et al.* (1985) observed the semen production from 9 Holstein Friesian x Hariana cross-bred bulls in different age groups (from less than 24 months to more than 36 months) in all seasons of the years (for the semen production parameters). Ejaculate volume, mass motility and live percentage of spermatozoa increased with growing age of the bulls, while the live abnormal percentage of spermatozoa increased simultaneously. Mass motility was poor during winter season, while sperm concentration was less was during summer season. The age groups had highly significant effect on all the semen characteristics except the concentration on spermatozoa in the semen.

Stalhammar *et al.* (1988) studied on 215 Swedish Red and White and Swedish Friesian bulls in 1983-85 in respect of libido, ejaculate volume; number of spermatozoa per ejaculate, sperm motility before and after the number of semen doses per collection was generally significantly affected by season and age of bull.

Stalhammar *et al.* (1989) reported on genetic and environmental factors influences semen quality and quantity for 215 young bulls of two main dual purpose breeds in Sweden, Swedish Red and White (SRB) and Swedish Friesian breed (SLB). The effects of bulls sire, season and bull's age at collection were estimated for ejaculate volume, motility, total numbers of sperm cells per ejaculate and numbers of semen doses per collection. Age of bull at collection affected ejaculate volume, total numbers of sperm cells and numbers of semen doses per collection. For the quantitative traits, 5-15% of the variation due to bull's age at collection. Total numbers of sperm cells collected per day doubled from 12 months to 30 months of age and older. The effects of bull's age at collection on the two motility characteristics were significant except for post freezing motility. There was significant seasonal effect on the semen characteristics. The summer months June, July and August were the best months for quantitative semen traits.

Barbosa *et al.* (1991) observed semen traits at 27 and 39 months of age in 7 Canchim and Nelore bulls. Volume was significantly greater in Canchim than in Nelore bulls at 27 months, but not at 39 months of age. There were significant breed differences or breed x age interactions for ejaculate volume or semen motility, concentration or abnormalities, but semen quality improved with increasing age bulls.

Verma *et al.* (1990) observed 96 ejaculates from 6 Jersey bulls in winter and summer. Season had no effect on live spermatozoa percentage in undiluted or diluted semen. Progressive sperm motility in undiluted semen was not affected by season, but higher in undiluted semen and in thawed semen collected in summer. In undiluted semen, total sperm abnormalities were not influenced by season.

Siratskii (1992) studied over a 20 years period from 642 bulls belonging to the pedigree breeding associations in Sumy, Chernigov and Kharkov Oblasts. The relationship between age of the bull and the volume of ejaculate and concentration of spermatozoa, mass activity were studied. Age effects were found in all these traits.

Zhang *et al.* (1993) evaluated 33 Holstein bulls at the Beijing bulls station were grouped by age (11 per group) 1-2 years, 2-4 years and over 4 years. The semen quality of each ejaculate volume, sperm concentration, total number of spermatozoa and sperm motility was recorded for 1 month. The result showed that scrotal circumference and ejaculate volumes were greater in last two groups ($P < 0.01$).

Rao and Sreemannarayana (1996) calculated the effect of age 3-14 years on seminal volume, sperm concentration, total sperm and semen doses per ejaculate, initial motility and post thaw motility was studied from 32 Murrah bulls at the Frozen semen bulls station, Visakhapatnam. The average volume, total sperm and semen doses per ejaculate increased progressively with advancement of age up to 14 years, while sperm concentration declined in older bulls. The quality of semen in terms of initial motility was better in young and aged bulls, while post thaw motility increased progressively with age.

Diarra *et al.* (1997) studied 294 young Holstein bulls between 10-18 months old. The sire effect was significant for the volume ($P < 0.01$), concentration ($P < 0.01$) and the number of motile spermatozoa per ejaculate ($P < 0.01$). Intraclass regression studies

indicated that each increase of 1 day of age in young bulls increase the volume of the ejaculate by 0.004%, the concentration and motility of the ejaculate by 0.024×10^8 spermatozoa per ml and 0.03% and the concentration of the ejaculate by 0.14×10^8 spermatozoa per ml.

Boge (1998) observed from two experiments carried out with semen of 30 progeny tested bulls of average 7.2 years, 67 bulls awaiting their progeny test results 4.9 years, 45 young bulls undergoing 2 progeny testing 1.2 years and 67 young bulls whose semen been used in test inseminations 1.8 years. In each experiment, 2 ejaculate of each bulls were examined. There was no significant difference between the 4 groups of bull in respect of sperm volume. Spermatozoa in a hypotonic medium had a volume 1.7-1.9 times greater than under isotonic conditions ($P < 0.05$).

Mathevon *et al.* (1998) studied 5644 ejaculates from 137 testing and 2023 ejaculates from 61 progeny tested Holstein bulls and to evaluate the importance of environmental, managerial, physiological and genetics on semen quality. Semen characteristics generally improved significantly affected age of young bulls. Season significantly affected all the semen traits in young bulls but did not significantly affect volume and sperm motility of mature bulls. Performance was better in winter than in summer. The highest number of motile spermatozoa per ejaculate were obtained with intervals if at least 4-5 days between collection.

Sarder (2001) studied 680 semen samples were collected from 8 bulls belonging 4 age groups in three different seasons at District AI centre, Rajshahi. Age groups had a significant effect ($P < 0.05$) on semen characteristics except sperm concentration. The highest volume of ejaculate, sperm concentration, total numbers of motile spermatozoa per ejaculate and total numbers of semen doses per ejaculate were found in age groups of 6.5<8.5 years and lowest in age groups of 2.5<4.5 years. The total numbers of semen doses per collection was highest ($P < 0.05$) in summer and lowest in winter season.

2.2.2 Puberty

Puberty may be defined as the age or time at which the generative organs become functional and reproduction may occur (Roberts, 1956). In bulls, puberty may be defined as the age at which spermatozoa are first evident in the ejaculate (Stewart *et al.*,

1980). Lustra *et al.* (1978) defined puberty as the age when a bull's ejaculate contains 50×10^6 spermatozoa with at least 10 percent progressive motility. The onset of puberty is a gradual process and is variable in times since it may be influenced within a species by the plane of nutrition, breed of animals, cross-breeding, methods of management, presence of chronic diseases and individual differences (Bellows *et al.*, 1964; Amann, 1981). The onset of puberty along with other sexual behaviors in young bulls is significantly hastened by rearing with other males or females and is retarded by rearing in isolation (Mather, 1980). Secondary sex signs, sexual desire, ability to copulate and presence of viable spermatozoa in the ejaculate characterize the onset of puberty in male. From birth to prepuberty, 10-12 weeks of age for Holstein bulls, the period is called infancy (Amann, 1983; Amann and Walker, 1983). The development of a bull during infancy is characterized by virtual absence of gonadotropin secretion and limited secretion of androstenedione or testosterone by the Leydig cells (Courot, 1978; Pelletier and Lacroix, 1980; Mostt *et al.*, 1984). Transition from the infantile stage to the prepubertal stage is evidenced by marked increase in frequency and amplitude of discharge of luteinizing hormone (Amann, 1986). Chenoweth *et al.* (1977) described puberty in bulls as a continuous and dynamic process mediated through the hypothalamo-pituitary axis and that it commences at, or before, birth. Others argued that puberty probably commences at 2 months and completed by 10 months of age in Holstein Friesian bulls (McMillan and Hafs, 1968).

The prepubertal sequence of events probably is initiation of spontaneous discharge of luteinizing hormones, differentiation of Leydig cells with increase secretion of androstenedione and subsequently testosterone, testosterone induced differentiation of Sertoli cells together with differentiation of gonocytes to prespermatogonia and A spermatogonia, increased sensitivity of hypothalamus anterior pituitary to negative feedback of gonadal steroids, diminished frequency and amplitude of luteinizing hormone discharge, formation of junction complexes between Sertoli cells and establishment of the blood testes barrier, formation of primary spermatocytes leading to spermatids and spermatozoa and continued increase in efficiency of spermatogenesis until sufficient spermatozoa are produced to provide the first ejaculation around 37 to 38 weeks of age (Amann, 1983; Coulter, 1986). Primary spermatocytes appear in the seminiferous tubules by 4 to 6 months, spermatids by 6-7 months and spermatozoa by

7-9 months of age in bulls; the seminal vesicle secretion from accessory glands appears by 5-6 months of age (Johnston *et al.*, 1982).

The post pubertal bulls, both androgen and estrogen participate in the control of luteinizing hormone secretion through negative feedback on the hypothalamo-pituitary axis (Schanbacher and Kinder, 1982; Schanbacher *et al.*, 1983). The concentrations of androstenedione, testosterone and estradiol in the serum from the bulls less than twelve weeks old are near similar to that from the older bulls (Karg *et al.*, 1976; Malak-Bedair and Thibier, 1979; McCathy *et al.*, 1979).

2.2.3 Factors influencing puberty

2.2.3.1 Breeds

Genetic variation exists between breeds of cattle for age at puberty (Bellows *et al.*, 1964; Amann, 1981; Gregory *et al.*, 1991). The dairy bulls, Holstein Friesian, Brown Swiss, Swedish Red and White, Avilena-Black-Iberian, to mention a few, reach puberty at between 9.5-12 months of ages. Deoni and Sahiwal bulls reach puberty at between 26-30 month of age (Tandel *et al.*, 1998; Shahjalal and Islam, 2001).

However, when these dairy cattle are closed with other breeds, their age at puberty varies. For example Holstein Friesian x Brahman bulls and Brown Swiss x Native Cross x Brahman bulls reach puberty in between 13-17 months of age (Madried- Bury *et al.*, 1993; Madried- Bury *et al.*, 1994). Likewise, the age at puberty in beef bulls Guzerat and Muturu varies from 11-19 months (Garcia *et al.*, 1987; Ezekwe, 1998). Crossbreed beef bulls like Brahman x Short horn Hereford x Africander reach puberty at 21.5 months of age (Perry *et al.*, 1990). The dual purpose cattle Nelore and Ongole reach puberty in between 12-17 months of ages (Silva *et al.*, 1999; Rao *et al.*, 2000).

2.2.3.2 Nutrition

Nutrition is one of the important factors that let animals reach at puberty in due time. A growing bull must receive adequate nutrition from birth through at least 8-10 months to reach puberty at the youngest possible age (Flipre and Almquist, 1961). Delayed puberty results from low plane of nutrition in young male animals (Bratton *et al.*,

1956). In a dairy breeding research centre, well-fed Holstein bulls achieved puberty at a mean age of 39-41 weeks (Amann and Almquist, 1976). However, *ad libitum* energy intake helped decreasing the age at puberty to 37 weeks (Almquist, 1982). In another related study, well-fed healthy Swedis Red and White bulls reached puberty by ten months of age but this period extended 12 months when the bulls were fed on a low plane of nutrition (Abdel-Raouf, 1960; Lunstra, 1982).

Table 2.1 Puberty in relation to measures of bull's reproductive indices

Breed	Type	Country	Age at puberty (month)	SC (cm)	BW(kg)	Ejaculate volume	Sperm motility	Sperm concentration	References
SRW	Dairy	Sweden	10	-	-	-	-	-	Abdel-Raouf (1960)
HF	Dairy	Columbia	9.5	-	-	-	-	-	Amann and Almquest (1976)
Guzerat	Beef	Brazil	19	-	253	4.25	-	-	Garcia (1987)
AfxBrxSHH	Beef	South Africa	21.5	-	308	-	-	-	Perry <i>et al.</i> (1990)
BSxNCx Br	Dairy	Venezuela	17	24	219	3.0	27	70	Madrid - Bury <i>et al.</i> (1993)
HFx Br	Dairy	Venezuela	13	28	355	2.0	19	-	Madrid-Bury <i>et al.</i> (1994)
ABI	Dairy	Spain	12	30.2	433	5	20	67	Gonzalez <i>et al.</i> (1997)
Deoni	Dairy	India	30	-	-	-	-	-	Tandel <i>et al.</i> (1998)
Muturu	Beef	Nigeria	11	-	91	-	-	-	Ezekwe (1998)
Nelore	Dual	Brazil	12	19.75	-	-	-	-	Silva <i>et al.</i> (1999)
Ongole Dual	Dual	India	27	27	268	5	20	67	Rao <i>et al.</i> (2000)
Sahiwal	Dairy	Bangladesh	26	--	-	-	-	-	Shajalal and Islam (2001)
HF	Dairy	Mexico	11	28	276	6.3	28	58.5	Jimenez-Severiano (2002)

HF=Holstein Frisian, BS=Brown Swiss, SRW=Swedish Red and White, ABI=Avilena-Black- Iberian, NC=Native Cross, Br= Brahman, Af= Africander, SHH= Short Horn Hereford, SC= Scrotal Circumference

2.2.3.3 Seasons

In certain animals such as horse and sheep which have limited breeding seasons, puberty occurs at the next available seasons when the animals are nearly grown (McDonald, 1975; Cole and Cupps, 1977; Hafez, 1980). But in cattle there is no such limitation. However, exposures to stress conditions especially during the cold months of the year delay the onset of puberty (Ginther 1968). Salisbury *et al.*, (1978) reported the seasonal variation because of varied nutrition due to seasonal availability has a profound effect on the age at onset of puberty. They reported that proportion of attaining puberty is high in spring and low in summer season due to possible changes of nutrition from season to season.

2.2.3.4 Body weight

In general, faster gaining breeds of large mature size reach puberty at a greater body weight than to slower gaining breeds of smaller size (Barth, 1997). Usually, dairy cattle reach puberty when the body weight is 30-40% of the adult weight, whereas in beef cattle this ranges between 45-50% (Roy *et al.*, 1975). It appears that dairy bulls, Holstein Friesian, Brown Swiss, Avilena-Black-Iberian reach at puberty when they weight about 276, 233 and 433 kg, respectively (Table 2.1). Accordingly, beef bulls Muturu and Guzerat reach at puberty when they weight about 91 and 253kg, respectively and Dual type bulls Nelore and Ongole reach puberty with a body weight around 268kg (Table 2.1).

2.2.4 Body weight of bulls

Pawlina *et al.*, (1989) reported on 66 bulls maintain in Animal breed and Insemination station during the period 1978-87. There was found the negative correlation between weight gain and spermatozoa content in ejaculate of crossbred but not pure breed bulls were stated.

Pawlina and Nowicki (1990) reported to influence of growth rate on semen production on 75 polish Black and White Lowland and 50 Polish Red and White lowland bulls. The negative effect of high growth rate during the rearing on semen production in bulls was stated.

Udala (1991) observed an experiment on 50 Black and White bulls in 13.5 months of life was carried out. Correlation between body weight as well as height in withers and chest and the semen volume, spermatozoa concentration in semen was calculated. The negative correlation between semen volume and growth traits in bulls was stated.

Siratskii (1992) studied over a 20 years period from 642 bulls belonging to the pedigree breeding associations in Sumy, Chernigov and Kharkov Oblasts were studied. A significant dependence of ejaculate volume and the total number of spermatozoa per ejaculate in live weight was found.

Asad (2001) reported that body weight groups 400-600kg, >600-700 kg, >700-900kg had significant ($P<0.01$) effect on volume of ejaculate, concentration, forward movement and non significant effect ($P<0.05$) on mass movement of the semen. The highest value of semen volume in 400-600kg and lowest value of semen volume in >700-900kg weight.

Sarder *et al.* (2001) studied that on 7 AI bulls at the deep frozen semen production laboratory, Rajabarihat, Rajshahi for a period from October 1998 to June 2000 to find out the phenotypic and genotypic variation in the semen traits. Body weight of bulls had significant ($P<0.05$) effect of volume of ejaculate, sperm concentration pre-freezing motility and post freezing motility except total number of sperm per ejaculate. The highest volume of ejaculate and total number of sperm per ejaculate was for 550-650kg and lowest for >650 kg. Scrotal circumference of the bulls significantly ($P<0.05$) affected all the semen traits. The highest volume of ejaculate was found in size of 36 cm scrotal circumference and lowest for <32 cm scrotal circumference.

Islam (2003) measured the body weight of bulls of three different age groups and concluded that the body weight of bulls increased with age. The body weight of bulls of 20, 23, and 25 months of age were 305, 340, 369 kg, respectively.

Shamsuddin (1996) measured the body weight and scrotal circumference of black Bengal bucks of two different age groups and concluded that body weight and scrotal circumference of buck increased with age. The body weight of bucks of 3.5 ± 0.4 months and 4.7 ± 0.7 months of age were 6.7 ± 1.0 and 7.6 ± 1.5 kg, respectively and scrotal circumference were 13.9 ± 0.9 and 15.7 ± 1.3 cm, respectively.

Gofuret *et al.* (2007) observed that the body weight of 4PI (permanent incisor) age group more than 2PI and it was 190.84 ± 9.52 kg in 4PI age group and 156.59 ± 8.34 kg in 2PI age group.

2.2.5 Scrotal circumference (SC) of bulls

Determination of scrotal circumference is an essential aspect of breeding soundness examination of bulls because of its significance as indicators of puberty, total sperm production, semen quality and pathological conditions of the testes (Ott, 1991).

Testicular size of the sire lines is an important traits in the selection of males practically those used in AI program (Ying and Johnson, 1996). Scrotal circumference has also been found to be correlated with fertility of bulls (Mateose *et al.*, 1978)

Young bulls with small scrotal circumferences have significantly lower fertility than the bull with larger scrotal circumferences. Bull with small testicles was shown to have semen with higher percentage of sperm defects (Achnelt *et al.*, 1964). Also reported that the scrotal circumference is smaller in summer than in winter (Worthern hemisspere) being negatively correlated with day length to a significant degree. The SC was positively correlated with fertility.

Troconiz *et al.* (1991) studied in 159 purebred Guzerat 8-110 months of age and 60 Nelore bulls 8-30 months of age were used in two traits to examine testicular development and consistency, body weight changes, puberty and semen traits. Age and body weight affected ($P < 0.01$) scrotal circumference in both breeds. Scrotal circumference is increasing ($P < 0.01$) linearly with age and body weight. However, scrotal circumferences tended to reach mature size more rapidly than did body weight in both bulls. Correlation coefficients between scrotal circumference and semen traits were positive ($P < 0.01$) ranging from 0.49-0.73 in the two breeds.

Thomson *et al.* (1992) reported the accuracy of scrotal circumference as a predictor of specific pathologic changes within testicular parenchyma was assessed by scoring 121 Hereford and Simmental bulls for scrotal circumference and semen quality. 32 cm was the minimal acceptable scrotal circumference to ensure both a low prevalence of tubules with irreversible loss of germinal epithelium and acceptable semen quality.

Scrotal circumference had a positive predictive value of 0.07 and a specificity of 0.89 for 25% grade 4+ tubules. For acceptable semen morphology, scrotal circumference had a positive predictive value of 0.08, a sensitivity of 0.06, and specificity of 0.88. For acceptable sperm motility greater than or equal to 30% progressively motile spermatozoa, scrotal circumference had a positive predictive value of 0.0, a sensitivity of 0.0, specificity of 0.87.

Laszczka and Wierzbowski (1993) reported that at 4 years an experiment on 119 bulls was carried out. Correlation coefficients $r=0.29$ between the volume and $r=0.34$ between the circumference of testes and the age of bulls were estimated. No correlation between the seasonal variation in testicular measurements as well as testicular volume and semen production in mature bulls was stated.

The relationship of scrotal circumference with puberty and measures of semen and bull is summarized in Table 2. Scrotal circumference varies with age and body weight in different breeds of cattle, because it is heritable (Lunstra *et al.*, 1978; Bourdon and Brinks, 1986).

Dairy bulls achieved scrotal circumference 26-30.2cm with body weight 276-433kg between 9.5-12 months of age (Gonzalez *et al.*, 1997; Jimenez-Siveriano, 2002). Dairy cross-bred bulls achieved scrotal circumference 24-28cm with body weight 219-355kg between 13-17 months of age (Madriet-Bury *et al.*, 1993; Madriet-Bury *et al.*, 1994).

Dual type cattle achieved scrotal circumference 25-27cm with body weight around 268kg between 12-27 months of age (Rao *et al.*, 2000). Scrotal circumference is favorably correlated with weekly sperm output in mature bulls and semen traits in young bulls (Almquist and Amann, 1961). Ham *et al.* (1979) noted that the scrotal circumference is a good predictor of speroutput in young bulls but it was not found worth in older bulls.

Bulls having higher scrotal circumference with larger testes produce greater semen volume with more spermatozoa during the first ejaculation (Randel *et al.*, 1994; Sundararaman *et al.*, 2002). Smaller testes produce lower quality semen and achieved poor calving rate (Randel *et al.*, 1994).

Joshi *et al.* (1990) did not find any correlation between scrotal circumference and semen volume but found positive correlation of testis length with semen parameters.

There is a relationship between the scrotal circumference and semen density of the first ejaculate and plasma concentration of sex steroids (Smith *et al.* 1989; Palaz *et al.*, 1994; Pinho *et al.*, 1999).

Table 2.2 Scrotal circumference in relation to measures of bull's reproductive indices

References	Observations
Almquist and Amann (1961)	Scrotal circumference had positive correlation with number of ejaculated spermatozoa with gonadal sperm reserve.
Hahn <i>et al.</i> (1969), Foote <i>et al.</i> (1970), Coulter and Foote (1976)	Scrotal circumference is highly correlated with testes weight and spermatozoa output in growing bulls.
Coulter and Foote (1979)	Bulls having greater scrotal circumference with large testes generally produce daughter that also mature early and may be more fertile.
Coulter (1986)	Scrotal circumference is a better predictor of puberty in bulls than either age or body weight, regardless of breed.
Smith <i>et al.</i> (1989), Palaz <i>et al.</i> , (1994); Pinho <i>et al.</i> , (1999)	Scrotal circumference has relationship with semen density of the first ejaculate and sex steroids concentration in the blood.
Bertschinger <i>et al.</i> (1992)	Scrotal circumference measurements are accurate predictors of sperm output in young bulls. Parity of dam, birth mass and various growth performance variables of bulls were correlated with scrotal circumference.
Swanepoel <i>et al.</i> (1993)	scrotal circumference had a significant relationship with epididymal sperm reserves in bulls
Randel <i>et al.</i> (1994)	The smaller testes produce lower quality semen and achieved poor calving rate. A yearling bull with a scrotal circumference greater than 36cm has very good testes size for its age, since most bull range between 32-36cm at this

References	Observations
	age.
Randel <i>et al.</i> (1994); Sundararaman <i>et al.</i> (2002)	Bulls with larger testes produce greater semen volume with more spermatozoa during first ejaculation.
Evans <i>et al.</i> (1995)	Scrotal circumference increase curvilinearly with age and body weight and subsequently correlate positively with semen quality.
Keeton <i>et al.</i> (1996)	Scrotal circumference is considered to be the most rapid method for bull selection. Bulls having big scrotal circumference have direct effect on the pre weaning growth.

The young bulls (18 months) with scrotal circumference more than 27 cm have higher concentration of testosterone and estradiol 17 β and denser semen than the counterpart bulls with scrotal circumference less than 23cm. Bulls with risk for subnormal testicular development have hereditary factors associated with abnormal spermatogenesis and low fertility (Aehnelt and Hahn, 1965).

As the bull matures, the scrotal circumference increases and that leads to an increase in the percentage of normal and live spermatozoa, sperm motility and the number of spermatozoa per ejaculate. The maturation of bulls and subsequent enlargement of testes results in the decreased proportion of proximal cytoplasmic droplets as well as knobbed and ruffled acrosomes (Evans *et al.*, 1995).

Keeton *et al.* (1996) reported that selection of sires for increased scrotal circumference provides a viable means for making genetic progress in the age at puberty and other predictors of inherent fertility in the population.

According to the society for Theriogenology (Ball *et al.*, 1983) minimal scrotal circumference for bulls irrespective of breeds should be 30cm at 15 months or less, 31 cm at more than 15 and less than 18 months, 32cm at more than 18 months and less than 21 months, 33cm at more than 21 months and less than 24 months of age. The

minimum acceptable sperm motility and spermatozoa with normal morphology should be 30% or fair and 70%, respectively. West Canadian Association of Bovine Practitioners (Barth, 1993) agreed with society for Theriogenology in general. They considered scrotal circumference varied with breed and age of the bulls and sperm motility 60% or be graded as good.

Gofuret *al.* (2007) observed that the scrotal circumference of bulls increased with the incresement of age of bull. Scrotal circumference of 4PI (permanent incisor) age group more than 2PI and it was 26.67 ± 0.73 cm in 4PI age group and 23.17 ± 0.73 cm in 2PI age group.

2.2.6 Factors influencing scrotal circumference

2.2.6.1 Breed

There are big differences between breeds of bulls in average scrotal circumference at any given age. In general, large milk producing breeds develop larger scrotal circumference at an earlier age and at maturity than smaller breeds of cattle that have lower milk production (Barth, 1997). The size of scrotal circumference of dairy, beef and dual purpose breeds at puberty are presented in the Table 1. Double muscle breeds such as Piedmonte, Belgian blue, Parthenaise, Blonde d'Aquitaine and Limousin have smaller scrotal circumference at puberty and at maturity (Barth, 1997). Bulls genetically superiors in scrotal circumference had a high rate of pre weaning growth rate.

2.2.6.2 Age and body weight

Scrotal circumference increased with age, following a pattern similar to body weight and testes and tended to reach mature size rapidly, this is indicated by the curvilinear relationship between scrotal circumference and body weight (Ocanto *et al.*, 1991; Wildeus, 1993; Madrid-Bury *et al.*, 1993). Body weight loss by any means had negative effect on the testicular growth leading to decreased scrotal circumference (Costa and Freneau, 1999).

2.2.6.3 Nutrition

Nutrition levels have a profound effect on the testicular development and scrotal circumference in young bulls. Bulls in high energy diet groups had a greater mean scrotal circumference at 12 months than the bulls in medium energy diet groups because of fat deposition in the subcutaneous tissue of scrotum (Randall, 1986; Greenough *et al.*, 1990).

2.2.7 Testicular parameters

2.2.7.1 Locations

Testes are extra abdominal organs and situated in the prepubic region, enclosed in a diverticulum of the abdomen termed the scrotum (Getty, 1975; Hafez, 2000).

The male gonads testes lie in sublumber region immediately caudal to the kidneys at primitive level. The testes travel a considerable distance from their point of origin to the scrotum under the influence of testosterone (Frandsen and Spurgeon, 1992).

Getty (1975) reported that the testes of bull are relatively larger than those of the stallion and have an elongated, oval outline. The long axis is vertical and the attached border being caudal. The medial surface somewhat flattened. Testes of an adult bull, in contrast, measures on the average about 10-12 cm in length excluding the epididymis. The width is about 6-8 cm and the craniocaudal diameter is about the same. The weight is about 300 gm. Often one testicle, more often the left is slightly the larger and lower in position, giving the scrotum an asymmetrical appearance. The surfaces are convex and smooth. The extremities, dorsal and ventral are blunt and rounded (Macleod *et al.*, 1964). The testes represented two surfaces, two borders and two extremities. The medial and lateral surfaces were convex. But the convexity of the lateral surface was much more than that of the medial surface. The free border was ventral and somewhat convex. The attached border was dorsal. The head extremity and tail extremity were rounded (Getty, 1975).

Getty (1975) stated that the testicles are situated in the prepubic region, enclosed in a diverticulum of the abdomen termed the scrotum. The long axis is vertical; the attached border being caudal.

McDonald and Pineda (1989) stated that the male gonads or testes are the primary organs of reproduction in the male. Normal adult males of domestic mammals have scrotal testes, and the scrotal position is essential to normal gametogenic function.

Dyce *et al.* (2009) investigated that the scrotum of the ram is concealed by wool. Rudimentary teats are common on the scrotum of both rams and bucks. The testis of the ruminants is large, elongated ellipsoids that hang almost vertically within the scrotum where they may be palpated. They are freely movable.

2.2.7.2 Shape and size of the testes

Getty (1975) reported that the testes of bull are relatively larger than those of the stallion and have an elongated, oval outline. The long axis is vertical and the attached border being caudal. The medial surface somewhat flattened. A testes of an adult bull, in contrast, measures on the average about 10-12 cm in length excluding the epididymis. The width is about 6-8cm and the craniocaudal diameter is about the same. The weight is about 300 gm. Often one testicle, more often the left is slightly the larger and lower in position, giving the scrotum an asymmetrical appearance. The surfaces are convex and smooth. The extremities, dorsal and ventral are blunt and rounded (Macleod *et al.*, 1964). The testes represented two surfaces, two borders and two extremities. The medial and lateral surfaces were convex. But the convexity of the lateral surface was much more than that of the medial surface. The free border was ventral and somewhat convex. The attached border was dorsal. The head extremity and tail extremity were rounded (Getty, 1975).

Getty (1975) reported that the testicles are elongated and oval in outline. Each testis represented two surfaces (medial and lateral), the medial surface is somewhat flattened; two borders, free (ventral) and attached (dorsal); and two extremities, head and tail extremities. The epididymis is adherent to the attached border of the testicle. This is the border by which the gland is suspended in the scrotum by the spermatic cord. The

smooth external surface of the testis is remarkable only for the winding pattern of the intracapsular vessels (Dyce *et al.*, 2009).

Macleod *et al.* (1964) reported in bovine animals that often the left testis is slightly larger and lower in position, giving the scrotum an asymmetrical appearance. The surfaces are convex and smooth. The extremities (dorsal and ventral) are blunt and rounded.

Table 2.3 Different gross parameters of testes of different animals (Frandsen and Spurgeon, 1992)

Parameters	Horse	Bull	Sheep	Pig	Dog	Cat
Weights	200 to 300 gm	250 to 300 gm	200 to 300 gm	150 gm	0.05-0.075% of body weight	-----
Shape	Oval	Elongated oval	Elongated oval	Elliptical	Round to oval	More rounded than dog
Superficial vascular patterns	Small vessels extending from free border toward body of epididymis	Very large and tortuous vessels extending from tail toward head	Vessels of different sizes extending from epididymal border. Tunica vaginalis and tunica albuginea are thicker than in bull and vessels are not visible through tunic	Many vessels extending from free border toward body of epididymis	Single vessels and branches extending from tail toward head of testis	Less extensive system than dog, but extending in similar direction

Parameters	Horse	Bull	Sheep	Pig	Dog	Cat
Parenchyma	Raddish gray	Yellow to creamy orange	Creamy white	Grayish to dark red	Reddish	Reddish

Archana *et al.* (2014) stated that the both testes were elongated in shape with their long axis oriented vertically. They were compressed latero-medially. But with the advancement of age as the size of the testis increased, they became oval and convex laterally and relatively flat along the medial surfaces. The anterior border was free and rounded. The epididymis and vas deferens was attached along its posterior border. Dorsal and ventral extremities were covered with caput and cauda of the epididymis, respectively. Centrally placed mediastinum testis was present as a pulpy small cord in one day old-kid. It is increased in size and appeared as a whitish fibrous cord extending right from the dorsal extremity of the testis up to the distal $3/4^{\text{th}}$ of its length in the later part of life of animal. In prepubertal goats, the average length of right was 5.39 ± 0.45 cm and average length of left testis was 5.49 ± 0.45 cm. In pubertal animals, the mean length, width and thickness of the right testis measured 6.66, 4.54 and 4.52 cm, respectively. The left testis measured 6.69, 4.59 and 4.64 cm, respectively. In post pubertal animals, the mean length, width and thickness of right testis measured 7.05, 5.03 and 5.29 cm, respectively. The overall mean length, width and thickness of the left testes were 7.06, 4.99 and 5.26 cm, respectively.

Raji and Ajala (2015) revealed that West African Dwarf (WAD) buck testis is ellipsoidal in shape, covered by tunica vaginalis (a serous membrane) and a finely arranged network of arteries and veins.

Getty (1975) stated that the testicles are relatively much larger; a testicle of adult ram may be 10 cm long. They are broader in proportion to their length. The testes in relation to the body and the epididymis in relation to the testes are relatively larger in small ruminants (Dyce *et al.*, 2009).

Islam (2001) found the average length of the right testis of the male goats of different ages of 15, 60, 120, 180, 360 and 720 days were 2.33 ± 0.21 , 3.10 ± 0.2 , 5.38 ± 0.16 , 5.73 ± 0.15 , 7.07 ± 0.12 and 6.63 ± 0.61 cm, respectively. The values for the left testis of those male goats were 2.27 ± 0.15 , 3.12 ± 0.10 , 5.57 ± 0.60 , 5.70 ± 0.26 , 7.20 ± 0.10 and 6.80 ± 0.6 cm, respectively. The average width of the right testis of those male goats were 0.97 ± 0.15 , 2.03 ± 0.25 , 3.17 ± 0.15 , 3.90 ± 0.10 , 4.67 ± 0.12 and 4.50 ± 0.26 cm, respectively and for the left testis 1.33 ± 0.49 , 2.13 ± 0.25 , 3.15 ± 0.05 , 3.45 ± 0.13 , 4.58 ± 0.16 and 4.08 ± 0.36 cm, respectively.

Siddique *et al.* (2005) found the biometrical values, including length, width, circumference and weight of each left and right apparently normal testes of 89 lambs and rams. The mean $\pm$ S.E. of length, width, circumference and weight in lambs for the left testes were 4.43 ± 1.01 cm, 3.26 ± 0.79 cm, 9.21 ± 2.19 cm and 25.36 ± 13.88 gm and for the right testes 4.45 ± 0.98 cm, 3.23 ± 0.70 cm, 9.20 ± 2.13 cm and 25.43 ± 14.12 gm, respectively. In adult rams, these values were 6.73 ± 1.59 cm, 4.68 ± 1.17 cm, 13.45 ± 2.95 cm, 77.29 ± 48.03 gm for the left testes and 6.62 ± 1.60 cm, 4.73 ± 1.18 cm, 13.45 ± 2.95 cm and 76.77 gm for the right testes, respectively. He compared the average length, width circumference and weight of testes of immature rams with those of mature rams were smaller than the later and this may be due to age factor, nutrition, management and age wise growth of the testis.

Gofur *et al.* (2007) stated the testes of bulls were elongated and had oval outline in shape. The testes represented two surfaces, medial and lateral; two borders, free and attached and two extremities head and tail extremities. With the increasement of age of bull, body weight and scrotal circumferences were increased as well as the length and breadths of testes were also increased. The length and breadths were higher in the bulls of 4PI age group than 2PI age group. The length and breadth of the left testis were higher than the right testis. The average length of left and right testis of bull's 2PI age group was 11.75 ± 0.14 and 11.17 ± 0.17 cm, respectively. The average length of left and right testis of bull's 4PI age group was 12.83 ± 0.44 and 12.0 ± 0.29 cm, respectively.

The size of the testes varies throughout the year in seasonal breeders (ram, stallion and camel). Removal of one testis results increase in weight up to 80% of the remaining gonad. In the unilateral cryptorchid, removal of the descended testis may be followed

by descent of the abdominal testis as enlarges (Hafez, 2000). Testicular size could be correlated for sperm output (Amann and Almquist, 1976). The curve of testis growth is highly related to sperm output potential of bulls ejaculate and this is particularly true in young bulls, where the correlation between estimated testis size and daily sperm output (Hahn *et al.*, 1969a; McMillan and Hafs, 1968).

2.2.7.3 Weight of the testes

Getty (1975) stated testes of an adult bull, in contrast, measures on the average about 10-12 cm in length excluding the epididymis. The width is about 6-8cm and the craniocaudal diameter is about the same. The weight is about 300 gm. Getty (1975) stated that the small ruminant, the genital organs resemble, in general, those of the bull. A testicle of an adult ram weighs about 250 to 300 gm. Getty (1975) stated that the average weight of horse testis is 225-300 gm.

Archana *et al.* (2014) stated that the right testis although longer (as in the new born kid) than the left, it weighed less than the left testis. In the prepubertal goats, the average weight of right testis was 73.71 ± 10.62 g but left testis weight was 75.28 ± 10.63 g. In pubertal animals, the testis although grew in size and weight both with the progressive age, the change in either of the parameters were not as abrupt as observed in prepubertal animals. On average the right and left testis of pubertal animals weighed 106.50 ± 5.73 gm and 108.5 ± 5.63 gm, respectively. In post pubertal animals the testis grew only very slightly in size. The mean weight of right and left testis was 114.60 ± 8.89 gm and 112.80 ± 9.61 gm, respectively.

Land (1973) suggested that changes in the weight and number of sperm in the testes and epididymis cannot be observed under experimental conditions, and cannot be used for estimating the potential number of ewes a ram could fertilize or for the selection of breeding sires unless accurate indirect methods for their estimation are developed.

The weight of the testis increased with the advancement of age of the animals. Islam (2001) reported in this contrast that the average weight of the right testis of the male goats of different ages of 15, 60, 120, 180, 360 and 720 days were 1.0 ± 0.2 , 9.67 ± 0.29 , 29.73 ± 0.64 , 30.90 ± 3.0 , 60.46 ± 0.9 , 59.60 ± 1.45 gm, respectively. The average weights

of the left testis of those male goats were 1.10 ± 0.1 , 9.97 ± 0.21 , 30.05 ± 0.54 , 31.47 ± 2.61 , 61.33 ± 1.33 , 60.67 ± 1.26 gm, respectively.

Lino (1972) found the high correlations between scrotal circumference *orin situ* length x diameter of the testes and weight of the testes or testes plus epididymis in rams. In bulls, these have been found by Willet and Ohms (1957); Hahn *et al.* (1969).

Ott *et al.* (1982) showed that the left testicular weight is slightly more than the right one in mature rams. The left testis was 10% larger than the right on subsequent examinations. In buffaloes and cattle, similar reports have been reported by McMillan and Hafs, (1968); Ahmed, (1984); Ahmed *et al.*, (1985); and Ali, (1989).

Swierstra (1966) showed that the production of sperm is primarily a function of the size of the testes. The correlation between testes weight and number of sperm in the testes was high in rams and bulls (Almquist and Amann, 1961).

Observations were made on the development of the testes and epididymis of thirty-two Clun Forest ram lambs. There were no significant differences between the weights and volumes of the left and right testes and epididymis. The rate of increase in testicular and epididymal weights was linear up to a body weight of 20.9 kg followed subsequently by a significantly greater rate of increase at higher body weights. Similar patterns of growth occurred in relation to age, although at a given age there was a wide range of testicular and epididymal weights. There was a close relationship between the development of the testis and the epididymis at body weights greater than 20.9 kg. There were two distinct phases in the dimensional growth of the testis. Increases in testicular length and breadth preceded at much the same rate at testis weights of below 20 gm. During the subsequent phase testicular length increased at a significantly greater rate than testis breadth (Colyer, 2009).

Gofur *et al.* (2007) stated the weight of the testes of 4PI (permanent incisor) age group was more than 2PI age group and in between the left and right testis of both age groups, the weight was more in the left than the right testis. The average weights of left and right testis of bulls of 2PI age group were 111 ± 1.48 and 107.63 ± 0.80 gm, respectively and the average weights of left and right testis of bulls of 4PI age group were 117 ± 1.47 and 112.97 ± 1.19 gm, respectively.

2.2.7.4 Blood Supply

The testes are richly supplied with blood by the testicular artery, a branch of the abdominal aorta (Getty, 1975; Macleod *et al.*, 1964; Frandson and Spurgeon, 1992). Near the testes this artery descends in a tortuous fashion on each surface, these give off small branches which enter the gland on the trabeculae and the septa. The veins, on leaving the testes, form a network (the pampiniform plexus) around the artery in the spermatic cord. The testicular vein, which originates from this plexus, usually joins the caudal vena cava on the right side and the left renal vein on the left side. The lymph vessels in general, follow the course of the veins and enter the lumbar lymph nodes.

In all farm animals including bulls, the testis is supplied with blood from the testicular artery which originates from dorsal aorta near the embryonic site of the testes, is a convoluted structure

In the form of a cone, the base of which rests on the cranial and dorsal pole of the testis. These arterial coils are intimately enmeshed by the so-called pampiniform plexus of testicular veins. Lymph from the testis passes to the lumbar aortic lymph nodes (Hafez, 2000).

Elayat *et al.* (2014) stated that the marginal part of testicular artery in buffalo and ram is too long in relation to the size and position of the testis the artery performs secondary tortuosity along its wavy course. The degree of tortuosity is found more pronounced in ram than in buffalo.

Hees *et al.* (1990) stated that the angioarchitecture of the testis plays important roles in the thermoregulatory and androgen transfer mechanism as well as in the transport of rete fluid to the epididymis.

Dhingra (1979) reported that the coiling of the intratesticular arteries, the clustering at the mediastinum testis and the presence of juxtaposed parenchymal veins seem to have some role in the hemodynamic and thermoregulatory mechanism of the buffalo testis.

In mammals including bull, the testicular artery is highly convoluted as it approaches the testis. Through the mediastinum, whereas others pass over the periphery of the testis

in the tunica vasculosa. Arterioles enter the septula from both the mediastinum and the periphery. Lymphatic vessels were numerous in the tunica albuginea and extend along the tubules. The testes have both a vasomotor and sensory innervations. Nerve from the spermatic plexus form anterior in the deeper part of the tunica albuginea and from their reach the walls of the tubules and the Leydig cells. The blood vessels and nerves do not penetrate into the seminiferous epithelium (Weiss and Greep, 1977).

The testes are well supplied by the nerves, derived from the renal and caudal mesenteric plexuses, from the testicular plexus (paired) of autonomic nerves, they run with the artery, to which they are chiefly distributed (Getty, 1975). The testicular plexus receives fibers from the second lumbar splanchnic nerve and another splanchnic nerve leaving the sympathetic trunk from intermediate ganglion between the second and third lumbar ganglia and the abdominal aortic plexus (Fischer, 1906).

The hemoglobin oxygen saturation in the proximal testicular vein is significantly higher than in the distal testicular vein. The single, very long and tightly coiled testicular artery is intimately associated with the pampiniform plexus (testicular veins) in the spermatic cord. To this vascular complex several functions have been assigned, such as temperature regulation of the testicular blood (Waites and Moule, 1961); reduction of the testicular arterial pulse (Waites and Moule, 1960) and transfer of steroids from the pampiniform plexus to the testicular artery. Transfer of steroids is considered to take place by passive diffusion down a concentration gradient in rats (Free and Jaffe, 1975). However, the blood of rats does not contain testosterone binding globulin. In the ram, contradictory evidence has been reported on testosterone transfer (Jacks and Setchell, 1973; Cooper and Waites, 1974).

Anatomical evidence has been presented for direct arterio-venous anastomoses (shunts) between the testicular artery and the pampiniform plexus in the spermatic cord of the bull, ram and boar by Wensing and Dijkstra, 1981; Wensing, *et al.*, 1981; Hees, *et al.*, 1984.

The testicular artery in mammals convolutes to a variable extent before reaching the testis, and is closely surrounded by the veins of the pampiniform plexus. This vascular mechanism has a thermoregulatory function for the testis. The vascular pattern in the

testis of ram is almost exactly the same as in the goat, except that the testicular artery is wider in calibre (1-3 mm.) and forms more tightly packed convolutions in the cord. The artery is also more convoluted, forming about eighty wide spirals near the testis, and are intimately related to the veins of the pampiniform plexus which have thin walls and anastomose profusely around the artery. The course of the artery on the surface of the testis is immediately on the deep surface of the tunica albuginea. From the convoluted part of the artery in the cord, or just above it, branches are given to all parts of the epididymis. In no case are any branches given off to the testis until the artery reaches the anterior surface of the gland (Harrison and Weiner, 1949). They found the degree of convolution (number of loops) of the testicular artery in ram is 80. Diameter of the testicular artery in ram is 1.3 mm.

Branches of the spermatic artery ramify in the mediastinum and tunica vasculosa. These send branches into the septa of the testis which give rise to a capillary network around the seminiferous tubules. The blood is collected by veins that accompany the arteries (Copenhaver *et al.*, 1978).

2.2.7.5 Thermoregulation of testis

Maloney and Mitchell (1996) found that in rams, the tunica dartos muscle is involved in moving the testes closer to the body by contracting under cold conditions and further by relaxing when hot. The body-scrotal temperature difference is greater in an environment of 6°C and less at 40°C, although during fever induced by lipopolysaccharide injection or during exercise, scrotal temperature remains constant while body temperature rises. More recent studies have shown that panting and reduction in body temperature following scrotal heating occurred in rams with a fever as well as under normal conditions (Maloney *et al.*, 2003). Scrotal surface temperature in stallions is increased during exercise (Staempfli *et al.*, 2005).

Setchell (2006) reviewed that the testes of many species of mammals descend either during fetal life or shortly after birth into a scrotum, where the temperature is appreciably lower than in the abdomen. In bulls, as in other animals with scrotal testes, the testes are cooled by countercurrent exchange of heat between the venous and arterial blood in the spermatic cord (Waites and Moule, 1961; Brito *et al.*, 2004).

Kastelic *et al.* (1996; 1997) showed that the temperature on the surface of the scrotum is lower at its base than near the neck, but the temperature inside the testis is almost uniform, even slightly warmer at the base. Heat is lost from the testis and scrotum to the environment through the scrotal skin, which is well endowed with sweat glands (Setchell and Breed, 2006).

Harrison and Weiner (1949) demonstrated that the testis temperature of the ram is lower than the body temperature. Variations in ambient temperature and humidity due to the weather together with the sheared or unsheared condition of the animal interacted to produce important changes in the body temperature of rams.

Foote *et al.* (1957) suggested that the effect of humidity was not uniform at the different ambient temperatures. The difference at 70°F was 0.22°, at 82°F it was 0.38°, and at 95°F it was 0.77°. The overall average temperature of the unshared rams was 103.62°F while that for the sheared rams was 102.90°F.

2.3 Histological parameters of the testis

2.3.1 Tunica vaginalis

Testis is a compound tubular gland and enveloped by a dense fibrous capsule, the tunica albuginea and a layer of peritoneum, the tunica vaginalis (Bacha and Wood, 1975; Johnson *et al.*, 1970; Copenhaver *et al.*, 1978; Trautmann and Fiebiger, 1952) which is so richly vascularized (Johnson *et al.*, 1970) in certain places of it and a connective tissue cord that extends through the testis on its long axis (Trautmann and Fiebiger, 1952) and gives off collagenous sheet in carnivores and pigs or strands in ruminants radiating the tunica albuginea.

A closed serous sac, the tunica vaginalis, surrounds the anterior and lateral surface of the testis. This cleftlike sac is a detached diverticulum from the peritoneal cavity. The testes have approximately the same relationship with this sac as they had with the peritoneal cavity before their descent into the scrotum. The visceral layer of the tunica vaginalis adheres as a smooth, glistening membrane to the tunica albuginea; the parietal

layer lines the inner surface of the scrotum. Both layers are lined by mesothelial cells. Posteriorly the serous sac is lacking, the testes lying behind and outside the tunica vaginalis (Copenhaver *et al.*, 1978).

Dellmann (1992) described that when the testis was removed from the scrotum, the parietal layer of the tunica vaginalis remained attached to the scrotum, whereas the visceral layer, the peritoneal covering of the testis (and epididymis), remained intimately associated with the underlying capsule of the testis, the tunica albuginea. The visceral layer of the tunica vaginalis consisted of a mesothelium and a connective-tissue layer that blends with the tunica albuginea (Dellmann, 1992).

The surface epithelium of the testis and the subjacent connective tissue is often called the visceral layer of the tunica vaginalis. At the posterior part of the testis, this layer is continuous with the parietal layer of the tunica vaginalis. Between the visceral and parietal layers is a cavity which is a remnant of the peritoneal cavity (Copenhaver *et al.*, 1978).

2.3.2 Tunica albuginea

The tunica albuginea is a solid capsule of dense irregular connective tissue. It consists predominantly of collagen fibers, a few elastic fibers, and myofibroblasts. Intratunical interstitial endocrine cells occur in the cat. Meandering branches of the testicular artery and a network of anastomosing veins constitute the vascular layer of the tunica albuginea (Dellmann, 1992). In bull, the tunica albuginea is thin; it contains many elastic fibers but no smooth muscle. The parenchyma is yellowish in color (Getty, 1975).

Copenhaver *et al.* (1978) described that the tunica albuginea testis was a tough, fibrous membrane that encapsulates the testes in man. It is about 0.5 mm in thickness. Externally, it is covered by mesothelium. During the early development of testis, this covering layer of epithelium is cuboidal and is the germinal epithelium. Beneath the basal lamina of the simple squamous epithelium, there is a layer of rather fine, closely woven collagenous fibers that blends with a deeper layer of interweaving, coarse collagenous fibers. This deeper layer forms the main component of the tunica albuginea. The inner part of the tunic is composed of looser connective tissue, the

innermost part of which is highly vascular and is called the tunica vasculosa. Just beneath the tunica vasculosa are seminiferous tubules and delicate connective tissue, the later forming the interlobular septa of the testis which are continuous with the tissue of the tunica vasculosa. The tunica vasculosa contains numerous quite large blood vessels. Branches of these penetrate the denser part of the tunica albuginea (Copenhaver *et al.*, 1978).

The tunica albuginea is a solid (Dellmann, 1992), thick, white (Hafez, 2000) capsule of dense irregular connective tissue. In the horse (Trautmann and Fiebiger, 1952) and in the camel (Singh and Bharadwaj, 1978), the tunica albuginea is rich in smooth muscle fibers and are reported to be derived cremaster internus and are continued in the septula testis. Smooth muscle fibers are present in the tunica albuginea of stallion, man, rabbit and rat (Bacha and Wood, 1990; Johnson *et al.*, 1970).

Goyal and Dhigra (1973) reported on fibrous capsule of buffalo composed of fibrous tunic and a loose connective tissue layer rich in blood vessels, the tunica vasculosa, which lies adjacent to the parenchyma of the testis. The tunica albuginea is composed of mostly collagenous fibers with a few elastic and reticular fibers. Scattered smooth muscle fibers and a few small venous spaces are also present. The animals ranging from 15-52 weeks in age demonstrate more or less the same histological picture with the exception of progressive increase in thickness of the capsule which occurs mainly due to the appearance of large number of blood vessels in the tunica vasculosa. The average thickness of the capsule in 15-16 weeks, 30-32 weeks and 50-52 weeks old testicles was 511 μm , 607 μm and 932 μm , respectively.

2.3.3 Septula testis and mediastinum testis

Dellmann (1992) stated that the tunica albuginea is continuous with connective tissue trabeculae, the so-called septula testis, which converges toward the mediastinum testis. These trabeculae are rather complete septa in the dog and boar, whereas in the other domestic animals, they are inconspicuous connective-tissue strands surrounding the large intra-testicular vessels, the septula testis divide the testicular parenchyma into a varying number of pyramidal (Copenhaver *et al.*, 1978) or cone shaped (Stiles, 1956) testicular lobules.

The septula testis is continuous with the mediastinum testis, a connective tissue area containing the channels of the rete testis and large blood and lymph vessels. The mediastinum testis of the stallion, cat, and many rodents is relatively small and located in a marginal position, whereas in ruminants, the pig, and the dog, it occupies a central position along the longitudinal axis of the gonad (Dellmann, 1992).

The mediastinum testis is an axial strand of connective tissue, which descends from the dorsal part of the attached border deeply into the gland. From it, the chief trabeculae radiate, but distinct interlobular septa are not present (Getty, 1975).

2.3.4 Interstitial tissue

Copenhaver *et al.* (1978) stated that, besides the usual connective tissue elements, the stroma contains characteristic cells known as the interstitial cells or cells of Leydig. These cells form the internal secretion known as testosterone. The interstitial cells occur in groups of various sizes. Small blood vessels are usually present in the groups. The interstitial cells are large and are ovoid or polygonal in shape. They have a large nucleus which is frequently eccentrically located. The cytoplasm of the interstitial cells is granular and fairly dense near the nucleus but peripherally it is vacuolated, and in usual preparations it stains quite lightly. This is largely due to the dissolving out of lipid granules and droplets. Most of the endoplasmic reticulum is of the smooth surfaced typed, and the mitochondria have tubular rather than shelflike cristae. These are characteristic but not universal features of cells that secrete steroid hormones (Copenhaver *et al.*, 1978). The interstitial cells also contain lipochrome pigment granules and crystalloids, the pigment granules increase in number in older man, and ultrastructural studies indicate that they may represent autophagic vacuoles. The crystalloids are rod-shaped structures and in cross section are oval or round. Their number and size vary greatly. They are formed of an albuminous substance and are quite resistant to solvents. Fixation soon after death is necessary to preserve them. The Leydig cells arise from fibroblasts and may revert to cells that are indistinguishable from fibroblasts. There are also numerous macrophages in the interstitial tissue interposed among the Leydig cells (Copenhaver *et al.*, 1978).

Between the tubules are masses of interstitial (Leydig) cell, which produce male sex hormone, testosterone (Hafez, 2000; Frandoson and Spurgeon, 1992).

Dellmann (1992) cited that the intertubular spaces contain loose connective tissue, blood and lymph vessels, fibrocytes, free mononuclear cells, and interstitial endocrine (Leydig) cells. Interstitial endocrine cells produce testicular androgens and in the boar, large amounts of estrogens as well. Two generations of interstitial cells, fetal and pubertal, develop from mesenchyme-like precursors. In some species, e.g., bovine and porcine, a third generation of interstitial cells (early postnatal) is encountered. Interstitial cells constitute approximately 1% of the entire testicular volume in the adult ram, approximately 5% in the bull and 20 to 30% in the boar. Interstitial endocrine cells occur in cords or clusters; not every cell in closer contact to a capillary. Between adjacent cells are intercellular canaliculi and gap junctions. High concentrations of steroids are found in testicular tissue and lymph (Dellmann, 1992). The interstitial endocrine cells are large polymorphous cells with spherical nuclei and smooth endoplasmic reticulum. Its membranes incorporate most of the enzymes necessary for steroid biosynthesis. The mitochondria possess tubular cristae and are involved in the first step of steroid hormone production, e.g., transformation of cholesterol to pregnenolone. The relatively small golgi complex does not participate in secretion of androgen. Release of androgen from interstitial cells is morphologically inconspicuous. Lipid inclusions are found in all species, but are particularly abundant in the cat (Dellmann, 1992).

The interstitial tissue often shows a lamellar structure and contains vessels and nerves. The Leydig cells occur singly or groups. They were more or less oval and have a large spherical nucleus with a distinct nucleolus. The cytoplasm contains yellow lipid granules, protein crystalloids (in the horse and cat) and pigment deposits which increase with age. The interstitial cells are probably derived from connective tissue cells. They are very labile elements and react to mild agents. They are most abundant in the boar and horse. Prior to the onset of spermatogenesis no typical interstitial cells are found in the horse (Trautmann and Fiebiger, 1952).

In ram, the interstitial tissue comprises about 15% of the testis and the Leydig cells comprise about 7.5% of the interstitial tissues, about 1% of the testis; the walls and

lumina of the blood and lymph vessels comprise about 375 of the interstitial tissue that is about 6% of the testis. A ram testis weighing about 250 gm, contains about 8×10^9 Leydig cells, each about $350 \mu\text{m}^3$ in volume (Monet-Kuntz *et al.*, 1988).

Leydig cells in mammals are in general relatively large, polyhedral, epitheloid cells surrounded by a typical plasma membrane which is usually covered with microvilli; each cell has a single, spherical or ovoid, eccentrically located nucleus with one to three large nucleoli. The cytoplasm contains moderate numbers of average sized mitochondria with mostly lamellar cristae and a well developed Golgi complex. The Leydig cells of some species contain large numbers of lipid droplets, however in rams, bulls, boars or stallions (Fawcett *et al.*, 1973).

Archana *et al.* (2014) reported that the undifferentiated Leydig cells spindle, elongated or ovoid shaped. Their cytoplasm appeared eosinophilic and granular. The nuclei of these cells were rounded or oval, eccentrically placed. They occurred in small groups of 2 to 3 in between the fibroblast. The nuclear membrane was indistinct. The typical differentiated Leydig cells were typical ovoid or roughly triangular and sometimes rounded in shape with highly vacuolated eosinophilic cytoplasm. Round or ovoid nuclei of these cells were generally present eccentrically towards the broader end of the cells. The nuclear membrane was distinct. They occurred singly or in groups. In prepubertal animals, the Leydig cells mostly dominated the cell types. They occurred in groups of variable sizes. The number and size of Leydig cells further increased in post pubertal animals in compared to pubertal animals.

Goyal and Dhigra (1973) reported on interstitial tissue of buffalo bulls at 15-16 weeks, the Leydig cells are few in number and are present singly or in groups in the stroma between the sex cords. These cells have characteristic rounded nuclei which measure 4-4.5 μm in diameters. The nucleus contains coarse chromatin granules arranged in the periphery of the nuclear membrane, with one prominent nucleolus in the centre. These cells are polyhedral in shape. The mesenchymal cells and fibroblasts present the same histological picture. At the 30-52 weeks of age, the interstitial tissue demonstrates irregularly arranged connective tissue and contains a large number of vessels. The Leydig cells have increased in number occur singly, in rows or in groups between the sex cords and along the blood vessels. The cytoplasm is lightly eosinophilic and

contains and small perinuclear granules in some of the cells, while in other it is vacuolated or empty. The Leydig cells range from 10-12 μm in length and 7-8 μm in width.

Mancini *et al.*, (1963) reported that Leydig cells in human testes are seen at the time of birth; later on they decrease in number and at the time of puberty their number again increased.

Hosney *et al.*, (1963) reported that the cells of Leydig of buffalo are arranged in groups between the seminiferous tubules or singly or in rows.

2.3.5 Sex cord

Kamal and Devi (2012) suggested that the progression of age, the gonocytes started migrating towards the basement membrane of sex cords. These cells were termed as prespermatogonia which could be seen in the sex cords of 2 months old kids. Such centrifugal movement of gonocytes to form prespermatogonia. Luminization of majority of sex cords initiated at 2 months of age and completed by 6 months of age in the Assam goats.

Abdel-Raouf (1960) stated that the luminization started at the 20-24 weeks of age in the sex cords of cattle.

Archana *et al.*, (2014) reported on sex cords in the Gaddi goats that sex cords were separated from each other by means of loose fibrocellular interstitial tissue. Each sex cord has a basement membrane surrounded by fine collagenous and reticular fibers. A single incomplete layer of fibroblast surrounded the sex cords beyond the fibro reticular layer. All the sex cords were filled with small indifferent basal cells and large primordial germ cells (PGC) designated as gonocytes. The small cells were spherical in shape, lay along the periphery and dominated the larger cells in number. They also stated that the large primordial germ cells were spherical in shape and mostly present in the cords. But more than often they were also found between the small cells in some of the sex cords. The cytoplasm was slightly acidophilic and usually vacuolated with distinct cell boundary. These cells were characterized by their large spherical nuclei. The chromatin material was clumped beneath the nuclear membrane. Typical Sertoli

cells and spermatogonia could not be identified in the sex cords of neonatal testis. With the advancement of age, the size of the sex cords increased. At 6 months of age, the sex cords constitute about 60% of the total parenchyma.

Baishya *et al.* (1987) reported that there were no significant differences in seminiferous tubules diameters between the age group's (0-15, 16-30, 31-60 and 61-90 days) or between the right and left testis. Gonocytes and prespermatogonia were found from birth to 60 days of age, spermatogonia and primary spermatocytes were first observed at 69 days of age.

Johnston *et al.* (1982) reported that primary spermatocytes appear in the seminiferous tubules by 4 to 6 months, spermatids by 6 to 7 months and spermatozoa by 7 to 9 months of age in bulls.

Malhi *et al.* (1999) stated that at birth, primitive Sertoli cell and gonocytes were observed in the seminiferous cords. The gonocytes migrated towards the basement membrane and on contact with basement membrane were classified as prespermatogonia. Primary spermatocytes were first seen at 6 months of age. Lumen formation in seminiferous cords was first observed at 1 year of age. The spermatogenic process was completely established by 3-3.5 years of age. The seminiferous epithelium cycle was divided into 8 stages.

Goyal and Dhigra (1973) reported on sex cords at 15-16 weeks of buffalo bulls are oval, solid and separated from each other by a moderate amount of interstitial tissue. The arrangement of small and large cells in the sex cords 83% and 17% of the total cell population respectively. At 30-32 weeks of age, solid sex cords are lined by small cells with irregular nuclei, small cells with rounded nuclei and large cells. The latter mostly occupy the centre of the cord and constitute 18% of the total cell population. Frequent mitotic figures and occasional degeneration of large cells are observed. Small cells with irregular nuclei constitute 71% of the total cell population in the sex cords. A few tubules show the presence of Sertoli cells which form 1% of the cell population. At the 50-52 weeks of age, the tubules become moderately tortuous and slight lumen formation is evident in all the tubules. Lumination is more developed in the tubules lying near the rete testis region than in the tubules of peripheral and central regions.

The tubules are lined by basal-indifferent cells, spermatogonia, Sertoli cells and large cells. Most of the large cells show degenerative changes. The average percentage of the basal-indifferent cells, spermatogonia and large cells in the seminiferous tubules is 67.3%, 28.5% and 1.5%, respectively. The Sertoli cells constitute 2.7% of the total cell population and are distinguished by their lightly stained columnar nuclei with folded nuclear membrane and acidophilic nucleolus. These cells lie vertical to the basement membrane but some horizontal nuclei are also observed.

Abdel-Raouf (1960) reported in bovine, the tubules lying at the periphery exhibit greater lumination than those lying near the rete testes region. The tubules do not show any change in the diameters in the different regions of the testis up to 32 weeks. It is indicated that no significant different exists in the tubular diameters in the different region of the testis. The tubular diameters increases 5 fold between birth and 71 weeks.

2.3.6 The seminiferous tubules

There are two to three (Trautmann and Fiebiger, 1952), one to four (Dellmann, 1992) convoluted seminiferous tubules reported to be present in each lobule. The convoluted seminiferous tubules in most mammals are tortuous two ended loops with a diameter between 150 and 300 μm . They are lined by the stratified germinal epithelium, surrounded by a lamina propria and connected at both ends to straight testicular tubules by a specialized terminal segment. Histologically, the seminiferous tubules have 3 components: lamina propria, somatic cells and spermatogenic cells (Dellmann, 1992).

The diameters of seminiferous tubules are between 200 to 250 μm , so that an average ram testis contains nearly 3000m of tubules. The tubules are surrounded by a specialized peritubular tissue which consist of several layers of non cellular material in domestic mammals and of myeloid cells, which are contractile and probably cause peristaltic contractions of the tubules to assists in the transport of spermatozoa (Setchell and Brooks, 1988)

Kamal and Devi (2012) suggested that the seminiferous tubules have prominent basement membranes supporting the germinal epithelium. A distinct network of reticular fibres interspersed with fine collagenous fibres were marked in the basement membrane of the tubules in the male goats. Elastic fibres were often found around the

blood vessels and in the internal elastic membrane. Increase in tubular convolution and decrease of intertubular space was marked as the age of the animals advanced. All the seminiferous tubules in the bucks from 6 months of age onwards have distinct lumina (Kamal and Devi, 2012).

The diameter of the seminiferous tubule is between 200 and 250 μm , so that an average ram testis contains nearly 3000 m of tubules. The tubules are surrounded by a specialized peritubular tissue, which consists of several layers of noncellular material in domestic mammals, and of myoloid cells, which are contractile and probably cause peristaltic contractions of the tubules to assist in the transport of spermatozoa (Setchell and Brooks, 1988).

Copenhaver *et al.* (1978) found that the wall of a seminiferous tubule consists of an outer capsule or tunica propria of fibroelastic connective tissue and flattened fibroelastic cells, which closely invest the tubule; a basal lamina and a lining of a complex stratified epithelium. The epithelium consists of two kinds of cells, the supporting cells or the cells of Sertoli and the spermatogenic cells.

The boundary tissue of seminiferous tubules consists of 4 layers. They are amorphous inner lamina, cellular inner lamina, amorphous outer lamina and cellular outer lamina. The outer lamina of boundary tissue of rat, mouse and hamster revealed in histochemical reactions meshes resembling honey combs. The wall of seminiferous canaliculies of bull and ram consists of more bigger and different structure than one at the outer laboratory animals. The different structure of boundary tissue in man was observed. The capillary vessels penetrate in the myofibroblastic layer, when compared to that found in other mammals on the surface of the mammals on the surface of the wall (Cieciura *et al.*, 1988). Each terminal segment of seminiferous tubules surrounded by a vascular plexus and may be subdivided into a transitional region, middle portion and terminal plug. The modified supporting cells of the middle portion and the terminal plug no longer display the typical Sertoli-Sertoli junctions seen in the transitional region and the seminiferous tubules proper. In the region of the terminal plug a distinct central lumen is generally not observed; spermatozoa and tubular fluid must pass through an intricate system of communicating clefts between the apices of the closely attached modified supporting cells. The vascular plexus investing the terminal segment

serves a dual purpose: it is regulatory device for fluid and sperm transport, as well as an area of increased diapedesis for white blood cells (Wrobel *et al.*, 1982).

2.3.7 Peritubular tissues

The lamina propria surrounds the seminiferous tubule. Its innermost layer is a basal lamina, often with club-shaped projections into basal infoldings of sustentacular cells and spermatogonia. Some collagen and elastic fibers connect the basal lamina to the flat peritubular cells, which form a stratum of one to five layers, depending on species (Dellmann, 1992). At birth, these peritubular cells resemble mesenchymal cells, which postnatally differentiate gradually into contractile cells. In some species (e.g., boar), they acquire all features of typical smooth muscle cells; in other species (e.g., bull), they represent myofibroblasts. The peritubular cells are responsible for tubular contractions. Thus, they participate in transport of tubular content and in spermiation. The outermost layer of the tubular lamina propria consists of fibrocytes and collagen fibrils. Free mononuclear cells (lymphocytes, monocytes) invade the lamina propria, but never invade the intact tubular epithelium (Dellmann, 1992).

Copenhaver *et al.* (1978) found that the flattened cells of the tunica propria are in multiple layers in primates. The layer closest to the epithelium usually assumes characteristics of smooth muscle and these cells are referred to as myoid cells. There is evidence that such cells in lower animals may assist the transport of sperm out of the tubules by their contractions. A basal lamina separates the epithelium from the tunica propria. Actually, the myoid cells are completely enclosed by a basal lamina-like layer (external lamina) as are typical muscle cells.

2.3.8 Sustentacular cells

Dellmann (1992) described that sustentacular cells are derived from undifferentiated supporting cells of the prepubertal gonad. These cells are mitotically active, contain large amounts of rough endoplasmic reticulum and produce the antiparamesonephric hormone, a glycoprotein that suppresses development of uterine tubes, uterus and vagina in the male. During puberty, sustentacular cell differentiation is accompanied by a morphologic transformation and loss of mitotic capability. The adult sustentacular cells are irregularly outlined, elongated cells. Their broad base rests on the basal lamina

and the remaining cytoplasm extends upward to the tubular lumen. They are rather evenly shaped; between 25 and 30 sustentacular cells are seen in a cross section of a seminiferous tubule (Dellmann, 1992).

Lateral and apical cytoplasmic processes of the sustentacular cells fill all the spaces between adjacent spermatogenic cells. The oval or pear-shaped nucleus is generally located in the broad basal portion of the cell and is often deeply infolded. Furthermore, a large nucleolus is present. It is vesicular in ruminants. The basal portion and the central trunk region of the sustentacular cell contain mitochondria, an inconspicuous golgi complex, abundant sER, little rER, free ribosomes, microtubules, actin and vimentin filaments, secondary lysosomes and lipid inclusions (Dellmann, 1992). Only a few organelles are found in the lateral and apical sustentacular cell processes. Shape of the cell, surface area and volume percentages of organelles change in accordance with spermatogenetic events (sustentacular cell cycle). Sustentacular cells form hemidesmosomes with the basal lamina. Temporary junctions with germ cells play a part in vertical displacement and release (spermiation) of the germ cells (Dellmann, 1992).

There is a network of tight junctions between the Sertoli cells which plays a critical role in the formation of the blood-testis barrier, and in the separation of the seminiferous epithelium into the basal and adluminal compartments (Pelletier and Byers, 1992). The tight junctions between the Sertoli cells provide a barrier preventing entry of immunologic cells into tubules as they may otherwise perceive haploid germ cells as abnormal cells. This is achieved by transporting the early meiotic spermatocytes from the basal to the adluminal compartment. Tight junctions, however, allow the passage of nutrients to reach more advanced germ cells. There is a special microenvironment, the stem cell niche, formed at the basal compartment where Sertoli cells support and regulate the self-renewal and differentiation of spermatogonial stem cells (SSCs) (de Rooij, 2009).

Adjacent sustentacular cells are joined by tight junctions associated with microfilaments and subsurface cisternae of the ER. The Sertoli cells are joined to each other by gap (communicating) junctions (Copenhaver *et al.*, 1978). These junctions separate a basal from an adluminal (apical) tubular compartment and constitute a

diffusion barrier. Renewal of spermatogenic stem cells and multiplication of spermatogonia take place in the basal compartment, to which intertubular tissue fluid has relatively free access. The blood-testis barrier selectively prevents many substances from entering the adluminal compartment, where the vital processes of meiosis and spermiogenesis take place in a controlled microenvironment. Sustentacular cells have nutritive, protective and supportive functions for the spermatogenic cells (Dellmann, 1992). In addition, they phagocytize degenerating spermatogenic cells and detached residual bodies of spermatids. They release the spermatozoa into the lumen of the seminiferous tubules (spermiation). They mediate the action of FSH and testosterone on the germ cells, participate in the synchronization of spermatogenic events, produce an androgen-binding protein and secrete constituents of the intratubular fluid, such as transferrin, androgen-binding protein and inhibin. Inhibin is reabsorbed from the lumen of the efferent ductules and the initial segment of epididymis. It then reaches the bloodstream and exerts a negative feed-back on hypophyseal FSH secretion. Paracrine signals from sustentacular cells modulate the activity of nearby interstitial endocrine cells (Dellmann, 1992).

The cells of Sertoli are tall, irregularly columnar cells that extend from the basal lamina to the lumen. Their sides are markedly uneven, showing pits and depressions into which fit the adjoining germ cells. The nucleus is ovoid and pale staining with finely dispersed chromatin, and it usually contains one or more prominent nucleoli. The nuclear membrane often shows a characteristic longitudinal groove (Copenhaver *et al.*, 1978).

The location of the nucleus varies in different Sertoli cells from the basal position to positions located at a considerable distance from the basal lamina. Electron micrographs show that the mitochondria, described as filamentous from light microscope studies, are unusually long and slender. In addition to the usual organelles, the cytoplasm contains lipid droplets, glycogen and, in man, a spindle-shaped crystalloid. The cell border is difficult to distinguish in routinely stained preparations, but the entire cell and its borders can be defined by silver methods. The Sertoli cells are the only ones that extend from the basal lamina to the lumen, and thus they give structural organization to the tubule (Copenhaver *et al.*, 1978). They rest on the basal

lamina in a patterned array that is readily seen in silver preparations of tangential sections of the tubule. Following the Sertoli cell from the basal lamina to the lumen, one finds that it is surrounded first by spermatogonia and then by different stages of spermatocytes and spermatids. The pattern of arrangement is seen best at the level of the primary spermatocytes, which are arranged in rings around the Sertoli cell. In ordinary preparations, it frequently appears that the spermatogenic cells are contained within the cytoplasm of the Sertoli cells (Copenhaver *et al.*, 1978). Studies with tracers indicate that these junctions are mainly responsible for a blood-testis barrier at the level of the preleptotene primary spermatocytes. Freeze- fracture studies have revealed even more strikingly the extensiveness of the occluding junctions. On effect these specializations establish two intercellular compartments within the seminiferous tubule. The basal compartment permits fairly free exchange of nutrients and waste products between the interstitial vasculature and the more primitive spermatogenic cells. The more apical compartment, beyond the level of the occluding junctions, is isolated from such direct exchanges. Thus, the Sertoli cells control the availability of substances presented to the more differentiated stages of the developing germ cells (Copenhaver *et al.*, 1978).

Fawcett (1975) and Setchell and Waites (1975) suggested that the Sertoli cells influence and modulate germinal cell development, first by forming and maintaining the seminiferous tubule barrier; second by producing and secreting particular components such as androgen binding protein (Fritz, 1978); plasminogen activator (Lacroix *et al.*, 1977); inositol (Robinson and Fritz, 1979); transferring (Skinner and Griswold, 1980, 1982) and other less well-defined proteins (Wright *et al.*, 1981; Kissinger *et al.*, 1982; De Philip *et al.*, 1982) and third by implementing the restructuring in the tubule which occurs during various stages of spermatogenesis (Russell, 1977).

The Sertoli cells are also responsible for phagocytosis of residual cytoplasm cast off during maturation of spermatocytes and for the synthesis of androgen- binding protein that helps to maintain the high androgen levels within the seminiferous tubule essential for proper term cell differentiation. Special staining methods show that the cytoplasmic processes of the Sertoli cells increase in number and size when the spermatids are

maturing. Thus, the Sertoli cells exhibit a cyclic activity that is correlated with the stage of spermatogenesis in any given region of the seminiferous tubule (Copenhaver *et al.*, 1978).

In bull, Sertoli cells are large and relatively clear except for the prominent, dark staining nucleolus. Cytoplasm is diffuse and its limits are indefinite (Hafez, 2000).

Sertoli cells or nurse cells are interspersed between and envelop developing spermatids and their precursors. The Sertoli cells are presumed to nourish the developing male sex cells and may transform testosterone into estrogen under the influence of FSH (Fradoson and Spurgeon, 1992).

Cigankova and Tomajkova (1987) reported that mitoses of Sertoli cells precursors were observed up to 20 days post partum, but mitoses of Sertoli cells were not observed in sexually mature animals.

2.3.9 The Spermatogenic Cells

Muller *et al.*, (1992) reviewed that based on the testicular consistency at palpation, each bull was assigned to one of three groups ranging from normal (Group A) to markedly reduced testicular consistency (Group C). In testes with a slight to moderate reduction of testicular consistency (Group B) degenerated primary spermatocytes and spermatids were common at all stages of seminiferous cycle. Abnormalities in the condensation of the chromatin, acrosomal defects and the presence of degenerated cells and cellular debris were the most common disturbances. In testes with a marked reduction of testicular consistency (Group C) the seminiferous epithelium of most tubule showed degenerative changes, thereby making it impossible to classify the stages of cycle. The changes were mainly restricted to spermatocytes and spermatids whose relative numbers were reduced. The number of cells undergoing meiotic division was also greatly reduced. Changes in the spermatids were also found including the appearance of severe acrosomal defects such as acrosomal pouches, the failure of nuclear elongation and defects in chromatin condensation.

The spermatogenic cells lie between the cells of Sertoli in an orderly manner, with four to eight layers occupying the space between the basal lamina and the lumen. In the

undeveloped testis, only the primitive germ cells or spermatogonia are present. With the onset of sexual maturity, the spermatogenic cells are represented in all stages of differentiation and are arranged in several more or less distinct layers (Copenhaver *et al.*, 1978).

The stratified epithelium of these seminiferous tubules contained type A and B spermatogonia; primary spermatocytes; secondary spermatocytes; round spermatids; elongated spermatids and spermatozoa. The intermediate type of spermatogonia could not be found. The spermatogonia were located close to the basement membranes, while their successive descendants were progressively closer to the lumina of the seminiferous tubules. The meiotic phases of the primary spermatocytes were recognized in the kids from 4 months of age onwards (Kamal and Devi, 2012).

At a given section of the seminiferous epithelium, depending on the species, there are 4-5 generations of germ cells at different stages of development. These stages appear sequentially throughout the seminiferous tubules with a helical or segmented arrangement. In the segmented arrangement, a wave-like release of spermatozoa occurs over time (Russell *et al.*, 1990).

Aire (1973) distinguished type-A and type-B spermatogonia based on the distribution pattern of chromatin and size of the spermatogonia in ram. Similar types of spermatogonia were also reported in rats (Clermont and Perey, 1957), stallions (Swierstra, *et al.*, 1974), bulls (Johnson, *et al.*, 1977), and goats (Bordooi and Dhingra, 1990). The criteria of these cells having irregular distribution of numerous Fulgent-positive chromatin granules in ovoid nucleus and their attachment to the nucleoli and nuclear membrane did not hold good in goat. Similar observations were also made by Bordoloi and Dhingra (1990).

Gondos *et al.* (1977) stated that the transformation of the gonocytes into spermatogonia as initiation of spermatogenesis. Prior to transformation of gonocytes into spermatogonia, an intermediate prespermatogonial stage occurred this had been ascertained as “quiescent” by Guraya (1980). The first appearance of spermatogonia in the seminiferous tubules was an important factor as it denotes the onset of spermatogenesis. The transformation of gonocytes to spermatogonia was observed in

the seminiferous epithelium at 4 months of age indicating the initiation of spermatogenesis at this age in Assam goat. However, Baishya *et al.* (1987) recorded the initiation of spermatogenesis at 69 days of which was much earlier as compared to 4 months of age. The age of initiation of spermatogenesis was reported to be variable in different animals, which was recorded as 70 days in the Dwarf Nigerian lamb (Aire, 1973), 120 days in Brown Swiss crossbred bulls (Raja and Rao, 1982).

The primitive germ cells, or spermatogenic from which all of the spermatozoa are ultimately derived, are located directly inside the basal lamina. They are spherical or cuboidal in shape, with a diameter of about 12 μm . They have a spherical nucleus with granular chromatin. They divide to maintain their own number and to provide the cells that differentiate into spermatocytes. Two main types of spermatogonia can be identified by special stains, namely, the A and the B spermatogonia. They differ in the intensity of cytoplasmic staining, and the A cells usually have one or two nucleoli at the inner surface of the nuclear envelope, whereas the B cells usually have a centrally located nucleolus (Copenhaver *et al.*, 1978). An A cell may divide either into two new stem cells or into two derivatives. The latter divide further before differentiating into spermatocytes. Electron micrographs show that the majority of divisions of the spermatogonia are incomplete regarding the cytoplasm, and that spermatogonia, spermatocytes and spermatids remain connected into spermatozoa is nearly finished. Only a small number of primitive type A spermatogonia have complete cytoplasmic divisions and thereby maintain a stem cell population of spermatogonia. The primary spermatocytes lie next to the spermatogonia on their inner side. They are large cells with a diameter of 17 to 19 μm and are formed from the inner most layer of the spermatogonia (Copenhaver *et al.*, 1978). The chromatin of the large vesicular nuclei has a variable appearance, depending on the stage of meiotic prophase. This stage of spermatogenesis is a lengthy one in which the critical events of genetic exchange between replicated strands of DNA occur prior to reduction in genetic content which is characteristic of germ cell development (Copenhaver *et al.*, 1978).

The primary spermatocytes were uniformly rounded cells with spherical nuclei. The chromatin substance was organized to form dense filamentous network which filled the nucleus completely. The nuclei were distinct spheroid shaped (Kamal and Devi, 2012).

Each primary spermatocyte gives rise to two smaller secondary spermatocytes that lie internal to the primary spermatocytes. Almost as soon as it is formed, each secondary spermatocyte divides to form two spermatids. The progeny of these spermatocyte divisions remain interconnected by cytoplasmic bridges resulting in clusters of 16 or more interconnected spermatids. The clusters of developing spermatids may be associated with more than one Sertoli cell. The spermatids adjoin the lumen of the tubule. They are about 9 μm in size. They undergo no further division but, by profound changes in their structure, they become directly transformed into the mature spermatozoa (Copenhaver *et al.*, 1978).

As the spermatids mature their flagellated tails extend out into the lumen of the tubule. When they are released from the Sertoli cell to become free in the lumen, they are called spermatozoa. Actually, free spermatozoa in the lumen of the tubule are relatively rare, for as soon as they are detached from the Sertoli cells they pass into the epididymis for storage and a variable amount of additional maturation. In animals with periodic rutting seasons, transverse sections through several seminiferous tubules during the rutting season show all stages of spermatogenesis. During the long intermissions between breeding seasons, the tubules revert to a prepubertal condition and contain only spermatogonia and Sertoli cells (Copenhaver *et al.*, 1978).

The spermatozoa are slender, motile, flagellate bodies having a total length of 55 to 65 μm . the mature spermatozoan consists of a head and a tail. The tail is composed of the middle piece, principal piece and end piece. The junction between the head and tail is known as the neck (Copenhaver *et al.*, 1978). Spermatozoa vary in length between approximately 60 μm (boar, stallion) to 75 μm (ruminants). With the light microscope, the spermatozoan appears to consist essentially of 2 portions- the head and the tail. With the electron microscope, the tail is seen to subdivide further into neck, middle piece and principal piece and end piece (Dellmann, 1992).

The head of the spermatozoan is a flattened, oval body with a length of 4 to 5 μm . The anterior portion is thinner than the posterior so that in profile it is pear-shaped. It consists chiefly of a nucleus, anterior portion of which is covered by the acrosomal cap, and the entire cell is covered by the cell membrane or plasmalemma (Copenhaver *et al.*, 1978). The shape of the nucleus determines the shape of the head of the spermatozoan.

At the caudal surface of the sperm head, the nuclear envelope lines an implantation groove where the tail is inserted in a joint-like manner (Dellmann, 1992).

The neck is a relatively short and narrow structure between the head and middle piece (connecting piece). It consists of a centrally located centriole and peripheral coarse fibers (Dellmann, 1992). The proximal centriole is located against the basal end of the nucleus at an angle of about 45° to the axis of the tail. The peripheral coarse fibers are continuous with the fibers of the middle piece (Copenhaver *et al.*, 1978).

The middle piece is about 5 to 9 µm in length and about 1 µm in width. It has a core with the typical structure of a flagellum. A sheath of longitudinally oriented mitochondria is a characteristic feature of the middle piece (Copenhaver *et al.*, 1978). A ring shaped thickening of the plasma membrane (annulus) of the middle piece marks the limit between middle piece and principal piece (Dellmann, 1992).

The principal piece is the longest portion of the tail, being 40 to 45 µm in length. It lacks the mitochondrial sheath of the middle piece (Copenhaver *et al.*, 1978). Semicircular ribs of structural proteins in a helicoidal arrangement fuse to two of the outer fibers to form the characteristic peripheral fibrous sheath of the principal piece (Dellmann, 1992).

The tail piece is relatively short and slender, being about 5 to 10 µm in length. It has the typical appearance of a flagellum, with two single microtubules surrounded by nine doubles (Copenhaver *et al.*, 1978). The termination of the fibrous sheath marks the beginning of the end piece. Distally, the peripheral doublets gradually become reduced to single and terminate at various levels (Dellmann, 1992).

Hafez (2000) stated that spermatogonia lie in the outermost region of the tubule; round nuclei appear as an irregular layer within surrounding connective tissue. Nuclei are small size and dark stain due to presence of large numbers of chromatin granules. Primary spermatocytes located just inside an irregular layer of spermatogonia and Sertoli cells; nuclei are larger than those of the spermatogonia and stain lighter. Secondary spermatocytes are not seen in the average tubule owing to the short duration of these stages. Spermatids lie internally to primary spermatocytes. Spermatozoa lie along the border of the lumen.

2.3.10 Spermatogenesis

Initiation of spermatogenesis starts in 4-month old kids. Luminization process is completed by 6 months of age with all the seminiferous tubules having well-developed lumina at this age. These seminiferous tubules contain all the spermatogenic cells of the adult testis. Onset of puberty is observed to be established at 6 months of age as evidenced by presence of spermatozoa adhering to the adluminal border of the Sertoli cells as well as in the tubular lumen (Kamal and Devi, 2012).

Spermatogenesis is the process of proliferation and differentiation of male germ cells to produce fertile spermatozoa. Spermatogenesis occurs in the seminiferous epithelium and consists of three phases. In the proliferative phase, a spermatogenic secondary cycle (SSC) starts to divide while others remain inactive as reserve. After more mitotic divisions, the daughters become A type spermatogonia which differentiate into A1-spermatogonia. Several divisions occur, dependent on the species, to produce more A1-spermatogonia, followed by intermediate-spermatogonia and ending with in B type spermatogonia. B type spermatogonia then divide to form preleptotene spermatocytes. The diploid primary spermatocytes pass through the Sertoli cell tight junctions to develop in the adluminal compartment and away from the body's immune system to undergo meiosis (de Rooij and Russell, 2000).

Primary spermatocytes develop into haploid spermatids through meiosis. Preleptotene spermatocytes become leptotene, zygotene and pachytene primary spermatocytes after DNA synthesis. The first meiotic division results in the formation of two secondary spermatocytes which undergo the second meiotic division fairly quickly to form four haploid round spermatids. The next step is spermiogenesis when the round spermatids give rise to elongated spermatids. There are several stages in spermiogenesis including acrosome appearance, nucleus condensation, cell elongation, flagellum formation and finally cytoplasmic resorption and spermiation (Russell *et al.*, 1990; de Rooij and Russell, 2000).

Copenhaver *et al.* (1978) stated that the nucleus of each spermatogonium, the stem cell from which all sperms are eventually derived, contains the somatic or diploid number of chromosomes. This number in man is 46, consisting of 23 pairs; one member of

each pair is of maternal and the other of paternal origin. After a number of ordinary mitotic divisions, by which each daughter cell receives the diploid number of chromosomes, the last generation of spermatogonia enters an intermitotic period characterized by growth and by nuclear change leading to differentiation of the cells to primary spermatocytes.

At the close of the growth period of the primary spermatocyte, long thread-like chromosomes can be seen in the nucleus that seem to be like those present in early prophase stages of spermatogonia. As the threads shorten and thicken, however, it can be seen that they are not single as in mitotic prophase, but double as the result of synapsis (pairing) of homologous chromosomes. Instead of 46 single chromosomes, there are 23 double threads. Next, each member of the pair, having replicated DNA prior to prophase, partially divides and a tetrad is formed. The tetrads have individual differences in size and form by which they may be identified. Even after they thicken and go on to the spindle, they retain individual characteristics (Copenhaver *et al.*, 1978). In the first maturation division, the chromosomes that paired during synapsis become separated, one member from each pair going to each of the resulting two secondary spermatocytes. This division differs from ordinary mitosis in that it does not consist of the longitudinal splitting of individual chromosomes after DNA replication, but whole, previously paired chromosomes are separated from each other, the daughter cells actually receiving only half the somatic number. It is a reductional or heterotypic division, in contrast to the ordinary equational or homeotypic division (Copenhaver *et al.*, 1978). It is the first nuclear division in the process known as meiosis, in contrast to mitosis. Each tetrad separates into two dyads, each dyad representing an original single chromosome whose DNA was replicated precociously for the second maturation division. The result of meiosis is the production of germ cells with a haploid number of chromosomes. Thus the maturing spermatids have a different genetic composition from typical somatic diploid cells (Copenhaver *et al.*, 1978).

The second meiotic division results in the haploid content of DNA in each definitive spermatid. The members of one pair of chromosomes (the sex chromosomes) are dissimilar in the male. One is known as the X chromosome and is of maternal origin. The other is the Y chromosome, of paternal origin. This differs from the condition in

the female, in which each ovum contains an X chromosome. Consequently, when a sperm carrying an X chromosome fertilizes an ovum, an XX combination is established and a female develops. On the other hand, when a sperm bearing a Y chromosome fertilizes an ovum, an XY combination is produced and the embryo develops as a male (Copenhaver *et al.*, 1978). The spermatids do not divide but mature to form the spermatozoa during which the spermatids are enveloped by the cytoplasmic processes of Sertoli cells, from which they apparently receive nourishment. Spermiogenesis produces a slender motile cell retaining only the essentials for fertilization and for transmitting hereditary material. It retains its nucleus, the golgi-derived acrosomal cap, a proximal centriole and mitochondria; the remainder of the cytoplasm is extruded (Copenhaver *et al.*, 1978).

2.4 Accessory genital glands

The accessory glands of the male sheep reproductive system correspond to ampulla, vesicular glands, bulbourethral or Cowper's glands and prostate. Their secretion contributes for the most ejaculate volume (Campos, 2003).

The contribution of each gland to sperm volume varies among species, due to differences in gland size and even absence of one or more of them, in some species (Gonzalez, 2002).

Noakes *et al.* (2001) noted that the accessory sex glands include the ampullae, prostate, vesicular glands and bulbourethral (Cowper's) glands. There is much variation between the anatomy of the accessory glands between species which is summarized Table 2.4.

Table 2.4 Accessory sex glands of the main domestic species

Species	Ampulla	Prostate	Vesicular gland	Bulbourethral gland
Cat		++		++
Dog	(+)	+++		
Horse	++	++	++	+

Cattle	(+)	++	+++	+
Sheep	(+)	++	+++	+
Pig		+	++	+++

The relative size and importance are indicated by the number of + symbols. Ampullary glands are present in species marked (+) but are not anatomically prominent.

Noakes *et al.* (2001) noted that the vesicular glands are prominent in ruminants, the stallion and boar. They are sac like in the stallion and the boar, and are firm, lobulated structures in the ram and bull. The glands are adjacent to the neck of the bladder and lateral to the ampullae. They open into the urethra just distally to the vasa deferentia. Their secretion, which is generally watery, contributes substantially to the volume of the semen.

2.5 Gross parameters of vesicular gland

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the glands were situated on the dorsolateral aspect of the neck of the urinary bladder and initial part of the pelvic urethra lateral to the ampulla of the vas differens. In one day old kids, the vesicular gland was small but glandular in appearance. Lobulation were indistinct. A small white cord like structure appeared at the point of union of ampulla with the urethra connecting both sides of gland. With the increase in age, the size of gland increased and lobulations on the surface became very much distinct. At six months of age it acquired the shape of sign of interrogation (?) and at 12 months of age; became 'S' shaped. The average weight of the vesicular gland in the neonatal Gaddi goats was 0.23gm on left side and 0.25gm on the right side which became 3.80gm and 3.82gm at 12 months age, thereby indicating a 25× increase in weight in the later part of prepuberty. The overall mean weight of the vesicular gland in prepubertal animals was 2.35 ± 0.26 gm. The weight of the gland grew at a very fast rate in the prepubertal animals and only slowly in the pubertal and post pubertal animals. The mean weight of the gland in pubertal and post pubertal animal was 3.05 ± 0.17 gm and 3.47 ± 0.14 gm, respectively. The average length, width and thickness of the right vesicular gland in one day old goats were 0.96, 0.59 and 0.53cm whereas in the left gland measured 1.10, 0.71

and 0.54cm, respectively. It grew for 2-3 times in all its dimension in the late prepubertal age. The overall mean length, width and thickness of the prepubertal gland was $2.44 \times 1.36 \times 1.06 \text{ cm}^3$. These parameters were $2.71 \times 1.62 \times 1.31 \text{ cm}^3$ and $2.97 \times 2.00 \times 1.17 \text{ cm}^3$ in pubertal and post pubertal goats, respectively. Thus indicating only approximately 50% increase in weight and size of the gland after the final attainment of maturity.

Gofur (2015) stated that the vesicular glands of buck situated on the caudodorsal aspect (near to the neck) of the bladder and the initial part of the pelvic urethra, lateral to the ampullae of the ductus deferens. The left vesicular gland situated 2-3 cm caudal than the right one. The vesicular glands grossly lobulated, irregular and more or less solid.

Getty (1975) stated in stallion, the seminal vesicles are two elongated and somewhat piriform sacs, which lie on each side of the caudal part of the dorsal surface of the bladder. They are partly enclosed in the genital fold and are related to the rectum dorsally. Their long axes are parallel with the ductus deferens and converge caudally. Each consists of a rounded blind end, the fundus; a middle, slightly narrower part, the body and a caudal constricted part, the neck or duct.

In the stallion, they are about 15-20cm long and their greatest diameter is about 5 cm. The vesicles chiefly retroperitoneal, but the fundus extends forward into the genital fold and hence has a serous covering. The excretory duct dips under the prostate and opens in common with or alongside of the ductus deferens in a pouch of the mucous membrane on the side of the seminal coliculus.

Getty (1975) stated in bull, the vesicular glands are not bladder like sac as in the horse, but are compact glandular organs with a lobulated surface. In the adult they measure about 10 to 12 cm in length, 5 cm in width in their largest part, and 3 cm in thickness. The dorsal surface of each faces dorsally and medially and is partially covered with peritoneum. The ventral surface faces in the opposite direction and is non-peritoneal. Each may be regarded as as consisting of a very thick walled, sacculated tube, bent on itself in a tortuous manner, this tube, if straightened out, would be about 25 cm in length. They are commonly unsymmetrical in size and shape. Short branches are often

given off from the chief tube. The excretory duct opens at the seminal colliculus just lateral to the ductus deferens.

Copenhaver *et al.* (1978) stated that the seminal vesicle are a pair of diverging secular structures about 5 cm in length situated behind the neck of the bladder and between the ampullae of the ductus deferens above and the prostate below. Their lower ends taper to a narrow straight duct that opens into the ampullae of the ductus deferens where these continue as the ejaculatory ducts.

Ghonimi *et al.* (2014) reported in buffalo bulls, the seminal gland was laterally attached to the terminal part of ductus deferens, where the excretory ducts of seminal glands opened on the dorsal aspect of pelvic urethra near the neck of the urinary bladder. The seminal gland was elongated, straight, tube-like structure, large in size, hard, tough and firm in consistency, pale –yellow in color, multilobulated and large lobules on the outer surface similar to grape –like appearance.

2.6 Histological parameters of vesicular glands

2.6.1 Capsule and interlobular septa

Gupta and Singh (1982) stated that the vesicular gland of goat was enclosed in a capsule of loose, irregularly arranged collagenous, reticular and smooth muscle fibers. The elastic connective tissue fibers were rare. Autonomic ganglia of variable sizes were often enmassed in the loose meshes of connective tissue fibers. The interlobular septa of variable thickness given off from the capsule divided the gland into numerous lobules.

Ghonimi *et al.* (2014) reported in buffalo bulls, the mature seminal gland was characterized by a stroma of well developed fibromuscular capsule and thick trabeculae plasmic projection. The capsule was mainly formed of smooth muscle bundles with inter-muscular irregular collagenous connective tissue. Thick well-developed fibromuscular trabeculae divided the vesicular glands into lobes of varying shapes, numbers and sizes. The secretory end pieces and ducts were surrounded with a highly vascularised connective tissue.

2.6.2 Tunica muscularis

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the tunica muscularis surrounded each lobule of the vesicular gland. It was composed of a thick layer of interwoven smooth muscle fibers. In one day old kids inner circular and outer longitudinal or oblique layers were distinct and the same pattern was observed in prepubertal goats. The average thickness of muscular layer in one day old kids was 29.40 μ m and its thickness increased with the advancement of age. In prepubertal goats its average thickness measured 78.20 μ m.

Sudhakar *et al.* (1986) stated that the vesicular gland of buffalo, the tunica muscularis was composed of mainly smooth muscle fibers and surrounded each lobules of the gland. Up to 2 years of age the muscle fibers were arranged in inner circular and outer longitudinal layers, but from 4 years onward only circular muscle fibers were seen. The layer was uniformly thick in all the 3 regions of the gland up to 2 years, but from 4 years onwards its thickness increased from cranial to caudal regions.

Marettova and Legath (2010) stated in bull vesicular gland, the muscular coat consists of an inner layer of circularly arrangement smooth muscle cells and an outer layer in which the smooth muscles cells have a longitudinal orientation. External to the muscle coat adventitia, consisting of loose connective tissue. The highly vascularised loose connective tissue of the lamina propria-submucosa is continuous with the dense connective tissue trabeculae. The interlobular septa are predominantly muscular, derived from the thick tunica muscularis. The capsule contains a few smooth muscle cells. Groups of smooth muscle cells were observed to arise from muscle layer and enter the mucosa layer. The trabeculae of the lamina propria of different thickness contain bands of smooth muscle cells which were seen to be localized centrally. Single smooth muscle cells forming incomplete layer were observed beneath the secretory epithelium. Numerous small blood vessels rich in smooth muscle cells were present in mucosa layer.

Elastic fibers in the vesicular gland were present within all layers-capsule, the muscular layer, submucosa and mucosa. Many elastic fibers occur in the muscular layer and were distributed among bands of the smooth muscle cells. In the submucosa and in the

mucosa layer, elastic fibers were present throughout the area of the lamina propria, forming thick and thin trabeculae. In the connective tissue of mucosa, elastic fibers were seen to be distributed regularly among the muscle cells and collagen fibers. An accumulation of elastic fibers occurred next to secretory alveolus to form thick elastic membranes beneath the secretory epithelium.

2.6.3 Tunica adventitia

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the tunica adventitia was comprised of loosely arranged meshwork of collagen, reticulin and elastin fibers. Many blood vessels, nerve fibers and autonomic ganglion were seen in the tunica adventitia. It measured 35.28 μm in thickness in one day old kids. In pre-pubertal animals its average thickness was 65.36 μm .

Sudhakar *et al.* (1986) stated that the vesicular gland of buffalo, the tunica adventitia consisted of loose meshwork of collagenous, reticular and elastic fibers. It also showed many blood vessels, nerves and autonomic ganglion.

2.6.4 Interstitial tissue

Gupta and Singh (1982) stated that the interstitial tissue was fibromuscular and filled the spaces in between the glandular alveoli. It contained collagenous and reticular fibers. The elastic connective tissue fibers were absent. The smooth muscle cells were generally condensed around the glandular alveoli as thin sheath of 1-2 layer thickness. The average thickness of the interstitial tissue was $16.3 \pm 0.8 \mu\text{m}$ in control glands.

2.6.5 Glandular end pieces

Ghonimi *et al.* (2014) reported in buffalo bulls, the parenchyma was formed of numerous secretory end pieces and secretory ducts. The secretory end pieces were of compound tubular and tubular-alveolar type with irregular shape and different size. Secretory end pieces were collected together in cluster where the alveoli in the gland apex were large in size and number. The secretory end pieces were lined by secretory epithelium of the simple columnar type with individual small basal cells arranged on the basement membrane among the attached basal parts of the columnar cells. Highly

eosinophilic vacuolated secretion was found to fill a acinar lumina giving a foamy appearance of luminal secretions. These vacuoles were observed in the cytoplasm of columnar and basal cells but were more abundant of the basal cells of mature seminal gland epithelium. The columnar cells were of secretory type. The free surface of these cells was projected into the lumen of secretory end pieces as an elongated or spheroidal cytoplasmic projection. Some of these cells had light, bleb-like apical projections. These projections were acidophilic had inverted pear shape-like structures. The cytoplasm of columnar cells was acidophilic and vacuolated. The basal cells were flat, trigonal, ovoid or lens-shaped cells, with ill-defined cell boundaries. These cells rested on the basement membrane and arranged individually, scattered in between secretory columnar cells, distributed irregularly and never reach luminal surface. The cytoplasm of the basal cells was lightly acidophilic, with many vacuoles of variable size that occupied mostly of the infranuclear position of the cells. Moreover, the cytoplasm was free from any secretory granules.

Dellmann (1992) stated that the paired vesicular gland is compound tubular or tubuloalveolar gland. The glandular epithelium is pseudostratified with tall columnar cells and small, spherical, often sparse basal cells. The intralobular and main secretory ducts are lined by a similar cuboidal epithelium, or by stratified columnar epithelium in the horse.

The highly vascularized loose connective tissue of the propria submucosa is continuous with the dense connective tissue trabeculae, which may subdivide the organ into lobes and lobules. A tunica muscularis of varying thickness surrounds the organ, followed by a tunica serosa or tunica adventitia.

In the stallion, the vesicular glands are true vesicles, with wide central lumina (ducts) into which open the short branched tubule-alveolar glands, separated by relatively thin connective tissue trabeculae with irregularly arranged smooth muscle cells.

In the bull, the vesicular gland is a compact, lobulated organ. Intralobular secretory ducts drain the slightly coiled tubular portions of the tubule-alveolar gland and in turn are drained by the main secretory duct. The secretory columnar cells have small lipid droplets and glycogen and give a positive alkaline phosphatase reaction. Some of the

columnar cells possess light, bleb like apical projections. The basal cells are characterized by large lipid droplets. The intralobular septa are predominantly muscular, derived from the thick tunica muscularis which is surrounded by a capsule of dense irregular connective tissue with a few smooth muscle cells.

The vesicular gland of the ram and buck is similar to that of the bull. Lipid droplets of the basal cells are absent in the ram but may be present in the buck. The epithelium of the vesicular gland of the buck is considerably higher during the breeding season than non breeding season.

The gelatinous, white or yellowish white secretory product of the vesicular gland amounts to about 25 to 30% of the total ejaculated volume in the bull, 10-30 % in the boar and 7-8% in the ram and buck. It is rich in fructose, which serves as an energy source for ejaculated spermatozoa.

Copenhaver *et al.* (1978) stated that the wall of the vesicles is composed of a connective tissue capsule with an underlying layer of smooth muscle that is thinner than that of the ductus deferens. Also stated that it is coiled or convoluted tube with numerous diverticula along its length that result in a lumen of labyrinthine complexity. In sections, the mucosa forms thin folds from which secondary and tertiary folds project into the lumen, bounding narrow recesses that may give a spurious appearance of being cross sections of epithelial lined tubules. However, all of these recesses open into a wider central portion of the lumen. The pseudostratified epithelium consists of low columnar or cuboidal cells with smaller numbers of rounded cells between their bases. The surface of the epithelium bears sparse microvilli and some of the cells have single flagellum projecting into the lumen from a pair of centrioles in their apical cytoplasm. The cells contain numerous slender mitochondria, a well developed granular cytoplasmic reticulum and abundant secretory granules. Small lipid droplets and aggregations of lipochrome pigment are commonly found in the basal cytoplasm. The pigment first appears at puberty and in older adults may be sufficiently abundant to give the interior of the vesicles a brownish color in the fresh state.

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the gland was compound tubule-alveolar gland. Each lobule of the gland showed folded mucosa

comprising of lamina epithelialis and lamina propria, tunica muscularis and tunica adventitia in one day old kids. The central collecting sinus of each lobule was lined with pseudo stratified columnar epithelium. Three types of cells have been identified, A, B and C cells. A type cells were tall columnar bottle like in shape with distinct cell boundaries. In one day old kids such cells measured $20.43\mu\text{m}$ in height and $4.54\mu\text{m}$ in width. Their vacuolated cytoplasm was finely granular and slightly basophilic in nature in the apical region. The nuclei were ovoid and their average height and width measured $5.68\mu\text{m}$ and $4.54\mu\text{m}$, respectively. In the late prepubertal goats 12months to less than 18 months theses cells contained eosinophilic cytoplasm which stained relatively more darkly in apical region. B type cells were spherical in shape and placed basally. The outlines of these cells were not distinct and their cytoplasm is clear. The centrally placed nuclei were either round or oval. The round nuclei were smaller than those of A cells and measured $4.34\mu\text{m}$ in diameters. C type cells were narrow columnar cells with darkly stained cytoplasm and hyperchromic nuclei. These cells were fewer in number as compared to A and B type cells. The number of C type cells was relatively fewer in pubertal animals which further decreased in the post pubertal animals.

The glandular unit or end pieces were luminated or non-luminated. In day old kids, the average diameters of the luminated glandular end pieces was $67.07\mu\text{m}$ while the non-luminated end pieces glandular end pieces measured $51.53\mu\text{m}$. The number of glands increased per unit area in prepubertal goats. At this stage, the average diameters of luminated and non-luminated end pieces were $124.91\mu\text{m}$ and $111.24\mu\text{m}$, respectively. In pubertal and post pubertal animals, the luminated acini was $193.72\pm 3.41\mu\text{m}$ and $193.84\pm 2.86\mu\text{m}$, respectively and the non-luminated acini was $169.59\pm 3.54\mu\text{m}$ and $166.96\pm 2.47\mu\text{m}$, respectively.

The glandular end pieces of the vesicular glands of Gaddi goats were lined by pseudostratified columnar epithelium. Occasionally, simple cuboidal or simple columnar was also encountered particularly when the alveoli was dilated. Similar to the epithelial lining of the mucosa. The size of the gland increased with age significantly. The secretory end pieces exhibited apocrine mode of secretion. The glands were surrounded by a connective tissue layer which contained collagen and reticular fibers in prepubertal, pubertal and post pubertal animals.

Sudhakar *et al.* (1986) stated that the vesicular gland of buffalo was a lobulated and compound tubule-alveolar gland. Each lobule consisted of tunica mucosa, tunica muscularis and tunica adventitia. The tunica mucosa was folded and consisted of a lamina propria covered with lamina epithelialis. The latter consisted of a pseudostratified columnar epithelium containing A, B and C types of cells. The A cells were of tall columnar type containing faintly eosinophilic cytoplasm with distinct cell boundaries. The B cells were placed at the basal lamina in between A cells. They had indistinct cell boundaries and round or oval nuclei. The C cells were tall columnar cells interpressed between the A cells but had contracted and elongated nuclei. C cells were numerous in the animals up to 2 years of age. From years onwards they rarely existed. Their epithelial height corresponded to those of A cells. Since the frequency of C cells was more in young animals and very less in adult buffaloes, it may be concluded that they represented an intermediate stage in the cycle undergone by the epithelial cells. The B cells might be the reserve cells.

In buffaloes 8 and 10 years old, the secretory end pieces were closely packed in the cranial part of the gland but loosely in the middle and caudal regions. The solid end pieces which were found at all stages of postnatal life of buffalo might be regarded as the reserve end-pieces. It might become active at the time of need. The alveoli, tubules and ducts were lined with epithelium similar to that found in the tunica mucosa. The alveolar diameter increased gradually with age and revealed apocrine mode of secretion.

Gupta and Singh (1982) stated that the glandular alveoli were of various shapes, measured $103.2 \pm 6.5 \mu\text{m}$ in mean diameters in goats. The alveoli of all sizes were luminized and lined with pseudostatified columnar epithelium of $22.5 \pm 0.6 \mu\text{m}$ of mean height. The tall columnar cells occupied the whole thickness of the epithelium. Their dark stained nuclei were placed in basal half of the cells. The free luminal surface of the surface of the cells was fringed with secretory streamers and appeared as provided with brush border. The basal cells of the pseudostratified epithelium were round or polyhedral in shape. Their centrally placed nuclei were spheroidal and dark stained.

Hib (2003) reported the men seminal vesicle is simple tubular gland, long which flows between the boundary of the ampulla of the ductus deferens and ejaculatory duct. The

glandular mucosa has a pseudostratified epithelium with high non-ciliated columnar cells, basal and oval cells. The lamina propria is surrounded by two layers of smooth muscle cells, an inner circular and an outer longitudinal one. Also the muscle layers are surrounded by loose connective tissue, which is rich in elastic fibers that constitute the adventitia.

2.6.6 Lamina propria

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the lamina propria comprised of loose areolar connective tissue containing secretory end pieces with varying number of alveoli. In one day old kids, the glands were only a few in numbers per unit area and the inter glandular connective tissue was preponderant. With the advancement of age, the number of alveoli per unit area increased and the amount of inter glandular connective tissue stroma was reduced. The fibro structural design comprised of the circularly arranged collagenous fibrous bundles with reticular fibers. The elastic fibers also occurred in the intralobular connective tissue. With the advancement of age, the collagenous and reticular fibers became coarser. Irregularly distributed elastic fibers were also recorded in the pubertal and post pubertal animals.

Sudhakar *et al.* (1986) stated that the vesicular gland of buffalo, the lamina propria consisted of a scant felt work of loose connective tissue enclosing alveoli, tubules, ducts and some solid end pieces. The muscle rich connective tissue septa divided the gland into smaller lobules indistinctly up to 2 years and clearly at 4 years and above. The septa thickened gradually from birth to 6 years and reduced later. The intralobular connective tissue which contained fibroblasts, smooth muscle, collagenous, reticular and elastic fibers and blood vessels decreased progressively as the age advanced.

2.6.7 Ducts

Archana *et al.* (2009) reported on the vesicular glands of Gaddi goats, the central collecting sinus of each lobule united to form the interlobular duct. The union of several such ducts, in turn formed the main excretory duct of the vesicular gland. The interlobular and main excretory ducts contained folded mucosa which was lined by pseudostratified columnar epithelium in one day old kids. In goats of later stages of

pre-pubertal age, the epithelium was composed of three types of cells, surface columnar cells, wedging plumpy/ cylindrical cells and basal cells.

Ghonimi *et al.* (2014) reported in buffalo bulls, the lumen of the intralobular ducts appeared highly evaginated and invaginated forming folds like structure similar to tongue in shape and lined by tall columnar cells with individual basal cells and acidophilic cytoplasm. The nuclei were spherical, central, lightly stained which lined with simple columnar cells with individual basal cells.

2.7 Gross parameters of prostate gland

Archana *et al.* (2012) reported on prostate gland of Gaddi goats mostly comprised of pars disseminata, but in some goats the existence of corpus prostate was also confirmed. In one day old Gaddi kids, a white colored band of tissue was observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. The pars disseminata of the gland occupied the entire wall of the pelvic urethra. The gland occurred in the urethra from its junction with the urinary bladder to the level of the ischial arch. It was surrounded by the urethral muscle which was thin dorsally, increased in thickness laterally and became thin ventrally. It was relatively sparse in the lateral and ventral walls of the pelvic urethra. In the middle of the pelvic urethra, the distribution of the gland was almost uniform encircled the whole pelvic urethra except in 1-2% of the very small ventral part. In the caudal segments of the pelvic urethra, the distribution of glandular elements was limited to the half of the pelvic urethra. In the pubertal and post pubertal animals, the corpus prostate was frequently observed in various samples as a distinct white color band below the cord joining the two vesicular glands. In postpubertal animals, it was approximately 0.56cm in height.

Gofur (2015) noted that the prostate gland is unpaired accessory reproductive glands in bucks and its consists only pars disseminates in goats which surrounds the pelvic urethra and corpus prostate absent in bucks.

Getty (1975) stated in stallion, the prostate is a lobulated gland which lies on the neck of the urinary bladder and the beginning of the urethra, ventral to the rectum. It consists of two lateral lobes and a connecting isthmus. The lateral lobes, right and left are somewhat prismatic in form and are directed cranially, laterally and somewhat dorsally. The deep surface of each lobe is concave and partly embraces the corresponding seminal vesicle. The dorsal surface is concave and is in relation with the rectum. The ventral surface is convex and lies on the obturatorious internus muscle and fat. The apex is pointed and lies near the caudal end of the ischiatic spine. The isthmus is a thin, transverse band, about 2 cm wide. It lies over the junction of the bladder with the urethra, the masculine uterus, and the terminal parts of the ductus deferens and the ducts of the seminal vesicles. Dorsally it is partly covered by the fibers of the urethralis muscle. A pars disseminata is absent.

Getty (1975) stated that the prostate is pale yellow in color and consists of two parts in the bull; they are, however, continuous with each other. The body is a small mass which stretches across the dorsal surface of the neck of the bladder and the origin of the urethra. It measures about 3.5 to 4.0 cm transversely and about 1.0 to 1.5 cm in width and thickness. The small ruminant has only pars disseminata, which completely surrounds the urethra in the male goat; in the ram the ventral side is free. The pars disseminata of the bull surrounds the pelvic part of the urethra; dorsally it forms a layer about 10-12 mm thick, but ventrally it is quite thin.

Nissar and Suri (2012) reported on Bakerwali (Kaghani) goat, the prostate gland was comprised of only disseminate part of prostate gland whereas corpus prostate was absent. The mean length of pars disseminata in pelvic urethra was 4.79 ± 0.14 cm. Width at proximal, middle and distal region was 1.41 ± 0.07 cm, 1.34 ± 0.05 cm and 1.42 ± 0.04 cm, respectively. Thickness was 1.15 ± 0.05 cm at proximal, 1.17 ± 0.05 cm at middle and 1.20 ± 0.05 cm at distal region, respectively. The weight and volume of pars disseminata in pelvic urethra was 8.11 ± 0.64 gm and 8.87 ± 0.48 cc, respectively.

2.8 Histological parameters of prostate gland

2.8.1 Capsule and interstitial tissue

Archana *et al.* (2012) reported on corpus prostate gland of one day old Gaddi goats was covered by a 63.14 μm thick fibrous capsule. A number of trabeculae approximately 6.58 μm thick arose from the capsule and divide the glandular tissue into lobes and lobules. The thickness of interlobular tissue and intralobular connective tissue measured 9.08 μm and 2.95 μm , respectively. The capsule comprised of collagen and reticular connective tissue fibers and smooth muscles. In one day old kids, mild to rich collagen concentration was observed in the capsular and interlobular connective tissue. The collagen also occurred in and extended along the wall of blood vessels. Reticular fibers were seen only sparsely in the stroma. The capsular thickness and interlobular connective tissue decreased with increased in age. The average capsular thickness in prepubertal animals measured 47.60 μm ; the interlobular and intralobular connective tissue thickness measured 7.72 μm and 2.27 μm , respectively.

The placement of corpus prostate in pubertal and post pubertal Gaddi goat was same as observed in the prepubertal goats. It was well separated and demarcated from the pars disseminata of the prostate by means of fibrous capsule. The thickness of capsule decreased in pubertal and further in post pubertal group however, the amount of connective tissue in the trabeculae containing smooth muscle, collagenic and reticular fibers increased. The interlobular connective tissue also reduced with the advancement in age from pubertal to post pubertal animals in the thickness in the pubertal animals. In post pubertal animals, the interlobular and intralobular connective tissue was sparse and scattered. The average capsular thickness in pubertal and post pubertal animals measured 35.66 μm and 25.87 μm , respectively.

Dellman (1992) stated that the prostate gland is surrounded by a capsule of dense irregular connective tissue that contains many smooth muscle cells around the internal portion which is also surrounded by the striated urethral muscle. Large trabeculae originate from the capsule and separate the external and internal portions into individual lobules. They are predominantly muscular in the external portion of the gland.

Copenhaver *et al.* (1978) stated that the prostate is the largest of the accessory glands of the male reproductive tract. Its secretion together with that of the seminal vesicles contributes to the volume of the ejaculate. The gland is about the size of a horse chestnut and surrounds the urethra at its origin from the urinary bladder. Dense connective tissue forms a thin capsule around the gland and extends into it as its stroma. Both capsule and stroma contain smooth muscle as well as fibroblasts and collagen. Septa of this fibromuscular stroma radiate from the colliculus seminalis towards the capsule portioning the parenchyma into more or less distinct lobes.

Sudhakar *et al.* (1985) reported on the Murrah buffalo bulls of post natal age to the 10 years of old, the pars disseminata was enclosed by a connective tissue capsule composed of collagenous, elastic, reticular and smooth muscle fibers. The capsule was surrounded by the skeletal muscle (M. urethralis). The thickness of the capsule increased constantly from birth to 10 years of age. In the buffalo, the urethralis muscle was surrounded by loose connective tissue which was rich in blood vessels and collagenic and reticular fibers.

The interlobular connective tissue trabeculae descended from the capsule of the gland dividing it into smaller lobules. These trabeculae were not compact and were indistinct in the young animals (up to 2 years of age), but were clearly seen in the adult buffaloes. The young animals had abundant connective tissue consisting of loose areolar connective tissue rich in blood vessels. The thickness of the interlobular connective tissue got reduced with the advancing age.

Trotter (1959) and Kainer *et al.* (1969) also reported that the presence of the capsule and the urethralis muscle in the pars disseminata of the bull.

Trautmann and Fiebiger (1957) described abundant of blood vessels in the connective tissue surrounding the urethralis muscle of bull.

Roy *et al.* (1985) reported that the prostate gland was encapsulated in fibromuscular capsule under the cover of urethralis muscle.

Chandrapal and Bharadwaj (1984) reported that in the prepubertal buffalo calves the capsule and interlobular septae arising from it, contained fine collagenous and reticular

fibers with smooth muscle cells. In prepubertal group, the capsule was relatively thicker and had numerous coarse collagenous and reticular fibers with few elastic fibers. In the post pubertal buffalo bulls, the capsule contained abundant smooth muscle fibers. The interlobular septae in pubertal and post pubertal animals become relatively thinner. Scattered striated muscle fibers were observed in some pubertal and post pubertal buffalo bulls.

2.8.2 Corpus prostate and pars disseminata

Noakes *et al.* (2001) noted that the prostate is intimately related to the pelvic urethra and in most species, is in two parts; its body surrounds the neck of the bladder and its disseminated part spreads around the pelvic urethra into which it has several openings. In the dog, the prostate is the main accessory gland and is relatively large, forming a discrete organ around the urethra. The prostatic secretion is watery and in the dog, contains large quantities of chloride ion, but citrate, fructose and inositol are not present in high concentrations.

Dellman (1992) stated that the prostate consists of varying number of individual tubuloalveolar glands derived from the epithelium of the pelvic urethra. Two portions may be distinguished, according more to topographic than to histologic features: the compact or external portion (*Corpus prostatae*) and the disseminate or internal portion (*Par disseminata prostatae*). The external portion either entirely surrounds part of the pelvic urethra at the level of the colliculus seminalis or covers part of its dorsal aspect. The disseminate portion is located in the propria submucosa of the pelvic urethra.

Dellman (1992) stated that in the carnivores, the external portion of the prostate gland is particularly well developed and separated into two distinct bilateral lobes. In the dog, these lobes completely surround the proximal portion of the pelvic urethra. In the cat, they are located on the lateral and dorsal aspects of the urethra. The internal portion of the dog consists of a few glandular lobules. In the cat, individual lobules of the internal portion are found scattered between the colliculus seminalis and the bulbourethral glands.

In the stallion, only the external portion of the prostate gland is present and consists of a right and left lobe, both connected by the narrow dorsal isthmus. The individual lobes

empty with 15 to 30 ducts into the pelvic urethra. The capsule, trabeculae and interstitial connective tissue are rich in smooth muscle.

The external portion of the prostate gland of the bull is relatively inconspicuous; it is absent in small ruminants. The particularly well developed internal portion encircles the urethra in the bull and buck; in the ram it is U shaped and the midline of the ventral aspect of the urethra is free of glandular tissue.

Sudhakar *et al.* (1985) reported on the Murrah buffalo bulls of post natal age to the 10 years of old, the pars disseminata occupied the wall of the whole pelvic urethra extending from body of the prostate to the ischial arch. In cranial portion of the pelvic urethra, it was mostly present in the dorsal wall; lateral and ventral walls had very sparse glandular distribution. In the middle and caudal portions of the pelvic urethra the distribution of the gland was comparatively more in the lateral and ventral walls than in the dorsal wall. The pars disseminata did not extend into the penile urethra.

The glandular components were loosely arranged in the young animals but were compact in the adult buffaloes. In the adult animals, the trabeculae were rich in collagenous and reticular fibers. The intralobular connective tissue was very much reduced in them due to the closely packed glandular end pieces, and at many places was formed of only thin reticular fibers laid along the basement membrane.

2.8.3 Glandular tissue

Archana *et al.* (2012) reported on corpus prostate gland of one day old Gaddi goats, the glandular tissue was represented by luminated as well as non-luminated secretory end pieces and ducts. The luminated secretory end pieces measured on an average 40.40 μm in diameters. They were lined by simple columnar to pyramidal shaped epithelium, which rested on distinct basement membrane. The mean height and width of the epithelial cells measured 13.62 and 4.54 μm , respectively. The cytoplasm of these cells was intensely eosinophilic. The centrally placed rounded nuclei measured on an average 4.42 μm in diameter. The average diameter of the non luminated secretory end pieces were 33.67 μm . The mean height and width of the lining simple columnar epithelial cells were 13.24 and 4.54 μm , respectively. The secretory end pieces increased in number in pubertal Gaddi goats as compared to prepubertal animals. The

luminated acini dominated over the non luminated acini. The average diameter of acini was 52.70 μm . These were lined by 15.20 μm high simple columnar epithelial cells. Their nuclei were either oval or rounded. The rounded nuclei were 3.98 μm in diameter and oval nuclei were 4.54 μm in height and 3.85 μm in width. The number of alveoli further increased in post pubertal animals, yet the alveolar diameter and epithelial height of alveoli did not change much over the preceding group of animals.

The luminated and non-luminated secretory end pieces of pars disseminata were distributed within the wall of the pelvic urethra. In one day old kids, the average diameters of luminated and non-luminated secretory end pieces were 43.69 μm and 30.19 μm , respectively. Both types of secretory end pieces were lined by single row of columnar or pyramidal shaped cells. The average height of the epithelium in both types of secretory end pieces was 12.93 μm . The average height and width of the nucleus in luminated end pieces measured 3.85 and 4.19 μm , respectively. The same parameters in non-luminated end pieces were 4.08 and 4.31 μm , respectively.

With the advancement of age, the number of luminated secretory end pieces increased over the non-luminated secretory end pieces. In prepubertal goats, the mean diameter of luminated secretory end pieces was 26.87 μm . Both types of secretory end pieces were lined by simple columnar or pseudostratified columnar epithelium. The glandular alveoli were mainly mucous type with only few serous ones placed in the peripheral part of the pars disseminata prostate in one day old kid. With the advancement of age, the numbers of serous acini increased. At 6 months of age, the alveoli of both serous and mucous type constitute the secretory parenchyma of the gland. Beyond 12 months of age, the serous alveoli were predominantly present in the glandular parenchyma.

Dellman (1992) stated that the secretory tubules, alveoli and intraglandular ducts of the prostate gland are lined by a simple cuboidal or columnar epithelium with occasional basal cells. The simple epithelium changes to stratified columnar or transitional epithelium towards the terminal portions of the ducts. Some of the epithelial cells give positive mucus reactions: the majority contains proteinaceous secretory granules. The tall columnar cell possesses microvilli and sometimes bleed like apical protrusions. Occasionally, concentrically luminated concretions of secretory material are found in the tubules and alveoli. The duct system of the prostate possesses secular dilatations in

which secretory material may be stored. Secretory portions and ducts of the prostate gland are surrounded by loose connective tissue containing smooth muscle cells which are particularly abundant in the external portion of the gland.

Dellman (1992) stated that the contribution of the prostatic secretions to the total volume of the ejaculate varies with the species. In ruminants, it is 4-6%, in the stallion 25-30% and in the boar 36-60%. One of the functions of the prostate is to neutralize the seminal plasma, made acid by accumulation of metabolic carbon dioxide and lactate and to initiate active movements of the ejaculated spermatozoa.

Copenhaver *et al.* (1978) stated that the prostate compound tubuloacinar glands, differing in length and degree of branching, are arranged in three concentric zones. Thirty to forty short glands immediately around the urethra discharge their secretion through separate openings on either side of the colliculus seminalis. These are called the mucous glands. Peripheral to these is a zone of longer, branching submucosal glands that share a smaller numbers of ducts. Outside of these are the main glands that make up the greater part of the prostate. They also stated that tubuloacinar units of the gland are quite variable in form, being narrow in some places, wider in others and sometimes having cystic dilatations. Branching folds or papillae of the mucosa project into the lumen. The epithelium is usually simple or pseudo stratified columnar but may be reduced to low cuboidal or even squamous in dilated or cystic regions. The cells contain abundant rough endoplasmic reticulum, a conspicuous Golgi complex and numerous secretory granules. The secretion of the prostate contains acid phosphatase, amylase and fibrinolysin. Ovoid or spherical bodies called corpora amylacea or prostatic concretions are often found in the lumen of the glands. These are glycoprotein in composition but may become a site of calcium deposition. They are more common in the prostate of older men.

Sudhakar *et al.* (1985) reported on the Murrah buffalo bulls of post natal age to the 10 years of old, the secretory elements of the pars disseminata consisted of solid and/ or luminated secretory end pieces distributed within the wall of the pelvic urethra. The undifferentiated secretory end pieces, most of which were solid in calves up to 2 years of age, were located between the cavernous spaces of the pelvic urethra. They become smaller and infrequent in the glands of adult buffaloes. The undifferentiated luminated

end pieces seen in neonatal animals became shorter in diameter up to 2 years. They disappeared from the glands of animals of 4 years and onwards as they were differentiated into either serous or mucous components.

The characteristic serous and the mucous components of the gland became evident at 4 years of age. In the cranial region of the pelvic urethra, the gland was predominantly serous in nature. A few mucous end pieces, however occurred near the urethral lumen. In the middle region the mucous lobules were present under the capsule on all sides of the pelvic urethra surrounding the serous lobules. In the caudal region the mucous lobules were predominant.

Sudhakar *et al.* (1985) also reported on the Murrah buffalo bulls of post natal age, the serous end pieces both alveoli and tubules of the pars disseminata were low columnar or cuboidal epithelium lined with distinct cell outlines and had finely granular eosinophilic cytoplasm which sometimes appeared foamy and stained with aniline blue in trichrome stain. The interlobular and intralobular ducts of the serous lobules were lined with simple cuboidal or low columnar epithelium with finely granular eosinophilic cytoplasm as in the glandular end-pieces.

The mucous end-pieces present in the pars disseminata were either in the form of the tubules or alveoli. They were lined with tall columnar epithelium having distinct cell outlines and clear or foamy cytoplasm which was strongly aniline blue reactive in trichrome. The nuclei were round, flat or oval and were placed along the basement membrane. The tubules and ducts were lined with the columnar epithelium of varying heights, having cytoplasm and nuclei similar to those of the alveoli. They were often secretory in nature.

Although the thickness of the capsule increased, the interlobular and interstitial tissue thickness decreased as the age advanced. This indicated that in younger animals the stroma was more than the parenchyma; in the adult buffaloes the tubules and alveoli were more than the connective tissue.

Chandramouly (1971) observed the extension of pars disseminata beyond the ischial arch in the adult buffalo. In the calves it was present only up to the middle third of the pelvic urethra. The parenchyma of the pars disseminata resembles in general the

histology of the compact part, while the stroma shows thicker connective tissue trabeculae. The epithelial lining of the ducts of the prostatic gland is of pseudostratified columnar in nature which gradually gets converted to the transitional type as the duct approaches the urethral lumen. The cytoplasm of these cells contains PAS positive granules. The nucleus is large and club-shaped, placed in the centre.

Chandramouly (1971) also reported that the corpus prostate consists of compound tubule alveolar glands with apparently run straight courses dorso-ventrally with tubular portions branching on the side. The intertubular stroma consists of collagen, elastic and smooth muscle fibers; the smooth muscle fibers and elastic fibers increase in the non-functional glands; the inter-alveolar connective tissue chiefly consists of reticular fibers and collagen fibers. The alveoli rest upon a distinct basement membrane as observed by the PAS technique. The alveolar epithelium is not folded. In the adult functional gland a majority of the alveoli are serous in nature but a few mucous alveoli could also be observed. Most of the alveoli consist of simple columnar epithelium. In a functionally active gland the cells are tall with a large oval nucleus placed towards the base of the cell; these cells show definite accumulation of basophilic material at the luminal margin of the cell. The cytoplasmic is eosinophilic by H and E method but stains blue by Mallory's triple stain. These cells show within the cytoplasm, varying stages of secretory activity with accumulation of basophilic granules followed by the appearance of numerous fine acidophilic granules both of which are PAS and Alcian blue positive; the nucleus is pushed towards the base of the cell but is still spherical. These cells correspond to type A cells as described by Martan and Allen (1965).

Roy *et al.* (1985) reported that the prostate gland was a compound tubule acinar type gland and was mainly consisting of pars disseminata portion. On the dorsal aspect of urethra a small and compact glandular portion was embedded within the urethralis muscle. It was part of ill-developed corpus prostate which remain inconspicuous when examine grossly. The glandular epithelium was simple, cuboidal to columnar with a few basal cells. The prostate ducts were lined with pseudostratified columnar epithelium which gradually transformed into transitional type towards urethral opening.

Chandrapal and Bharadwaj (1984) reported that in the prepubertal buffalo bulls, the secretory end pieces had intensely eosinophilic, PAS reactive secretory mass in their

lumina. They were lined by columnar, pyramidal and pseudo stratified epithelium. The epithelial cells rested on PAS positive basement membrane. The pubertal and post pubertal buffalo bulls, the corpus prostate was composed of serous and mucus gland. Serous secretory end pieces in the corpus prostate of pubertal buffalo bulls were lined by simple columnar to pyramidal epithelium which rested on discrete basement membrane. The lining epithelium cells contained finely granular, eosinophilic cytoplasm. Mucous glands were relatively few in number and lined by columnar epithelium. The lining cells had faintly basophilic, foamy cytoplasm which showed intense PAS reaction. In the post pubertal buffalo bulls the serous and mucus glands were lined by simple columnar or pyramidal and simple columnar epithelium respectively. In serous portion, the cytoplasm contained coarser PAS reactive granules as compared to the pubertal buffalo bulls. The mucus secretory tubules were relatively more in number. Some secretory end pieces had different sized spherical, basophilic corpora amylacea which were intensely reactive for PAS and colloidal iron staining.

2.8.4 Ducts

Archana *et al.* (2012) reported on pars disseminata prostate gland of Gaddi goats, each lobule contained luminated ducts which opened into the pelvic urethra. The mean diameter of these ducts was 28.68 μm in one day old Gaddi kid. These ducts were lined by stratified cuboidal to transitional epithelium of an average 11.35 μm in height and 8.96 μm in width. The cytoplasm of epithelial cells was homogenously eosinophilic. A few large clear cells were also observed in the epithelium of the ducts. In prepubertal animals the ducts were lined by multilayered epithelium with distinct basement membrane formed of thick reticular fibers. The average diameter of the duct in prepubertal goats was 32.62 μm . The average diameters of duct in pubertal and post pubertal goats measured 44.22 μm and 46.44 μm , respectively. Towards the lumen of urethra these were lined by pseudo stratified columnar epithelium.

The diameters of corpus prostate measured 40.86 μm . These were lined by stratified cuboidal epithelium with rounded nuclei resembling those of secretory end pieces. The average height of the epithelium was 12.64 μm and their nuclei measured 4.54 μm in diameter. In prepubertal animals the average diameter of acini measured 44.98 μm and height of their epithelium was 12.89 μm .

Sudhakar *et al.* (1985) reported on the Murrah buffalo bulls of post natal age to the 10 years of old, the ducts of the pars disseminata had wider lumen and were lined by stratified cuboidal epithelium with distinct cell membranes. They were surrounded by mesenchymal cells and immature fibroblasts in young buffalo calves. The excretory ducts of the glands which opened into the lumen of the urethra were lined with transitional epithelium near their opening.

Chandrapal and Bharadwaj (1984) reported that the corpus prostate opened into numerous ducts in the pelvic urethra. The intralobular ducts or collecting sinuses had irregular lumen and contained in-dippings which termed the secretary end pieces. These sinuses were lined by pseudostratified columnar epithelium in the prepubertal animals and simple columnar epithelium in pubertal and post pubertal buffalo bulls. The epithelium of collective sinuses and indippings resembled in staining characters to the serous and mucus glands, respectively. The interlobular ducts in prepubertal animals were lined by simple columnar epithelium. Finely granular cytoplasm was moderately PAS reactive. In pubertal and post pubertal buffalo bulls, these ducts contained folded mucosa and were lined by simple columnar, pseudostratified columnar or stratified cuboidal epithelium.

2.9 Gross parameters of bulbourethral gland

Gofur (2015) stated that the bulbourethral glands consists of right and left club-shaped independent lobes in bucks lie on the dorsal surface of the caudal part of the pelvic urethra at the level of the ischial arch under the covering of a layer of fibromuscular tissue and closely related to the bulb of penis. The bulbourethral glands are well visible and somewhat round bodies. The length and weight of left bulbourethral glands significantly ($p < 0.05$) higher than the right in black Bengal goats.

Noakes *et al.* (2001) noted in the stallion, bull and ram, the bulbourethral glands are small, rounded structures lying between the anus and urethra. Their watery secretion discharged prior to coitus and is considered to cleanse the urethra of urine. In the boar, the bulbourethral glands are large, cylindrical structures lying along each side of the intrapelvic urethra.

Getty (1975) stated in stallion, the bulbourethral glands (Cowper's gland) are two in number and are situated on either side of the pelvic part of the urethra close to ischial arch. They are covered by the urethralis muscle, from which muscle, from which bundles enter the larger trabeculae of the gland. They are ovoid in form, somewhat depressed dorso ventrally, and their long axes are directed obliquely craniad and laterad. In the stallion they may measure about 4 cm in length and about 2.5 cm in width.

Getty (1975) stated in bull that the bulbourethral glands are somewhat smaller than the stallion. They are covered by a thick layer of dense fibrous tissue and also partially by the bulbospongiosus muscle. Each has a single duct which opens into the urethra under cover of a fold of the mucous membrane. The forms a blind pouch about 1.25 cm deep on the wall of the urethra.

Nissar and Suri (2012) reported on Bakerwali (Kaghani) goat, the bulbourethral glands paired ovoid structure, were located on either side of the pelvic urethra close to the ischial arch. The mean length of right and left bulbourethral glands was 1.16 ± 0.10 cm and 1.18 ± 0.10 cm, respectively. Width of bulbourethral glands of right and left were recorded as 1.18 ± 0.07 cm and 1.19 ± 0.07 cm, respectively.

Archana *et al.* (2011) noted that the bulbourethral gland of Gaddi goats were paired, rounded or ovoid in shape, located on either side of the pelvic urethra close to the ischial arch. Each gland was covered by a distinct fibrous capsule which was ensheathed by thick bundles of striated bulbocavernosus muscle. In new born kids, average weight of bulbourethral gland measured 0.09gm. The average length, width and thickness were 0.68cm, 0.54cm and 0.45cm, respectively. A significant in various parameters took place in the first six months of post natal life. At the six months of age the glands weights 1.60gm. The average length, width and thickness measured 1.50cm, 1.50cm and 0.90cm, respectively.

2.10 Histological parameters of bulbourethral gland

2.10.1 Capsule and interlobular septa

Dellmann (1992) stated that the gland is ensheathed by a fibroelastic capsule containing a variable amount of striated muscle cells. Trabecula, extending from the capsule, consists of dense irregular connective tissue and some smooth and striated muscle fibers. The interstitium consists of loose connective tissue and a few smooth muscle fibers.

Gupta and Singh (1982) reported that the bulbourethral gland of goats of each size was covered by the bulbocavernosus muscle. A thin fibromuscular tissue below the muscle invested the gland as true capsule. Rarely the continuity of capsule over the gland was interrupted by the bundles of bulbocavernosus muscle penetrating the parenchyma of the peripheral lobules. The interlobular septa of variable thickness radiated from the capsule to divide the gland into lobules of different sizes. The average thickness of the capsule and interlobular septa measured $132.7 \pm 8.0 \mu\text{m}$ and $27.5 \pm 2.5 \mu\text{m}$, respectively in normal gland.

Suri *et al.* (2012) described in the Gaddi goat and Gaddi sheep, the fibrous capsule ensheathed the gland composed of collagen and a few reticular fibers. From the capsule, thin septae descended into the gland dividing its parenchyma into distinct lobules. The overlying loose connective tissue contained fibroblasts, blood vessels, nerves and occasionally autonomic ganglia. The thickness of the capsule and septae were minimum during autumn season and maximum during summer season in goat and sheep. The septae also showed moderate reaction for PAS. The capsule, septae and interlobular connective tissue did not show seasonal variation for PAS and cholesterol staining in both the species.

Archana *et al.* (2011) noted that the bulbourethral gland of Gaddi goats, each gland was enclosed by true capsule which measured $78.40 \pm 0.2 \mu\text{m}$ in thickness in new born kids. It gave off $45.08 \mu\text{m}$ thick interlobular connective tissue septae which divide the glands into lobes and lobules of different sizes. The capsule was rich in collagen, but the interlobular and intralobular connective tissue had mild concentration of collagen fibers. Only very fine elastic fibers were visible in the capsule. The overlying

connective tissue contained fibroblasts, blood vessels, nerves and occasionally autonomic ganglia. In pre-pubertal Gaddi goats, the capsule had circularly arranged collagenous bundles, abundant reticular and few elastic fibers. The elastic fibers were frequently observed in the walls of the blood vessels and very fine elastic fibers were noticed in the connective tissue trabeculae. With the increase in age, the connective tissue stroma vs parenchyma ratio decreased. The percent ratio of connective tissue and parenchyma was 80: 20 in one day old kids, which became 43: 57 in late pre-pubertal animals after 12 months of age. The thickness of capsule increased with age in pubertal and further in post pubertal animals. The collagen and reticular fibers of capsule became coarser. The amounts of elastic fibers were increased with age. The thickness of interlobular and intralobular connective tissue were decreased consequently the parenchyma of the gland increased with age.

2.10.2 Glandular alveoli

Dellmann (1992) stated that the paired gland is located dorsolaterally from the bulber portion of the urethra. It is compound tubular (boar, cat, buck) or tubuloalveolar (stallion, bull, ram) gland. It is absent in the dog.

The secretory portions of the gland are lined with a tall columnar epithelium and occasional basal cells. They open into collecting ducts either directly or through connecting pieces lined by simple cuboidal epithelial cells with dark cytoplasm..

In the cat, the gland consists of spacious, sinus like intraglandular ducts and short, narrow, mostly unbranched tubular end pieces. The secretory surface of the cells is increased by a well developed system of inter cellular canaliculi.

In the stallion, the bulbourethral gland is completely surrounded by the bulbocavernous muscle. Three to four bulbourethral ducts are present.

In ruminants, the gland is also surrounded by the bulbocavernous muscle. In the bull and ram, short connecting pieces link the secretory portions to the collecting ducts which are lined by a simple cuboidal epithelium (sometimes also secretory). In the buck, the secretory portion empty directly into these ducts. Smooth muscle cells are particularly abundant within the interstitium.

The mucus and proteinaceous secretory product of the bulbourethral gland is discharged prior to ejaculation in ruminants, where it apparently serves to neutralize the urethral environment and to lubricate both the urethra and vagina. In the cat, the secretory product is mucus and also contains glycogen. In the absence of vesicular gland, this bulbourethral glycogen may be the source of feline seminal fructose. It may provide energy for the metabolism of the spermatozoa.

Copenhaver *et al.* (1978) stated that the paired bulbourethral glands (Cowper's glands), partially embedded in the muscle of the urogenital diaphragm, are compound tubuloalveolar glands less than 1 cm in diameter. Each opens by a single duct into the floor of the proximal portion of the cavernous urethra. Their secretion is emitted at the onset of ejaculation and makes a small contribution to the initial fraction of the semen.

The gland consists of branched and contorted tubules whose blind ends are often dilated to form alveoli. The tubules comprising the small lobules of the gland converse on wider channels called ampullae. The duct arises from confluence of the ampullae of the several lobules. Prominent connective tissue partitions between the lobules contain collagenous and elastic fibers and both striated and smooth muscle fibers. Some of the later extend into the lobules.

The glandular epithelium is quite variable in appearance. In the alveoli, the cells are cuboidal, whereas in the tubules, they are pyramidal or low columnar. Their nuclei are irregular in outline and displaced towards the base by the large number of secretory droplets in the apical cytoplasm. The abundant secretory droplets have the staining properties of an acidic mucin.

The secretion of the bulbourethral glands in the human is clear, mucus-like, viscous fluid that, on contact, can be drawn out into a long thin thread. It is thought to have a lubricant function.

Gupta and Singh (1982) reported that the bulbourethral gland of goats mainly mucus type. A few serous or mixed secretory end pieces occurred only at the periphery of superficial lobules. These differed in shape, size and staining behavior in different or even in the same part of the gland.

The mucus alveoli in normal gland measured $50.0 \pm 1.8 \mu\text{m}$ in average diameter. A gradual but significant decrease in their size noticed with increase in castration period. The alveoli were lined with simple columnar epithelium with an average height of $11.4 \pm 0.5 \mu\text{m}$ in normal glands. The serous alveoli in normal glands measured $32.8 \pm 0.9 \mu\text{m}$ in average diameter. The simple columnar epithelium lining them measured $12.5 \pm 0.2 \mu\text{m}$ in height in normal glands. The secretory material contained in the lumina of both types of alveoli was PAS positive.

Suri *et al.* (2012) described in the Gaddi goat and Gaddi sheep, the bulbourethral glands were compound, mixed (sero-mucus and predominantly mucus), tubule-alveolar glands, consisting of mostly luminated and few solid end pieces. The glandular parenchyma was composed of alveoli, tubules and solid end pieces. The alveoli and tubules were predominantly mucus in nature. A few serous end pieces were also observed scattered between mucus end pieces or formed a separate lobule in the center of the gland in both species. The solid secretory end pieces had round or oval nuclei. The diameter of solid end piece was maximum during autumn season followed by spring, winter and minimum during summer season. The diameter varied significantly between seasons in both the species. The mucus end-pieces (alveoli and tubules) were lined with tall columnar or pyramidal cells having oval or flat nuclei in basal parts. They contained finely granular cytoplasm, which stained light basophilic. The cytoplasm was sometimes appeared foamy or vacuolated. The diameter of alveoli and tubules was largest during autumn season followed by spring, winter and smallest during summer season. The epithelial height of alveoli and tubules was recorded highest during autumn season and minimum during summer season. In both species, epithelial cells were seen to discharge secretion which was more during autumn season and very less during summer season, as most of alveolar lumina and ducts were filled during autumn season. The cytoplasm of mucus epithelial cells was intensely PAS positive during autumn season and weak was observed during summer season. The serous end-pieces were few and were present either under the capsule or in the center of the gland between mucus lobules singly or in groups forming separate lobules. The serous alveoli and tubules were lined with cuboidal epithelium with rounded nuclei. The alveolar and tubular diameter was recorded maximum during autumn season and minimum during summer season in both the species. The height of alveolar and tubular

epithelia was maximum during autumn season. The ducts were also lined by epithelium similar to that of alveoli and tubules. A few of them contained eosinophilic, PAS reactive.

Archana *et al.* (2011) noted that the bulbourethral gland of Gaddi goats was compound tubuloalveolar type. In new born kids, the solid and luminated secretory end pieces were arranged in a small groups around the widely luminated ducts. The average diameters of solid secretory end pieces were 36.43 μ m in one day old kids which became 47.57 μ m in late pre-pubertal animals. The respective parameter of luminated end pieces 38.47 μ m in one day old kids and 51.68 μ m in post pubertal animal. Mucus as well as serous secretory alveoli were present in the bulbourethral gland, however the mucus alveoli more than serous alveoli. The typical mucus acini were lined with simple columnar epithelium with distinct cell membrane. The cytoplasm was clear with basally placed nuclei. The darkly stained nuclei of these cells varied from spherical, oval to flatten in shape and contained distinct nucleolus. The height and width of the lining epithelium of the luminated secretory end pieces in new born kids measured 14.75 μ m and 5.33 μ m, respectively. The respective parameters of non-luminated end pieces were 15.89 μ m and 4.19 μ m, respectively. The respective parameters of pre-pubertal animals were 18.16 μ m, 4.76 μ m and 14.50 μ m, 4.50 μ m, respectively. The nuclei of the both type end pieces were round, oval, angular or flattened in shape. With the advancement of age, the glands became highly lobulated in pubertal animals and further in post pubertal animals. The number of luminated end pieces increased in pubertal animals in compared to preceding age group, however both type of secretory end pieces were observed in pubertal as well as post pubertal animals. These were dominantly mucus in nature. The diameter of luminated and non luminated secretory end pieces increased with age. The glands were lined with simple columnar epithelium, the height of the lining cells increased significantly in pubertal and post pubertal animals.

2.10.3 Ducts

Copenhaver *et al.* (1978) stated that the small ducts and ampullae are lined by cuboidal or columnar epithelial cells. Their luminal surface is irregular and bears short microvilli. The basolateral surfaces are corrugated and interdigitated with similar irregularities on adjacent cells. The larger excretory ducts of the bulbourethral gland are

lined by pseudostratified epithelium resembling that of the urethra, but here too patches of mucous cells may be found.

Dellmann (1992) stated that the collecting ducts lined by a simple cuboidal or columnar epithelium, unite to form larger intra glandular ducts lined by a pseudostratified columnar epithelium. These in turn open into a single bulbourethral duct with a lining of transitional epithelium.

Suri *et al.* (2012) described in the Gaddi goat and Gaddi sheep, the ducts of mucus lobules (both inter and intralobular) and lumina of alveoli and tubules were lined with epithelium similar to that of alveoli. The ducts were completely filled with basophilic secretions during autumn season whereas appeared empty during summer season in both the species. In Gaddi goat, dark eosinophilic granules were observed scattered uniformly in the secretions. The secretions were moderately PAS positive and remained unaffected.

Archana *et al.* (2011) noted that the bulbourethral gland of Gaddi goats, in new born kids, the ducts were very few and short. They were seen originating as an out pocket of cisterns. Their diameter measured 32.92µm. These were lined to simple cuboidal to low columnar epithelium. The diameter of duct increased with the increasing of age.

CHAPTER III
MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Place of Research

The research work was carried out in the post graduate laboratory of the Department of Anatomy and Histology, Sylhet Agricultural University, Sylhet and Post graduate laboratory of the Department of animal Husbandry and Veterinary science, University of Rajshahi during July 2012 to June 2014. Collaboration was required with other Agricultural Universities of Bangladesh for technical help or assistance.

3.2 Study area and selection of animals

The physically sound and healthy bulls were selected for this purpose. The research area includes greater Sylhet region that represent the indigenous bulls. Bulls were divided into three major categories such as prepubertal group, pubertal group and post pubertal or adult group. Prepubertal group bulls were examined properly for the determination of age. The age of this group was determined below 1 year. Pubertal group of bulls were selected after examination of scrotal circumference, semen population counting, and motile semen % and through libido behaviors. The age of this group was 2-3 years. Bulls having age 3.5 years and above were included in adult group or post pubertal group which are usually used for breeding purpose. Prepubertal group having 4 bulls, pubertal group having 16 bulls and adult 8 bulls, total 28 bulls were used in the present study. The research requires 28 bulls were bought from the market for the purpose of collection of fresh samples. Then the bulls were reared for two weeks for recording the physical characteristics under good nutritional condition. The details information related to the purchase of bull and collection of samples were recorded.

3.3.1 Parameters considered for study

Body weight and scrotal circumferences of the bulls before Halal methods of slaughtering. Topographic location of the testis, shape and size of the testis. Gross parameters of testis (Left and right) involved testicular weight (with and without

epididymis), testicular circumference, testicular length (with and without epididymis), testicular width and testicular volume.

Topographic location of the accessory genital glands, shape and distribution of glandular tissue involved for the gross and microscopic study. The parameters of weights, length, width and thickness of the vesicular gland (Left and right) and bulbourethral gland (Left and right).

Histological parameter of testes were seminiferous tubular diameter, tubular morphology and germ cell type; thickness of tunica albuginea and Leydig cells. The thickness of capsule (tunica muscularis), thickness of interstitial tissue, diameter of the lumen (Maximum and minimum) acini and epithelial height of the glandular end pieces, diameter of the duct and epithelial height of the duct of vesicular and bulbourethral gland.

The parameters of prostate glands were included thickness of interlobular CT septa, diameter of the secretory lumen (Maximum and minimum) acini and epithelial height of the glandular end pieces, diameter of the duct and epithelial height of the duct.

3.4 Sample collection

Each testicle, left and right was taken in Bouin's fluid (Gridley, 1960) immediately after slaughtering and brought to the laboratory. The externally present fat and fascia of each testis were removed. The gross biometrical values of testis were recorded after dissecting the spermatic cord.

After opening the pelvic cavity, the root of the penis separated from pelvic part of the urethra with the urinary bladder and vesicular gland taken in Bouin's fluid (Gridley, 1960) immediately after slaughtering and brought to the laboratory. The externally present fat and fascia of each vesicular gland and prostate were removed. Bulbourethral glands were opened by dissecting urethralis muscle at the root of the pelvic urethra. The gross biometrical values of vesicular, prostate and bulbourethral glands were recorded.

3.5 Observation of gross parameters

3.5.1 Body weight

The measurements of body weight were recorded by using a measuring tape applying the formula of Rahman *et al.* (2004) before slaughtering the animals.

Live body weight (W) = $L \times G^2 / 300$ pounds

Where, L = Length from the point of shoulder to pin bone in inches

G = Chest girth in inches

Then, the body weight were converted from pound to kg, dividing by 2.2

3.5.2 Scrotal circumference

The scrotal circumference was measured as per method stated by the Society of Theriogenology (Ball *et al.*, 1983). The testes were retracted into the lower part of the scrotum for measurement of scrotal circumference. The thumb and other fingers were placed on the sides rather than on the front or back of the scrotum to prevent separation of the two testes. Then a flexible metal tape (Scrotal tape; Lane manufacturing company, Denver, USA) was looped and placed around the greatest diameter of the scrotum and pulled snugly so that the tape was firmly in contact with the entire circumference. Repeated measurement was done and the mean of the measures were recorded to ensure the accuracy.

3.5.3 Measurement of gross parameters of the testes

3.5.3.1 Testicular weight

Testicular weights were measurement individually by using electronic balance immediately after removing the testicular covering, the tunica vaginalis. The weights of testis with the epididymis were recorded first. The epididymis has been trimmed off from each of the testis. Then only testicular parenchyma was measured.

3.5.3.2 Testicular circumference

Testicular circumference was measured with a measuring tape which was passed round the broadest point of the scrotum. Repeated measurement was done and the mean of the measures was recorded to ensure the accuracy.

3.5.3.3 Testicular length and width

The testicular lengths were measured with the epididymis and without epididymis by using a vernier caliper. The breadths of the each testis were measured by using vernier calipers.

3.5.3.4 Testicular volume

The volume of the each testis was recorded, using Archimedes principle of water displacement theory.

3.5.3.5 Measurement of gross parameters of the accessory glands

3.5.3.5 .1 Vesicular gland

The measurement of length of the vesicular gland (antero-posterior), width (latero-medial) and thickness (dorso-ventral) of the right and left gland were recorded separately using the help of vernier calipers in the situ position of the gland. Weights of the gland were recorded by using electronic balance.

3.5.3.5 .2 Prostate

Topographic location of the gland, shape and distribution of glandular tissue involved for the gross and microscopic study.

3.5.3.5 .2 Bulbourethral gland

The measurement of length of the bulbourethral gland (antero-posterior), width (latero-medial) and thickness (dorso-ventral) of the right and left gland were recorded separately using the help of vernier calipers in the situ position of the gland. Weights of the gland were recorded by using electronic balance.

3.6 Histometric studies

The testes were cut mid-sagittally and the tissue samples were taken from each half of each testis. A part of each testicular samples were fixed in more than 20 times the volume of each in Bouin's fixatives for 24 hours, dehydrated in series of submerging progressively more concentrated ethyl alcohol (70%, 80%, 90%, 95%, 100%). The tissues were kept for 3 hours in each grade of ethyl alcohol. Tissues were then transferred in a hydrophobic clearing agent, xylene in two changes to remove the ethyl alcohol. In both cases the tissues were kept for 40 minutes. Infiltrated the tissues through 2 changes of liquid paraffin in the oven taking 2 hours in each step to remove the xylene from tissues. When the tissues were dehydrated, cleared and infiltrated, they were placed in liquid paraffin for embedding, which were then allowed for hardening. This was achieved by cooling in the room temperature.

The hardened tissue blocks containing the tissue samples were then sectioned at 6 micron thickness using microtome (Microm GmbH, type HM 325, Germany) and the sections were floated in a water bath at 45°C for flatten out on water. Then floated sections picked up carefully with clean slides, which have been smeared with Mayer's egg albumin and dried in the slide warmer. The successful sections were chose and to ensure the different position of tissue about every 21st section, the tissue examined were not in the same portion.

The sectioned tissue were deparaffinized by transferring them through xylene for two changes; 15 minutes in each case. Then these were transferred through descending grade of alcohol (100%, 95%, 90%, 80%, and 70%). Afterward these were passed through the normal water and distilled water. Tissues were stained with Hematoxyline and Eosin (Gridly, 1960). The tissue then dehydrated by transferring through baths of progressively more concentrated ethyl alcohol (70%, 80%, 90%, 95%, and 100%) and passed through xylene. Each slide was clean- blotted and mounted in Canada balsam under a cover slip. Four slides with four tissue sections in each slide made up per tissue sample were prepared for each bull.

Histometric studies of vesicular gland, prostate and Bulbourethral glands were processed as stated similarly to the testes.

3.7 Histometric observations

The stained tissue sections of testis of bull were studied under compound binocular microscope (Abota Corporation, USA). The evaluation included seminiferous tubular diameter, tubular morphology and germ cell type present in each tubular section; tunica albuginea and Leydig cells.

The evaluation of vesicular glands were included thickness of tunica adventitia, thickness of tunica muscularis, thickness of interstitial tissue, diameter of the luminated (Maximum and minimum) acini and epithelial height of the glandular end pieces, diameter of the duct and epithelial height of the duct.

The evaluation of prostate glands were included thickness of interlobular CT septa, diameter of the secretory luminated (Maximum and minimum) acini and epithelial height of the glandular end pieces, diameter of the duct and epithelial height of the duct.

The evaluation of bulbourethral glands were included thickness of capsule, thickness of interlobular connective tissue, diameter of the secretory luminated (Maximum and minimum) acini and epithelial height of the secretory end pieces, diameter of the duct and epithelial height of the duct.

3.8 Ocular photo microscopy

For histological observations of the testes, images were captured from random areas. Photograph from the selected specimens were prepared and placed in this thesis for illustrations of the results.

3.9 Histometric measurements

Tubular diameters of seminiferous tubules were determined by measuring twenty approximately round tubules per slide with a microscope, having its eyepiece already calibrated with a stage micrometer. Two measurements at right angles to each other were taken on each tubule and the average recorded (i. e., four slides per testis). Twenty tubules were measured in each of the four slides to observe a total of eighty tubules per animal.

Microscopic measurement of stained sections of the vesicular gland, prostate and Bulbourethral glands were recorded as above stated in testes.

3. 10 Data analysis

The data compiled in Microsoft excel sheet from this experiment were processed for further analysis. Mean value, Standard Error (SE), level of significance, phenotypic correlations and correlation coefficient were estimated with the help of Statistical Analysis System (SAS, 1998).

3.11 Research designing plan and time schedule

Time chart	Research plan		
	1 st year	1 st quarter	Selection of animals, sample collection, tissue processing (fixation, dehydration, clearing and infiltration, tissue blocking and sectioning) for prepubertal and pubertal bulls.
		2 nd quarter	Hematoxylin and Eosin staining for histological study. Periodic Acid Schiff, Van Gieson's and Verhoeff's staining for histochemical study.
		3 rd quarter	Digital photo microscopy, digital labeling, light microscopic data collection,
		1 st Seminar on research methodology and 1 st year result	
	2 nd year	1 st quarter	Selection of animals, Sample collection, Tissue processing for adult bulls.
		2 nd quarter	Hematoxylin and Eosin staining for histological study. Periodic Acid Schiff, Van Gieson's and Verhoeff's Elastic staining for histochemical study.
		3 rd quarter	Digital photo microscopy, digital labeling, ocular microscopic data collection
		Data analysis, production and preparation of analysis	
	3 rd year	1 st quarter	Data analysis, data interpretation, statistical analysis, designing the table , diagram chart etc.
		2 nd quarter	Thesis writing
		3 rd quarter	Final seminar on research findings
		Thesis submission for evaluation	

CHAPTER IV
RESULTS

CHAPTER IV

RESULTS

4.1 Body weight and scrotal circumference

As body weight and scrotal circumference correlate with the testicular parameters. Bodyweight and scrotal circumference at different age of bulls were recorded in this study. The body weights of prepubertal, pubertal and adult bulls were 82.39 ± 5.72 kg, 205.34 ± 11.27 kg and 319.22 ± 9.75 kg, respectively. The body weights of bulls were higher in adult group than in prepubertal and pubertal groups which indicated that the body weights of bulls increases with the advancement of age up to adult. (**Figure 1**) The testes were suspended in a vertical direction by spermatic cord and were housed in a long, ovoid cutaneous diverticulum at the prepubic region termed as scrotum. The average scrotal circumferences of prepubertal, pubertal adult bulls were 19.04 ± 0.90 cm, 30.65 ± 0.97 cm and 35.00 ± 0.49 cm, respectively. The scrotal circumferences changes significantly with the incensement of age and body weight.

4.2 Topography and gross parameters of the testis

The shape of the testes of bulls was elongated and had an oval outline with their long axis oriented vertically. The average circumference of the left testes and right testes of prepubertal bulls were 8.78 ± 0.44 cm and 7.78 ± 0.52 cm, respectively. In pubertal bulls, average circumference of the left testes and right testes were 12.30 ± 0.39 cm and 11.66 ± 0.33 cm, respectively. In adult bulls, the average circumference of the left testes and right testes were 14.01 ± 0.29 cm and 13.13 ± 0.31 cm, respectively. The left testis is slightly larger than the right in each bull and the circumference increased with the advancement of ages.

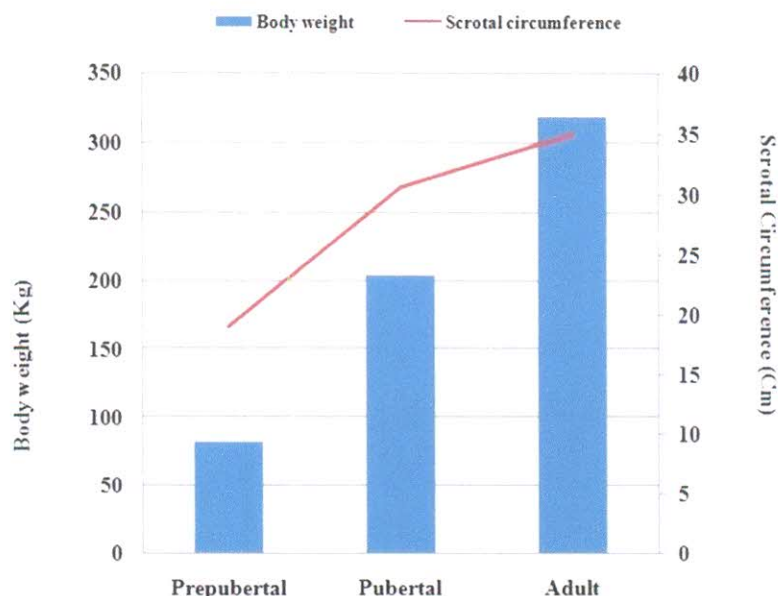


Figure 1. The bar chart showing the body weight and scrotal circumferences of Indigenous bulls.

The left testicular circumferences are always higher in percentage of the scrotal circumferences than that of the right. The percentage of testicular circumference decreases along with the advancement of age as the percentage thickness of skin and connective tissue increases with advancement of age (**Table 4.2**). A relationship between body weight and scrotal circumference are shown in **Figure 1** which shows that scrotal circumference increases along with the increment of body weight.

Each testis represented two surfaces (medial and lateral), two borders (free borders or ventral borders, attached borders or dorsal borders) and two rounded extremities (head and tail). The lateral surface was more convex than the medial one, the medial surface was somewhat flattened because of the contact with the well defined scrotal septum.

Table 4.1 Gross testicular parameters of indigenous bulls (n=28)

Sl. No.	Parameters		Pre pubertal	Pubertal	Post pubertal/adult
1	Testicular circumference	Left (cm)	8.78±0.44**	12.30±0.39**	14.01±0.29**
		Right (cm)	7.78±0.52**	11.66±0.33**	13.13±0.31**
2	Testicular weight (gm)	Left	With epididymis	22.35±1.52**	147.78±22.14**
			Without epididymis	18.57±1.40**	129.22±19.89**
		Right	With epididymis	19.38±1.77**	130.65±14.12**
			Without epididymis	16.10±1.64**	123.86±19.53**
3	Testicular length (cm)	Left	With epididymis	6.92±0.22**	11.53±0.74 ^{NS}
			Without epididymis	4.97±0.12**	10.03±0.64 ^{NS}
		Right	With epididymis	6.72±0.21**	11.20±0.69 ^{NS}
			Without epididymis	4.85±0.10**	9.67±0.59 ^{NS}
4	Testicular width (cm)	Left	2.77±0.11 ^{NS}	4.23±0.25 ^{NS}	6.05±0.48 ^{NS}
		Right	2.58±0.12 ^{NS}	4.20±0.28 ^{NS}	5.53±0.43 ^{NS}
5	Testicular volume (mm ³)	Left	27.92±1.05**	114.75±28.24**	211.16±8.77**
		Right	25.31±1.06**	107.87±27.21**	194.50±11.25**

Asterisk (*) indicates significant difference (*p<0.05, **p<0.01 and NS= non significant)

Table 4.2 Testicular circumference and thickness of skin and connective tissue have shown as percentage of the scrotal circumferences of the indigenous bulls.

Type of bulls	Scrotal circumference (Average mean in cm)	Left testicular circumference in % of scrotal circumference	Right testicular circumference in % of scrotal circumference	Thickness of skin and connective tissue %
Prepubertal	19.04	46.11	40.86	13.03
Pubertal	30.65	40.01	38.04	21.94
Adult	35.00	40.02	37.51	22.46

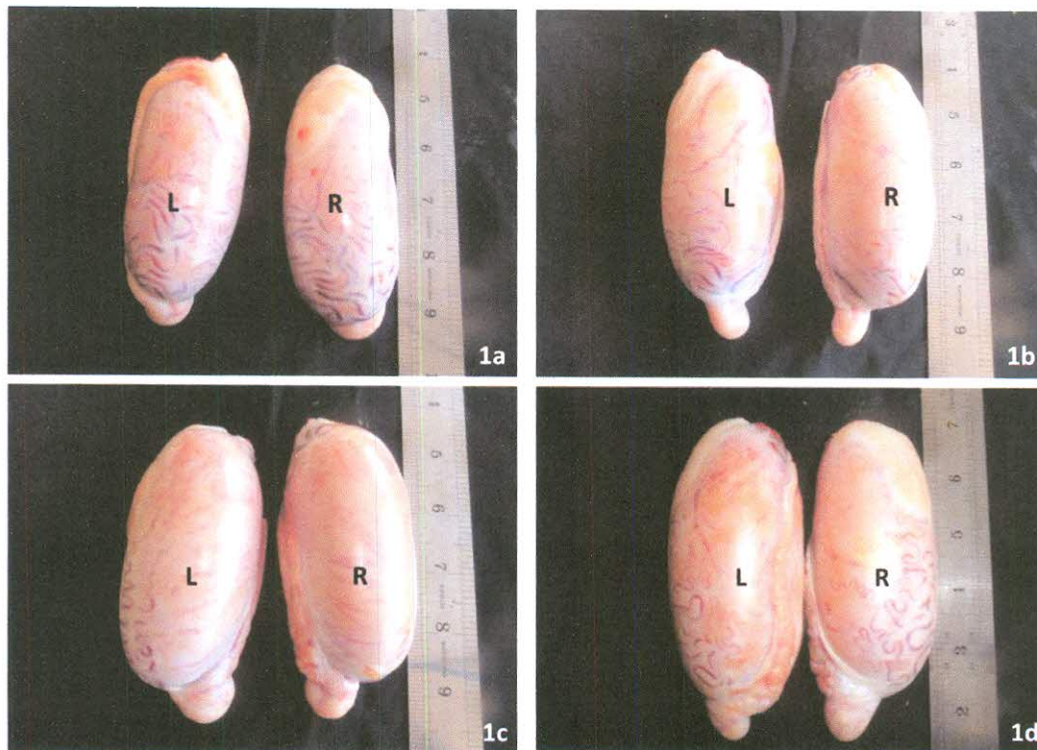


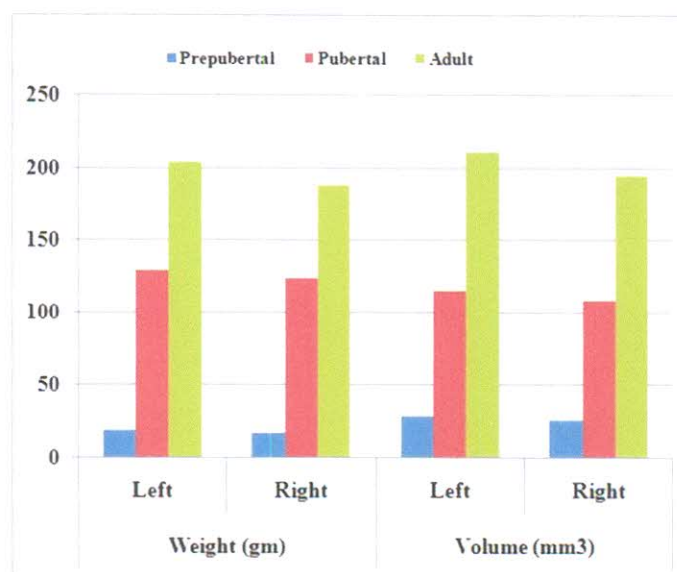
Figure 2. The gross picture of the left(L) and right(R) testis of pre-pubertal (1a, 1b) pubertal (1c) and post pubertal or adult (1d) bulls.

The size of the testes varied in different age groups and even in between left and right testes of the same age group. The size of the left testis of indigenous bulls was slightly larger than the right in all three groups. With the advancement of age the size of the both left and right testis increased (**Figure 2**), the shape become oval with convex lateral surface and relatively flattened medial surface. The average left and right testicular weights (without epididymis) were 18.57 ± 1.40 gm and 16.10 ± 1.64 gm, respectively in prepubertal bulls, 129.22 ± 19.89 gm and 123.86 ± 19.53 gm respectively in pubertal bulls and 204.36 ± 14.10 gm and 188.41 ± 13.59 gm respectively in adult bulls (**Table 4.1**). The weights of the left and right testes of bulls were consistently unequal and the left testis was significantly ($p < 0.01$) heavier than the right in all age groups. The relationship of testicular weight with body weight of bulls was shown in **Table 4.3**.

Table 4.3 Testicular weights as a percentage of the body weights of the indigenous bulls

Type of bulls	Body weight (Average mean in kg)	Left testicular weight in % of body weight	Right testicular weight in % of body weight
Prepubertal	82.39	0.022	0.019
Pubertal	205.34	0.062	0.060
Adult	319.22	0.064	0.059

The left and right testicular weight (without epididymis) showed gradual increment except adult right testis (**Table 4.3**). Whereas, the left and right testicular volume (without epididymis) showed gradual increment with the advancement of age (**Figure 3**).

**Figure 3.** The bar chart showing the testicular weight (without epididymis) and volume of Indigenous bulls.

In adult bulls, the left and right testicular weights were 204.36 ± 9.75 gm and 188.41 ± 9.75 gm without epididymis, respectively. The weights of the left and right testes of bulls were unequal and the left testis was significantly ($p < 0.01$) heavier than the right. The testicular parameters were presented in **Table 4.1**. The left and right testicular weights of prepubertal bulls (without epididymis) were 0.022% and 0.019% of the body weight; 0.062% and 0.060% of the body weight of the pubertal indigenous

bulls, but in adult conditions, it was 0.064% and 0.059% of the adult weights presented **Table 4.3**. In the present study the testicular weight varied in different age groups and even in the right and left testes of the same animal.

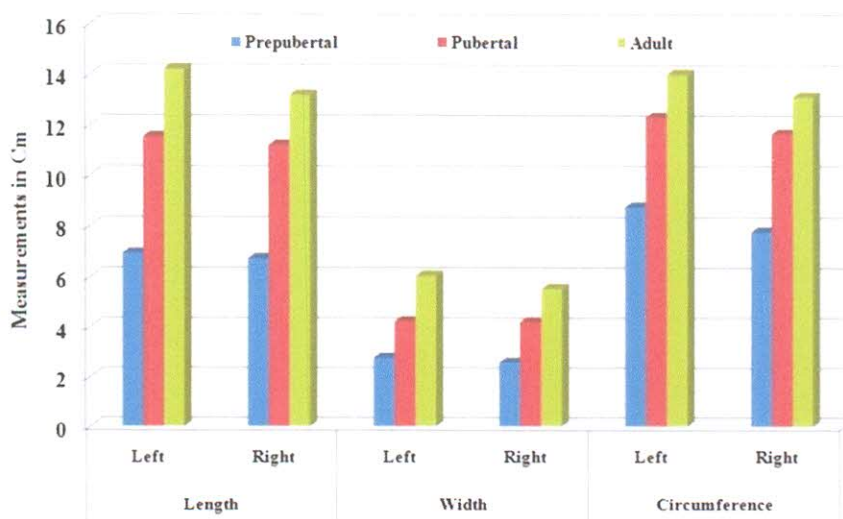


Figure 4. The bar chart showing the testicular length (without epididymis), width and circumferences of Indigenous bulls.

The average lengths of the left and right testes (without epididymis) were 4.97 ± 0.12 cm and 4.85 ± 0.10 cm respectively in prepubertal bulls, 10.03 ± 0.64 cm and 9.67 ± 0.59 cm respectively in pubertal bulls and 11.93 ± 0.31 cm and 11.76 ± 0.40 cm respectively in adult bulls. In all three groups of bulls, the length of testes increases along with the advancement of age where left testis is slightly heavier than the corresponding right testis. (**Figure 4**).

The average breadths of the left and right testis (without epididymis) were 2.77 ± 0.11 cm and 2.58 ± 0.12 cm respectively in prepubertal bulls, 4.23 ± 0.25 cm and 4.20 ± 0.28 cm respectively in pubertal bulls and 6.05 ± 0.48 cm and 5.53 ± 0.43 cm respectively in adult bulls (**Table 4.1**). In all three groups of bulls, the breadth of testes increases along with the advancement of age where left testis is slightly heavier than the corresponding right testis. (**Figure 4**).

The average volumes of the left and right testes without epididymis were 27.92 ± 1.05 mm³ and 25.31 ± 1.06 mm³, respectively in prepubertal bulls, 114.75 ± 28.24 mm³ and 107.87 ± 27.21 mm³, respectively in pubertal bulls and 211.16 ± 8.77 mm³ and

194.50±11.25 mm³, respectively in adult bulls (**Table 1**). In all three groups of bulls, the volumes of testes were increased significantly ($p<0.01$) with the advancement of ages and left testis is bigger in volume than right one.

4.3 Phenotypic relations of body weights and scrotal circumferences

Phenotypic correlations of the body weights and scrotal circumferences with testicular parameters of bulls were presented in **Table 4.4**. The body weight was not significantly correlated with all testicular parameters in all three groups of bulls. In prepubertal bulls, the body weight was significantly correlated with testicular weight with ($r=0.95$, $p<0.05$) and without ($r=0.97$, $p<0.01$) epididymis. In pubertal bulls, the body weight was significantly correlated with testicular circumference in the left testis ($r=0.95$, $p<0.05$). In adult bulls, the body weight was not significantly correlated with testicular parameters. The scrotal circumference was not significantly correlated with all testicular parameters in all three groups of bulls. In prepubertal bulls, the scrotal circumference was significantly correlated with testicular circumference with ($r=0.98$, $p<0.01$) right testis. In pubertal bulls, the scrotal circumference was significantly correlated with testicular circumference in the right ($r=0.96$, $p<0.01$) testis and in the left ($r=0.98$, $p<0.01$) testis. The testicular volume was also correlated with scrotal circumference in the left ($r=0.96$, $p<0.05$). In adult bulls, the scrotal circumference was significantly correlated with testicular circumference with ($r=0.95$, $p<0.05$) left testis.

Table 4.4 Phenotypic co-relation coefficients of body weight (BWT) and scrotal circumference (SCRF) with testicular circumference of right testis (TSCRT) and left testis (TSCLT), testicular weight of right testis with epididymis (TWRWI) and left testis (TWLWI), without epididymis right testis (TWRWO) and left testis (TWLWO), testicular length of right testis with epididymis (TLNRWI) and left testis (TLNLWI) and without epididymis right testis (TLNRWO) and left testis (TLNLWO) and testicular width of right testis (TBDRT) and left testis (TBDLT) and testicular volume of right testis (TVRT) and left testis (TVLT) of prepubertal (Pr), pubertal (Pu) and adults (A) bulls.

		TSCLT	TSCRT	TWLWI	TWLWO	TWRWI	TWRWO	TLNLWI	TLNLWO	TLNRWI	TLNRWO	TBDLT	TBDRT	TVLT	TVRT
Pr	BWT	0.76	0.86	0.95*	0.97**	0.88	0.92	0.89	0.75	0.83	0.09	0.83	0.78	0.84	0.79
	SCRF	0.89	0.98**	0.91	0.91	0.90	0.94	0.76	0.65	0.72	-0.19	0.78	0.83	0.88	0.93
Pu	BWT	0.95*	0.91	0.85	0.86	0.81	0.85	0.86	0.72	0.69	0.76	0.80	0.87	0.92	0.92
	SCRF	0.98**	0.96**	0.82	0.84	0.89	0.82	0.72	0.79	0.80	0.82	0.81	0.87	0.95*	0.94
A	BWT	0.91	0.65	0.80	0.80	0.51	0.61	0.50	0.75	-0.42	-0.66	0.80	0.64	0.66	0.36
	SCRF	0.95*	0.92	0.71	0.66	0.75	0.75	0.07	0.43	-0.40	-0.76	0.75	0.78	0.67	0.67

Correlation coefficients with different superscripts differ significantly at *P<0.05, **P<0.01

4. 4 Histological parameters of the testes

The visceral layer of the tunica vaginalis consisted of mesothelium and connective tissue layer. The tunica albuginea contains mainly the collagen fibers with a few elastic and reticular fibers. The tunica vasculosa is the inner layer of the covering contains mainly blood vessels which lie adjacent to the parenchyma to the testis. Connective tissue septa radiate from the mediastinum towards the capsule and divided the parenchyma of the testis into a number of incomplete pyramidal lobules. The thicknesses of tunica albuginea were presented in **Table 4.5**. The mean thicknesses of tunica albuginea of prepubertal, pubertal and post pubertal bull testes were $150.00 \pm 3.76 \mu\text{m}$, $332.50 \pm 30.19 \mu\text{m}$ and $573.75 \pm 29.66 \mu\text{m}$, respectively. The thicknesses were increases with the advancement of ages (**Figure 5**).

4.4.1 Septula testis

Septula testes were indefinite strands surrounding the large intra testicular vessels. These testicular septula divide the testicular parenchyma into a varying number of pyramidal or cone shaped testicular lobe and lobules. It was composed of collagen fibers and continuous with the mediastinum testis.

Table 4. 5 Histological parameters of testes of indigenous bulls (n=28).

Sl. No.	Parameters (μm)	Prepubertal	Pubertal	Post pubertal/adult
1	Thickness of tunica albuginea	$150.00 \pm 3.76^{**}$	$332.50 \pm 30.19^{**}$	$573.75 \pm 29.66^{**}$
2	Cross sectional length of seminiferous tubules	$566.50 \pm 16.02^{**}$	$637.90 \pm 18.75^{**}$	$728.50 \pm 13.78^{**}$
3	Cross sectional breadth of seminiferous tubules	$144.50 \pm 21.52^{**}$	$165.25 \pm 23.03^{**}$	$159.50 \pm 19.31^{**}$

Asterisk (*) indicates significant difference (* $p < 0.05$, ** $p < 0.01$ and NS= non significant)

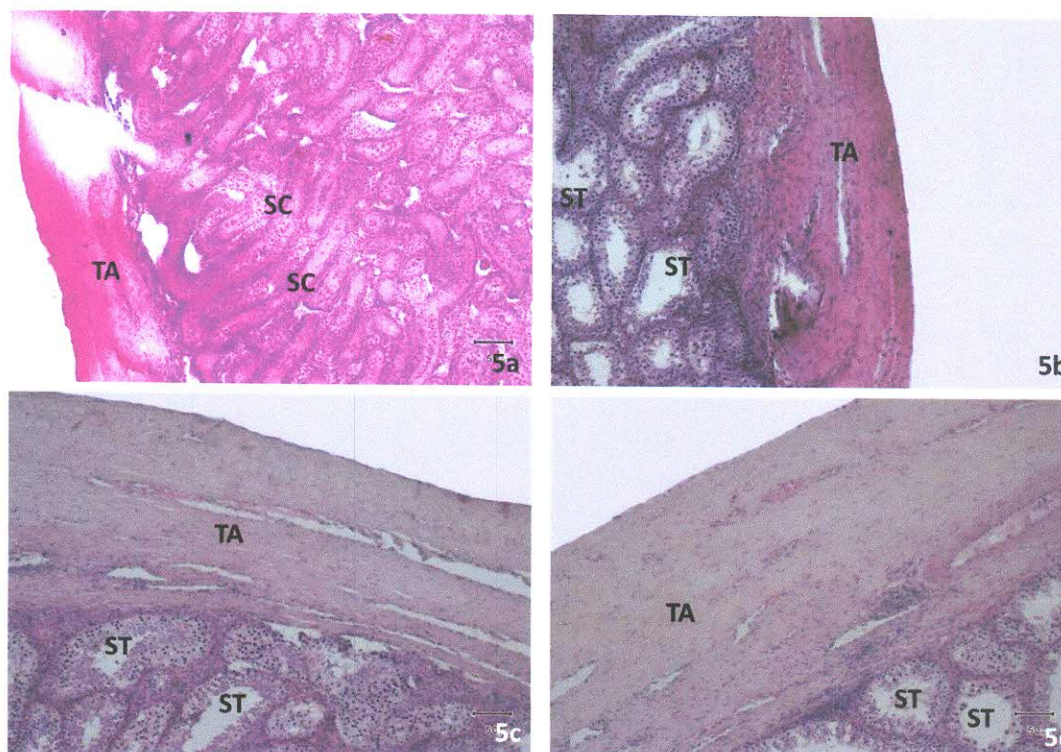


Figure 5. Thickness of tunica albuginea (TA) and Sex cord(SC)/Seminiferous tubule(ST) in prepubertal (5a), pubertal(5b), and postpubertal or adult (5c, 5d) testes. HE 10X

4.4.2 Interstitial tissue

The intertubular or inter-sexcord tissue was fibro cellular consisting of fibroblast, mesenchymal cells and differentiated Leydig cells with varying numbers. The fibrous component of the interstitial tissue consisted of mainly collagenous fibers. In prepubertal bulls, usually Leydig cells were observed in a large group (> 50 cells) in the present study, but sometimes observed few cells in small groups (5-15). The numbers of the Leydig cells were arranged in large groups (**Figure 6a**). The Leydig cells were rounded, ovoid or roughly triangular shape with round or ovoid nuclei generally present eccentrically towards the broader end of the cell. The nuclear membrane was distinct.

In pubertal bulls, the interstitial tissues consisted of loose connective tissue materials, blood and lymph vessels, fibrocytes, free mononuclear cells and especially the interstitial (endocrine) cells of Leydig present in the collagen fibrous network in between the seminiferous tubules. The Leydig cells were usually larger than those of

the prepubertal bulls. Polymorphs, ovoid or polygonal in shaped cells occurred in a smaller groups (**Figure 6b, 6c**) with spherical nuclei including granular cytoplasm.

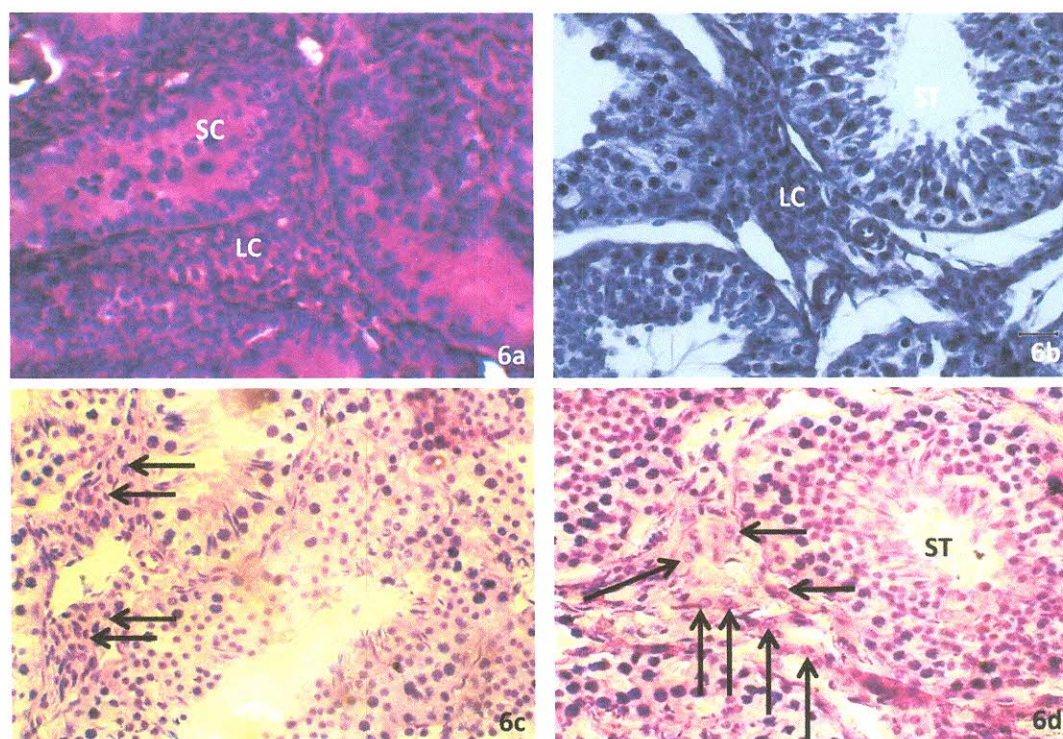


Figure 6. Changes in the primitive sex cords (SC) into true seminiferous tubules(ST) and the interstitial tissue containing numerous interstitial cells (arrow) of Leydig (LC) in prepubertal (6a), smaller groups in pubertal (6b, 6c), and singly dispersed in adult(6d) testes. HE 40X

In adult bulls, the cells of Leydig located in the interstitial tissues as a single dispersed form within the connective tissue materials as a large, polymorph cells. The cells were usually single (**Figure 6d**) or sometimes in a small group. The Leydig cells varied in number and arrangement with in different age group of indigenous bulls. The number of Leydig cells apparently decreases where as their size increase with the advancement of age may be due to rapid enlargement of the seminiferous tubules. The arrangement of Leydig cells were usually large groups in prepubertal, small groups or few cells in pubertal and singly dispersed form in adult indigenous bulls (**Figure 6**).

4.4.3 Sex cord

The seminiferous tubules without lumen was generally called sex cord. In the present study, the testicular parenchyma of prepubertal bulls consisted of solid, non-luminated, tubular structures arranged as coiled or straight appearance. The solid tubular seminiferous tubules were surrounded by fine collagenous connective tissue fibers (**Figure 7**). The intertubular tissues were fibro cellular. The sex cord having basement membrane and the cords were filled up by the centrally placed large primordial germ cells (PGC) designated as gonocytes and small indifferent basal cells (**Figure 8**). The basal cells were spherical in shape lying along the periphery of the sex cord and thus were more numerous than the PGC of the cord. The PGC had large spherical shape with distinct cell boundary contain rounded large nucleus, located in between small basal cells. The cytoplasm was slightly acidophilic. The typical spermatogenic cells and Sertoli cells could not identified in the present study of the prepubertal bulls. The large PGC showed cytoplasmic degeneration and nuclear fragmentation. The centrally grouped PGC sometimes distributed all over the cord in the apical portions of the basal cells. The gonocytes or PGC numbers varied from cord to cord. The PGC or gonocytes which migrated centrifugally cells called prespermatogonia of the cord.

4.4.4 Seminiferous tubules

With the advancement of ages, prepubertal bulls become pubertal and the solid sex cord became luminized true seminiferous tubules. The testicular parenchyma mainly consisted of seminiferous tubules containing three components, basement membrane, somatic cells and spermatogenic cells. The tubules were two ended loops; round and oblong in outline varying in appearance due to the complex coiling of the tubules at the different angles and levels. The numbers of the seminiferous tubules were increased with the advancement of ages. The size of the seminiferous tubules increased with the age of the animals. A distinct network of reticular fibers interspersed with the collagenous fibers was marked in the basement membranes supporting the germinal epithelium of the tubules (**Figure 7**). The intertubular space decreased with the advancement of age whereas the tubular convolution increased with the advancement of age. The cross sectional diameters of the seminiferous tubules were presented in the

Table 4.5. The average cross sectional length and breadth of seminiferous tubules were $566.50 \pm 26.52 \mu\text{m}$ and $144.50 \pm 26.52 \mu\text{m}$ in prepubertal bulls, respectively.

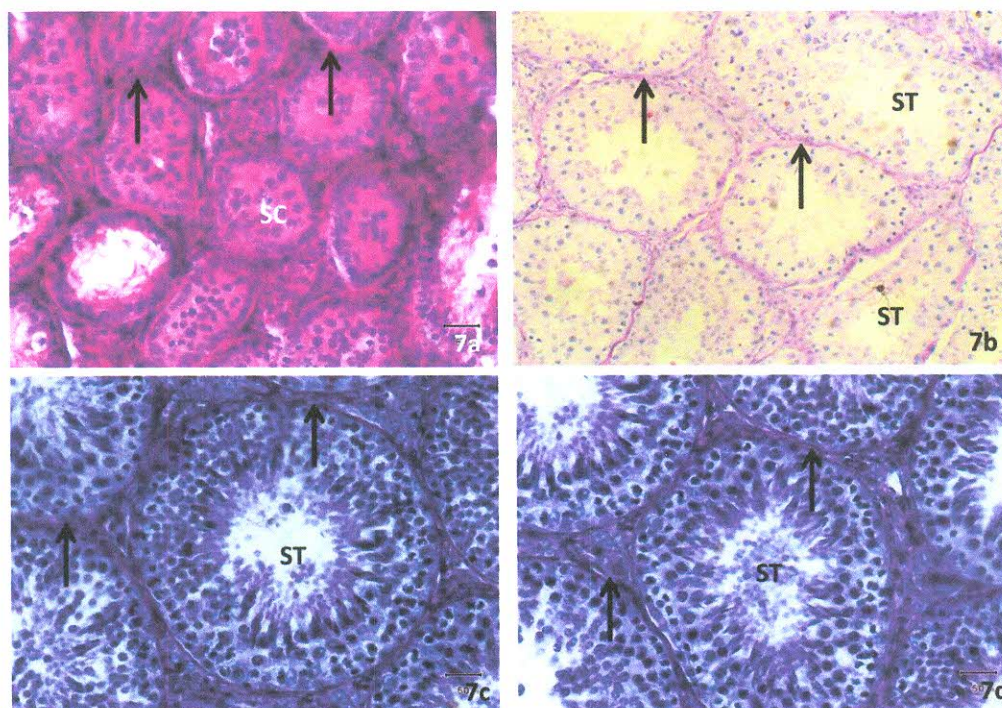


Figure 7. The primitive sex cords (SC) and true seminiferous tubules (ST) and having basement membrane consisting of collagenous connective tissue (arrow headed) in prepubertal (7a), pubertal (7b), and adult (7c, 7d) testes. HE 40X

In pubertal bulls, the average cross sectional lengths and breadths were $637.90 \pm 18.75 \mu\text{m}$ and $165.25 \pm 18.75 \mu\text{m}$, respectively. But in adult bulls, the average cross sectional lengths and breadths were $728.50 \pm 18.75 \mu\text{m}$ and $159.50 \pm 18.75 \mu\text{m}$, respectively. The parameters showed the cross sectional diameters increased with the advancement of age (**Figure 7, 8**).

The epithelium of the seminiferous tubules developed to form spermatogenic cells. The lining complex stratified epithelium was composed of spermatogenic cells arranged in chronological lineage (**Figure 9**) and sustentacular or Sertoli cells in the tubules. The spermatogenic cells were appeared in different stages of growth, proliferation and differentiation in the seminiferous tubules.

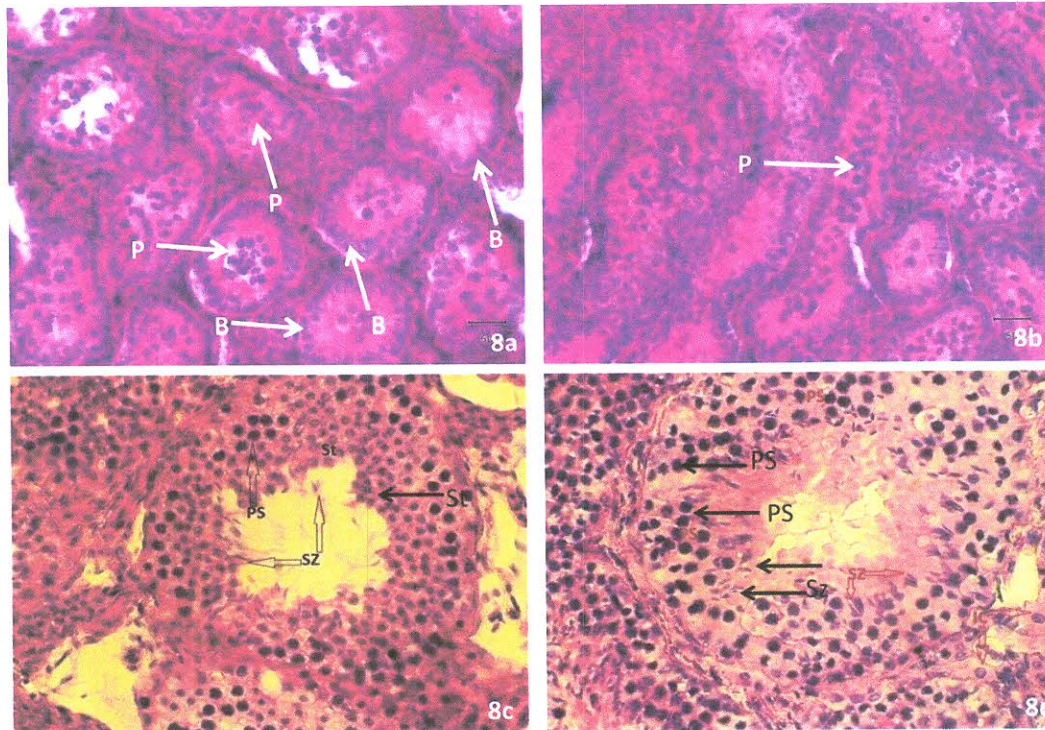


Figure 8. The cross sectional breadth and length of pre-pubertal (8a, 8b) bull with centrally placed primordial germ cell (P), basal cells (B), pubertal (8c) and adult (8d) showed primary spermatocytes (PS), spermatid (St), spermatozoa (SZ) and interstitial cells of Leydig (IC) in the seminiferous tubules.. HE 40X

The spermatogenic cells were located in between the Sertoli cells in an orderly manner with 4-8 layers occupying the space between the basal lamina and the lumen, while their successive descendants were progressively closer to the lumina of the seminiferous tubules, called the spermatogenic lineage. The spermatogenic layers divided into 5-8 in case of pubertal and 3-5 in case of adult bull (**Figure 9**). The spermatogenic cells were four categories, the primitive germ cells or spermatogonia, the primary spermatocytes, the secondary spermatocytes, spermatids or spermatozoa. All four types of cells were found within the fully lumined seminiferous tubules at the 18 month of early pubertal bulls. The primary spermatocytes, secondary spermatocytes and spermatids were almost developed within the 12-18 months period of time.

The primitive germ cells or spermatogonium were the smaller cells adherent to the basement membrane between the Sertoli cells (supporting cells). The cells were fixed, spherical or cuboidal or oval in shape with lightly acidophilic cytoplasm and distinct

spherical nuclei. The transformation of gonocytes into spermatogonia occurred in the prepubertal indigenous bulls.

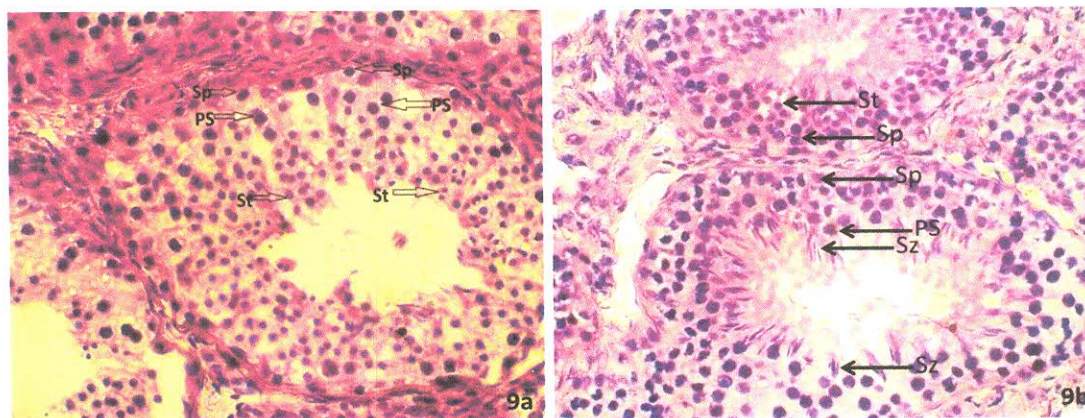


Figure 9. The sections of seminiferous tubules of pubertal (9a) and adult (9b) testes showing spermatogenic lineage of chronological cell display of spermatogonium (Sp), primary spermatocytes (PS), spermatid (St) and spermatozoa (Sz) of indigenous bulls. HE 40X

The primary spermatocytes were large spherical or oval shaped cells with large round or spherical centrally placed nucleus just next to the spermatogonia at inner side (**Figure 9**). They were biggest cells with large rounded or oval nuclei than the other spermatogenic cells. The cells showed different stages of proliferations.

Secondary spermatocytes were located internally and smaller than the primary spermatocytes, but larger than the round spermatids. They were rounded in shape and contained eosinophilic granular cytoplasm and the nuclei were spherical, centrally placed with distinct nuclear membrane. The numbers of secondary spermatocytes were fewer in numbers in the present study than the much greater primary spermatocytes and round spermatids at all three stages of indigenous bulls (**Figure 9**).

Spermatids were smaller cells located adjoined to the lumen of the seminiferous tubules. But in the transformational stages of the spermatids were seen usually as an early or cap spermatids or round spermatids and late or flagellated or elongated spermatids. The round spermatids were the smallest cells of the spermatogenic series. They possessed spherical nuclei in their cytoplasm. The elongated or flagellated spermatids observed elongated nuclei with condensed nuclear materials stained deeply

and remain attached to the apical part of the Sertoli cells, looked migrated centripetally towards the lumina of the seminiferous tubular lumen.

Spermatozoa were the final form of spermatids usually found attached to the apical part of the Sertoli cells at their luminal border and sometimes found in the tubular lumen (**Figure 9**). Spermatozoa also observed within the spermatogenic lineage just nearer to the apex of the Sertoli cells. The head was elongated or elongated oval in shape with flagellated tail occurred pubertal and adult bulls, but the early pubertal bulls showed smaller numbers of spermatozoa than the late pubertal and adult bulls. The spermatozoon was appeared first at or after 18 month of age.

The cells of the spermatogenic lineage especially spermatogonium, primary and secondary spermatocytes had more staining affinity than the spermatids and spermatozoa in the routine staining technique in the present study (**Figure 9**). The distribution and populations of spermatogenic cells in different seminiferous tubules were variable.

Sertoli cells were tall, irregularly columnar cells with broad basal portion laid on the basal lamina and extended to the lumen of the seminiferous tubules. The cells were looking transparent other than the ovoid nucleus in the routine staining methods in the tubules of pubertal and adult bulls. The nuclei were spherical, oval, elongated in shape, located near the basement membrane. Sertoli cells were located singly (**Figure 10**). The cell boundaries were not distinct.

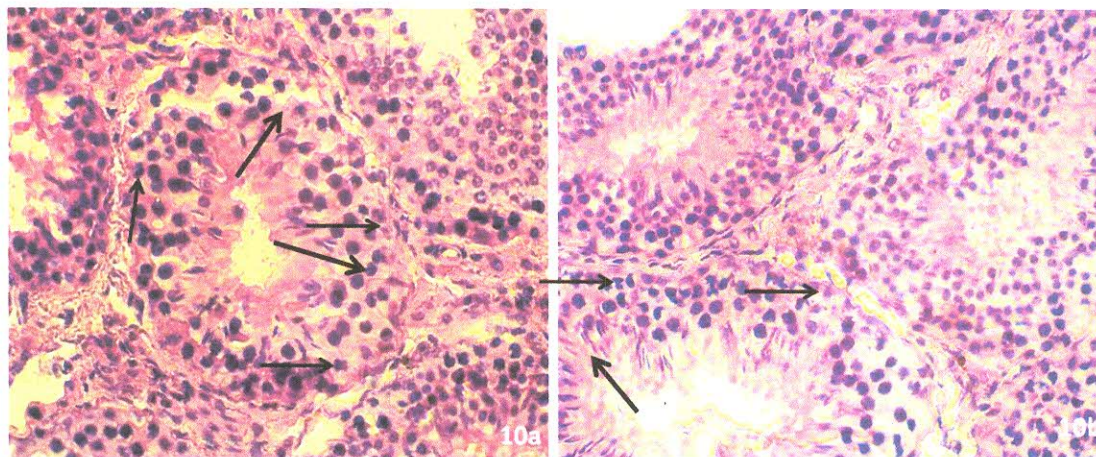


Figure 10. Seminiferous tubule showing spermatogenic lineage and singly placed Sertoli cells (arrow head marking) of adult bull (10a, 10b), 40x

4.5 Gross parameters of vesicular gland

The vesicular glands of indigenous bull were located on the dorso-lateral aspect of the neck of the urinary bladder (**Figure 11a**). It was lateral to the ampulla of vas deferens and the beginning part of the pelvic urethra (**Figure 11a**). The glands were paired, compact and multi lobular. Its surface was covered by a thick capsule of fibrous connective tissue. The lobulations of vesicular gland were distinct small area of fibrous cord present. At the junction of left and right vesicular gland with the union of ampulla and urethra for connecting the gland. The right and left glands of each bull were unsymmetrical, multilobulated in size and shape. From the pre pubertal stage to adult, the sizes become larger gradually and lobulation become more distinct (**Figure 11d**). The excretory duct opens at the seminal colliculus just lateral to the ductus deferens.

Table 4.6 Gross morphological parameters of vesicular gland (Mean $\pm$ SE) of indigenous bull from pre pubertal to adult stage (n=28)

Sl no.	Parameters	Pre pubertal		Pubertal		Post pubertal	
		Left	Right	Left	Right	Left	Right
1	Weight(gm)	3.87 $\pm$ 0.15 **	3.71 $\pm$ 0.21 **	13.82 $\pm$ 2.07 **	12.65 $\pm$ 2.00 **	20.37 $\pm$ 1.45 **	18.58 $\pm$ 1.46 **
2	Length(cm)	4.27 $\pm$ 0.41 **	4.01 $\pm$ 0.37 **	7.55 $\pm$ 0.36 **	7.23 $\pm$ 0.38 **	9.18 $\pm$ 0.70 **	8.86 $\pm$ 0.60 **
3	Width(cm)	0.75 $\pm$ 0.07 NS	0.75 $\pm$ 0.06 NS	1.95 $\pm$ 0.10 **	1.90 $\pm$ 0.10 **	2.52 $\pm$ 0.15 **	2.33 $\pm$ 0.34 **
4	Thickness(cm)	0.72 $\pm$ 0.03 NS	0.68 $\pm$ 0.01 NS	1.41 $\pm$ 0.11 **	1.28 $\pm$ 0.10 **	1.58 $\pm$ 0.09 **	1.51 $\pm$ 0.07 **

Asterisk (*) indicates significant difference (*p<0.05, **p<0.01 and NS= non significant)

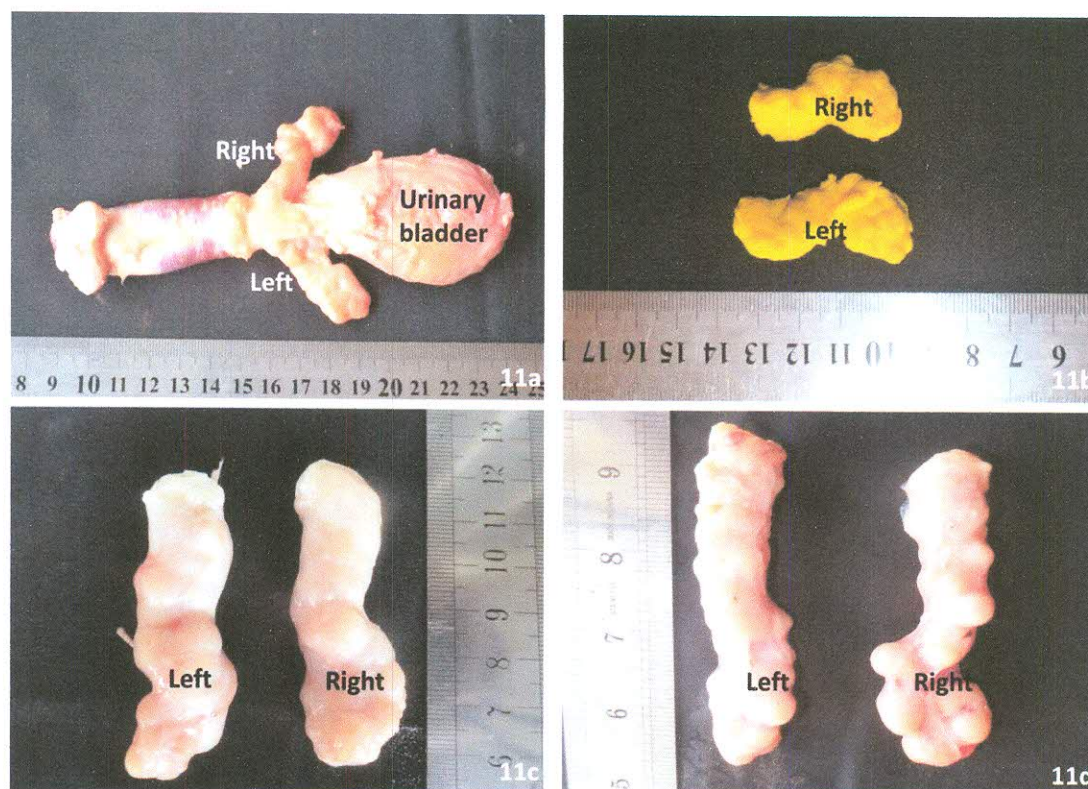


Figure 11: The right and left vesicular glands having multiple lobulations becomes distinct with the advancement of age and left one comparatively larger than the right of prepubertal (11a, 11b), pubertal(11c) and adult (11d) bull.

The gross morphological parameters of the vesicular gland of indigenous bull presented in **Table 4.6**. In the prepubertal bull, the average weight, length, width and thickness of left vesicular glands were $3.87 \pm 0.15\text{gm}$, $4.27 \pm 0.41\text{cm}$, $0.75 \pm 0.07\text{cm}$ and $0.72 \pm 0.03\text{cm}$ and right vesicular glands were $3.71 \pm 0.21\text{gm}$, $4.01 \pm 0.37\text{cm}$, $0.75 \pm 0.06\text{cm}$ and $0.68 \pm 0.01\text{cm}$ were respectively. In the pubertal bull, the average weight, length, width and thickness of the left vesicular glands were $13.82 \pm 2.07\text{gm}$, $7.55 \pm 0.36\text{cm}$, $1.95 \pm 0.10\text{cm}$ and $1.41 \pm 0.11\text{cm}$ and right vesicular glands were $12.65 \pm 2.00\text{gm}$, $7.23 \pm 0.38\text{cm}$, $1.90 \pm 0.10\text{cm}$ and $1.28 \pm 0.10\text{cm}$ respectively.

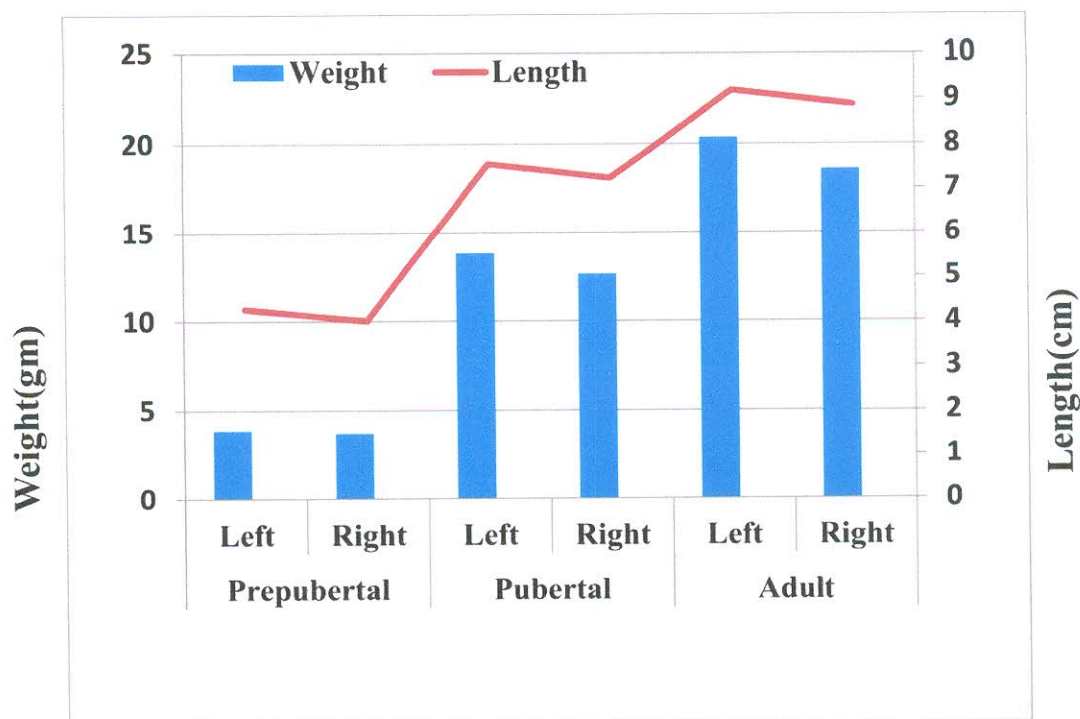


Figure 12. The bar chart showing weight(gm) and length (cm) of the vesicular glands of prepubertal, pubertal and adult indigenous bulls

In the adult bull, the average weight, length, width and thickness of the left vesicular glands were 20.37 ± 1.45 gm, 9.18 ± 0.70 cm, 2.52 ± 0.15 cm and 1.58 ± 0.09 cm and right vesicular gland were 18.58 ± 1.46 gm, 8.86 ± 0.60 cm, 2.33 ± 0.34 cm and 1.51 ± 0.07 cm, respectively (**Table 4.6**). Left vesicular glands were slightly higher than the right in all parameters (**Figure 11,12**). The weight, length, width and thickness of the vesicular gland grew faster during pre-pubertal to pubertal stage than pubertal to adult stage of bull's growth. All parameters of the vesicular gland of pubertal and adult group were significantly increased.

Table 4.7 Vesicular weights as a percentage of the body weights of the indigenous bulls

Type of bulls	Body weight (Average mean in kg)	Left vesicular weight in % of body weight	Right vesicular weight in % of body weight
Prepubertal	82.39	0.0046	0.0045
Pubertal	205.34	0.0067	0.0061
Adult	319.22	0.0063	0.0058

The left and right vesicular weights were 0.0046% and 0.0045% of the body weight of the prepubertal indigenous bulls. The left and right vesicular weights were 0.0067% and 0.0061% of the body weight of the pubertal indigenous bulls, but in adult conditions, it was 0.0063% and 0.0058% of the body weight of the adult bulls presented **Table 4.7**. In the present study the vesicular weight percentage was highest in the pubertal bull in case of both left and right vesicular gland. Percentage was always slightly higher in the left than the right in all groups.

Table 4.8 Weight gains of vesicular gland and length increases in pubertal and adult bull from the prepubertal indigenous bull calculated in percentage.

Sl. no	Parameters	Prepubertal weight(gm) and length (cm) in mean		Pubertal	Adult
1.	Weight gain %	Left	3.87	357	526
		Right	3.71	340	500
2.	Length gain %	Left	4.27	176	214
		Right	4.01	180	220

The left and right vesicular glands weights and length were increased from the prepubertal bull to pubertal and prepubertal to adult condition were calculated in percentage presented **Table 4.8**.

4. 6 Histology of vesicular gland

The vesicular gland of indigenous bull was a multi lobulated compound tubulo-alveolar gland. The glandular parenchyma divided into lobules with irregular shape and different size. The left and right vesicular gland had similar histological inspection.

4. 6.1 Capsule and interlobular septa

The capsule of the vesicular gland was mainly composed of smooth muscle bundles with inter-muscular irregular collagenous connective tissue (**Figure 13**). The well-developed fibro-muscular trabeculae (**Figure 14**) divided the vesicular glands into lobes of varying shapes, numbers and sizes. The secretory alveoli or end pieces and ducts were surrounded by a connective tissue. The loose connective tissue layer was

continuous with the dense fibro-muscular trabeculae. The thick tunica muscularis gave off interlobular fibro-muscular septa into the gland proper was predominantly muscular (**Figure 14**). Groups of smooth muscle cells were observed to arise from muscle layer and enter the mucosa layer (**Figure 17**). Single or groups of smooth muscle cells forming incomplete layer were observed beneath the secretory epithelium (**Figure 15**).

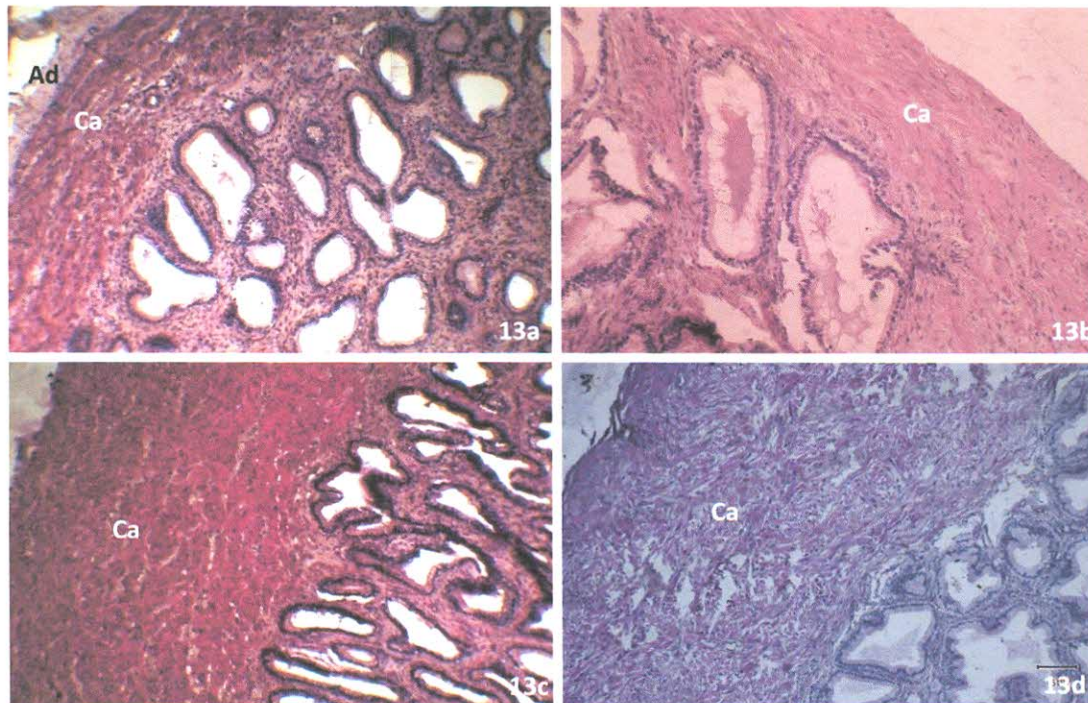


Figure 13. Vesicular gland showing adventitia(Ad) and thickness of fibro-muscular capsule (Ca) of prepubertal bull (13a), pubertal (13b, 13c) and adult (13d) bull. HE x10

The muscularis layers were present in the covering of the vesicular gland surrounded the gland proper mainly the smooth muscle bundles arranged circularly in all three age group of indigenous bull (**Figure 13**). With the advancement of age the layer significantly increased in diameters (**Table 4.9 and Figure 13**). The tunica adventitia composed collagenous connective tissue (**Figure 13a**).

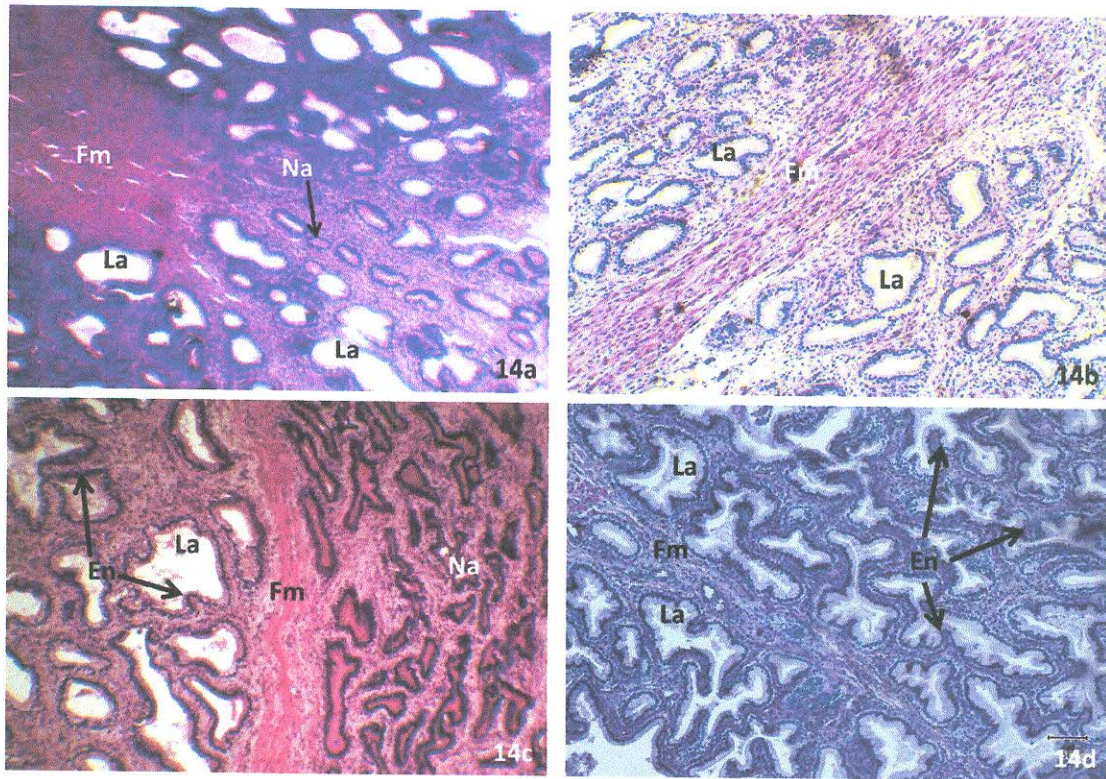


Figure 14. Thickness of interlobular fibro muscular septa (Fm) consisting of smooth muscle bundle separating the glandular parenchyma and luminated secretory alveoli (La) with the mucosal enfolding (En) with secretion and non-luminated alveoli (Na). Prepubertal(14a, 14b), pubertal (14c) and adult (14d) bull. HE x10

Many blood vessels, nerve fibers and autonomic ganglions were observed in the tunica adventitia. The histometric data of tunica adventitia showed that the continuous and progressive and significant increased from prepubertal to adult stage of bull (**Table 4.9**). Tunica muscularis has similar trend except the interstitial tissue changes were seen more prominent from the prepubertal to pubertal stage than pubertal to adult stage of development.

Table 4.9 Microscopic measurement of various component of vesicular gland
(Mean±SE) in indigenous bull of different age group (n=28)

Sl no.	Parameters (µm)	Pre pubertal	Pubertal	Post pubertal
1	Thickesses of tunica adventitia	431.50±22.15**	514.00±36.20**	701.00±22.49**
2	Thickesses of tunica muscularis	463.00±20.12**	659.00±15.27**	693.00±8.69**
3	Thickesses of interstitial tissue	219.00±22.64**	118.00±17.95**	70.00±8.68**
4	Glandular end pieces			
	a) Minimum diameter of luminated acini	72.50±4.54**	110.00±4.64**	155.00±3.28**
	b) Maximum diameter of luminated acini	200.00±5.99**	256.25±4.61**	340.00±4.64**
	c) Epithelial height	10.00±0.64 ^{NS}	21.10±0.93 ^{NS}	21.50±0.88 ^{NS}
5	Duct			
	a) Diameter of duct	162.50±5.91**	220.00±7.34**	331.25±12.91**
	b) Epithelial height	11.00±0.40 ^{NS}	20.80±0.68 ^{NS}	21.30±0.59 ^{NS}

Asterisk (*) indicates significant difference (*p<0.05, **p<0.01 and NS= non significant)

4. 6. 2 Glandular lobules and secretory end pieces

The glands were compound tubule-alveolar appeared as luminated and non luminated. Occasionally, the luminated and non-luminated secretory lobules were located separately by the interlobular fibro muscular septa (**Figure 14**). The secretory end pieces or alveoli were observed luminated or non-luminated in the same lobules or sometimes separated (**Figure 14a, 14c**). The numbers of glandular units were more in per unit area in the pre pubertal bull than the pubertal and adult. In the prepubertal bull, minimum and maximum diameters of glandular end pieces were 72.50±4.54 µm and 200.00±5.99µm, respectively in the luminated acini. In the pubertal bull, minimum and maximum diameters of glandular end pieces were 110.00±4.64 µm and 256.25±4.61µm, respectively in the luminated acini. In the post pubertal or adult bull,

minimum and maximum diameters of glandular end pieces were $155.00 \pm 3.28 \mu\text{m}$ and $340.00 \pm 4.64 \mu\text{m}$, respectively in the luminated acini (**Table 4.9**). These observations indicated that the maximum and minimum diameters of luminated glandular end pieces increased gradually and significantly with the advancement of age. The glandular size was increased significantly with the advancement of age and reached maximum at adult stage. The alveolar diameter increased gradually with the age and exposed apocrine mode of secretion.

Secretary end pieces or alveoli were arranged together in a group where the alveoli of the gland apex were large in size. In the prepubertal bull, the small and large rounded and/or oval secretary alveoli were observed in a same lobule (**Figure 14a, 14b**). In the pubertal bull, the small and large rounded, oval and multigonal secretary alveoli were observed in a same lobule (**Figure 14c**). In the adult bull, the shape of the small and large rounded, oval and multigonal secretary alveoli were grouped in a same lobule (**Figure 14d**). The alveoli of each lobule of the gland showed gradually folded mucosa in pubertal and adult bull, whereas, in prepubertal bull, almost no mucosal enfolding observed (**Figure 14**). The alveoli usually lined by cuboidal to columnar or pseudostratified columnar epithelium. In sections, the mucosa forms thin folds projects into the lumen. The free surface of epithelial cells was projected into the lumen of secretary end pieces as an elongated or spheroidal cytoplasmic projection. Some of the cells had light, bleb-like apical projections. These projections were acidophilic inverted pear shape structures. The highly eosinophilic vacuolated secretion was found in the lumina of the secretary acini.

4. 6. 3 Glandular epithelium

The lining epithelium of end pieces of vesicular gland was generally pseudostratified columnar epithelium. In the pre pubertal bull, the epithelium showed simple cuboidal or simple columnar type (**Figure 15a, 15b**). Pseudo stratification was clear at the pubertal and adult bull in the vesicular gland (**Figure 15c, 15d, 15e and 15f**). The lining cells were A, B and C type. Type A cells was tall columnar cells having distinct cell boundaries with the oval, round or elongated nucleus (**Figure 15a, 15b**). The cytoplasm of the cells was finely granular and faintly eosinophilic cytoplasm. Type B cells or

basal cells were located in between the basal lamina and the type A cells having round or oval nucleus with indistinct cell boundaries (**Figure 15a, 15b, 15e, 15f**).

The shape of the cell was spherical, trigonal, ovoid or lens-shaped and their cytoplasm was clear (Figure 15a, 15b, 15e, 15f). The basal cells were observed from prepubertal to adult stage. Type C cells were narrow columnar cells interspersed between A cells with darkly stained cytoplasm with hyper chromic nuclei (Figure 15a, 15b). They were fewer than the type A and B cells. The observation of type C cells was numerous in the pre pubertal bull, but rarely existed in pubertal and post pubertal bull (Figure 15a, 15b).

The epithelial height of glandular acini and duct were $10.00 \pm 0.64 \mu\text{m}$ and $11.00 \pm 0.40 \mu\text{m}$ in the prepubertal bull, $21.10 \pm 0.93 \mu\text{m}$ and $20.80 \pm 0.68 \mu\text{m}$ in the pubertal bull and $21.50 \pm 0.88 \mu\text{m}$ and $21.30 \pm 0.59 \mu\text{m}$ in the adult bull, respectively (**Table 4.9**). The present study indicated that epithelial height of the duct and alveoli increases with the advancement of age, but no significantly difference among them. It was remarked that chief secretary cells A were not grow structurally rather mature functionally at puberty or adult. The cytoplasm of cells were mostly vacuolated, eosinophilic with spherical to ovoid shape nuclei in all three types (A, B and C) cells.

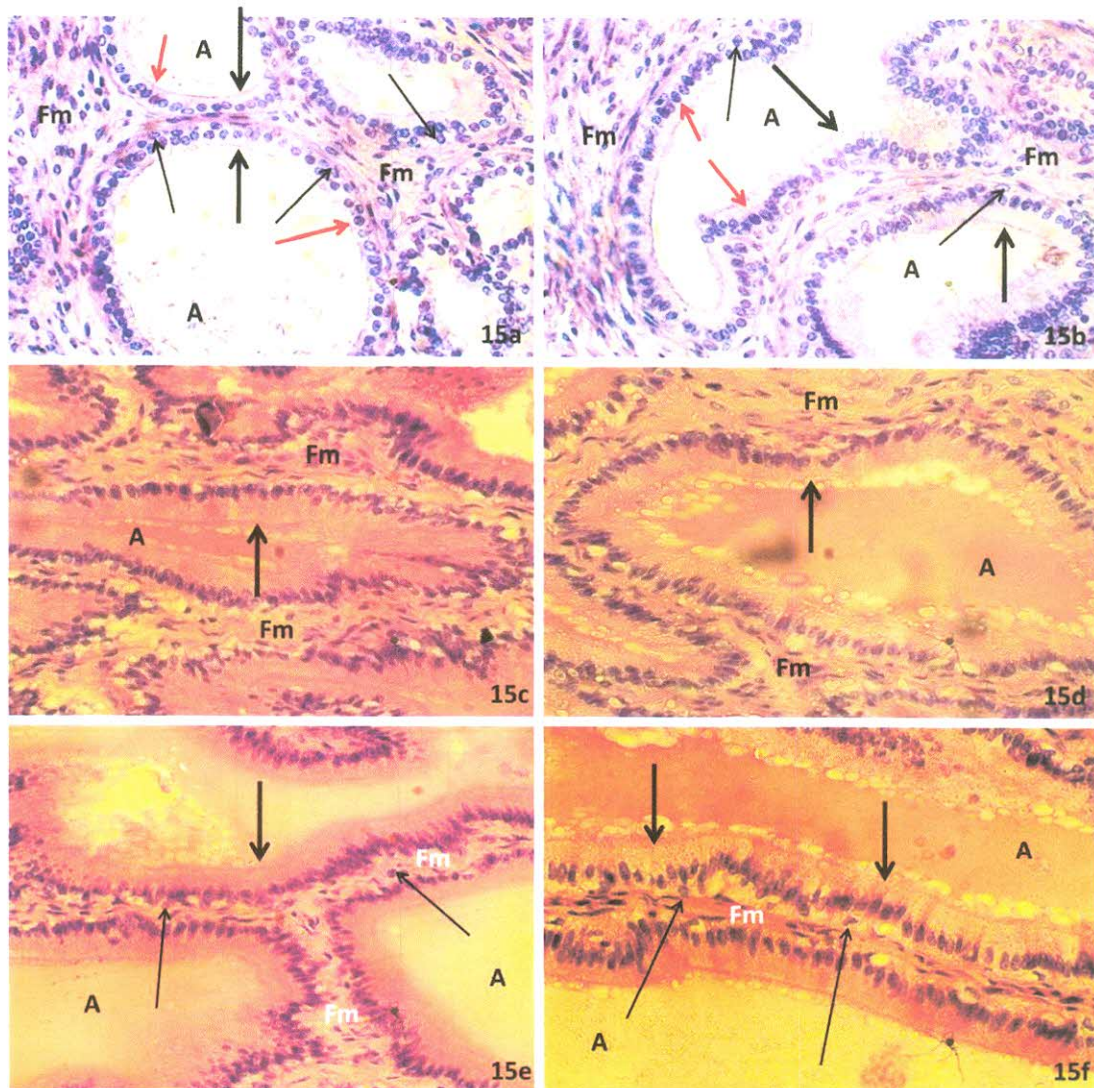


Figure 15. Vesicular gland showing thickness of interstitial fibromuscular tissue septa (Fm), secretory alveoli (A) and height of lining pseudostratified epithelium, secretion in the luminal space with type A cells (double black arrow), basal cells B(single black arrow) and C cells(red arrow). Prepubertal (15a, 15b), pubertal(15c, 15d) and adult(15e, 15f) bull. HE x40

4. 6. 4 Ducts

The lumen of the intralobular ducts appeared highly evaginated and invaginated forming primary mucosal folds like structure elongated in shape especially in pubertal and adult bulls and lined by tall columnar or pseudo stratified columnar cells (**Figure 17**).

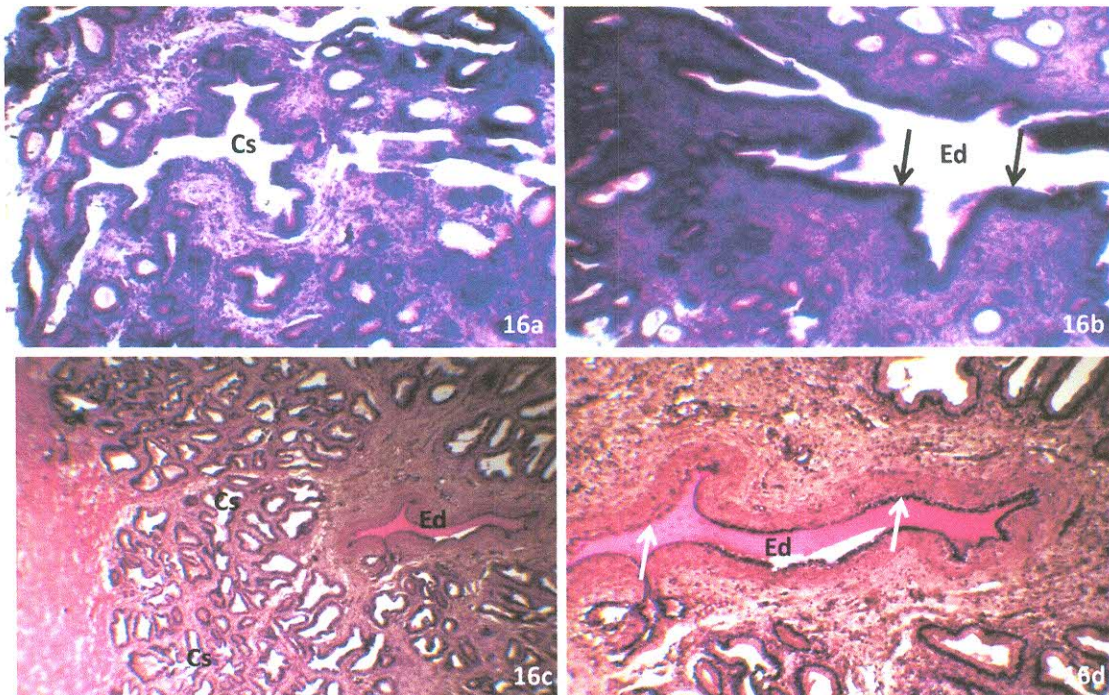


Figure 16. Vesicular gland showing developing collecting sinus (Cs) in the glandular lobules, main excretory duct (Ed) lined by pseudostratified columnar epithelium (arrow headed) of prepubertal (16a, 16b) and pubertal bull (16c, 16d). HE x10, 5c (HE x4).

In prepubertal bulls, the luminal surface of the intralobular duct or collecting sinus less invaginated compared with the adult bull and lined by simple columnar epithelium (**Figure 16a**). The nuclei were spherical, central, lightly stained. The average diameters of the duct and the height of the lining epithelium were $162.50 \pm 5.91 \mu\text{m}$ and $11.00 \pm 0.40 \mu\text{m}$, respectively in prepubertal bulls. The average diameters of the duct and the height of the lining epithelium were $220.00 \pm 7.34 \mu\text{m}$ and $20.80 \pm 0.68 \mu\text{m}$, respectively in pubertal bulls. The average diameters of the duct and the height of the lining epithelium were $331.25 \pm 12.91 \mu\text{m}$ and $21.30 \pm 0.59 \mu\text{m}$, respectively in adult bulls. It is evident that, the diameters of the duct and epithelial height increased with the advancement of age.

The intralobular duct or central collecting sinus of each lobule united to form the interlobular duct. The union of several such ducts, in turn formed the main excretory duct of the vesicular gland. The intralobular duct, interlobular and main excretory ducts contained folded mucosa which was lined by pseudostratified columnar epithelium

(Figure 16a, 16b, 17). Three types of cells were also identified in the pseudo stratified epithelium containing A, B and C cells as glandular epithelium.

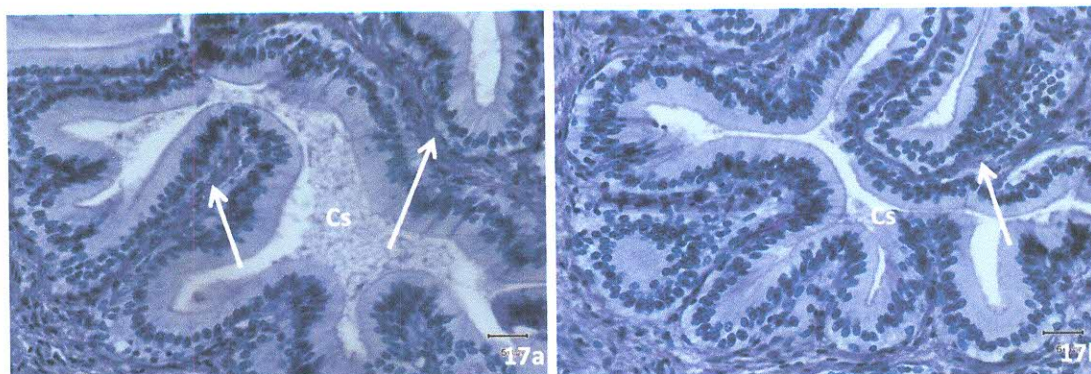


Figure 17. Vesicular gland showing collecting sinus (Cs) with secretion enfolded mucosa lined by pseudostratified columnar epithelium supported by smooth muscle (arrow headed) enter the mucosa layer of adult bull. HE x40

4. 7 Gross anatomy of prostate

The prostate glands of bull consisted of two portions, external portion or corpus prostate and internal or pars disseminata portion. The two portions were distinguished, compact external portion or corpus prostate was observed dorsal of the pelvic urethra at the level of the colliculus seminalis. The pars disseminata portion was located along the entire wall of the pelvic urethra. The corpus prostate was a white colored band of tissue observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. The corpus prostate was found in all three groups of bulls. In prepubertal, pubertal and adult bulls, the corpus prostate was frequently observed in various samples. In adult bulls, it was observed approximately higher in raised area of the gland with dorsal depressing line (**Figure 18b**), whereas in prepubertal bull dorsal surface was convex (**Figure 18a**).

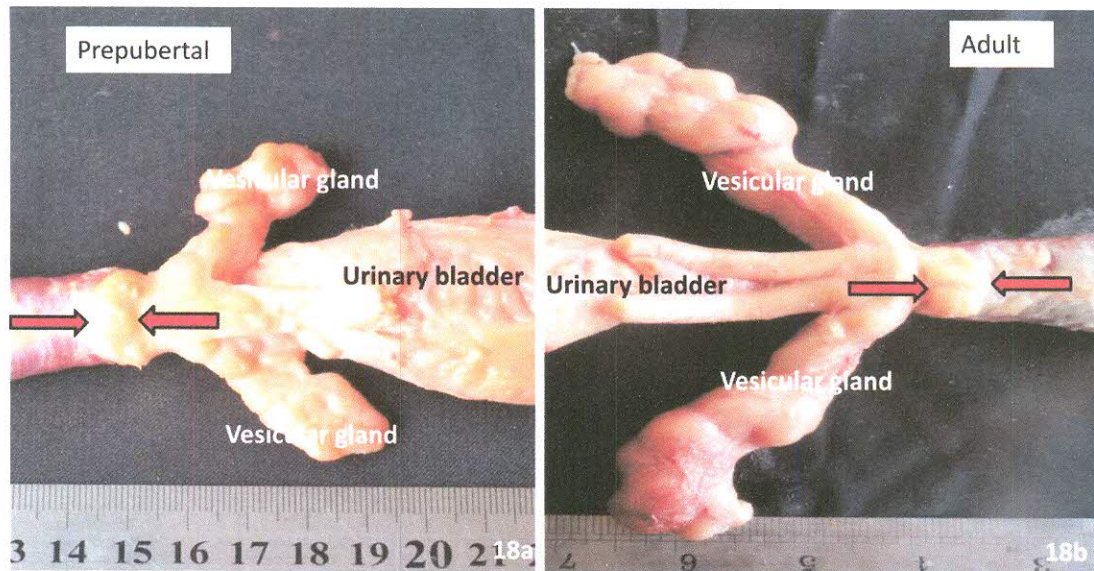


Figure 18: The raised arrowhead structures white colored band of tissue adjoined the two vesicular glands at the junction of urinary bladder with pelvic urethra are the corpus prostate convex (18a) and with dorsal depressing line (18b).

Internal portion of the gland occurred in the urethra from its junction with the urinary bladder to the level of the ischial arch. It was surrounded by the urethral muscle which was thin dorsally, increased in thickness laterally and became thin ventrally.

4. 8 Histological parameters

The corpus portion and disseminate portion of the prostate gland has similar histological structures except the histometric parameters. The corpus prostate was well demarcated with the pars disseminate prostate by the fibro muscular connective tissue septa. The corpus capsule enclosed by the connective tissue composed of collagenous fibers along with smooth muscle cells (**Figure 19**). The smooth muscle fibers were observed predominantly in the capsule, trabeculae and interstitial connective tissue septa than the fibrous components (**Figure 19**). The capsules gives off trabeculae and the trabeculae gives off interlobular septa and the glandular parenchyma divided into lobe and lobules. The thickness of capsule and trabeculae were varies with the location of the gland. Usually, capsular thickness was higher than the trabecular and finally interlobular septa lesser than the trabecular (**Figure 20**).

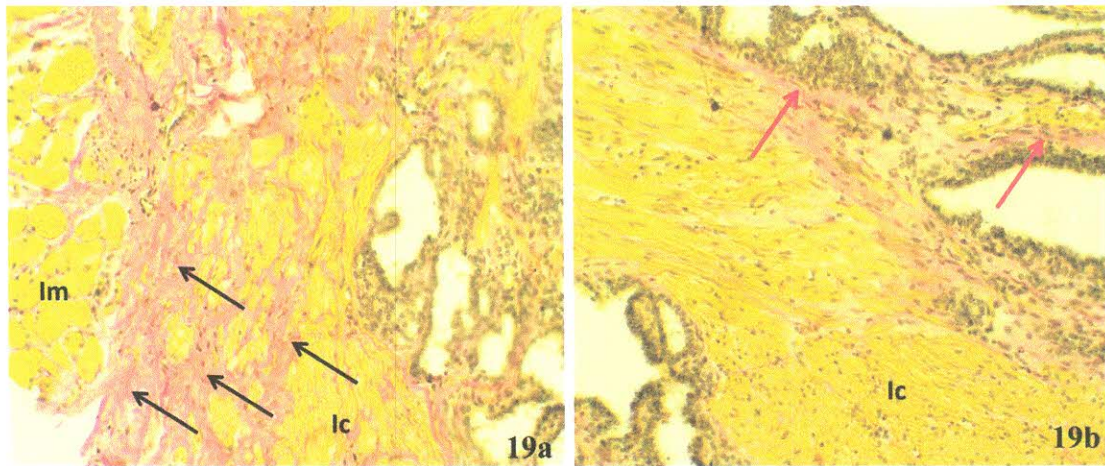


Figure 19. Prostate gland showing the fibro muscular capsule with collagen fibers pink color (arrow headed), yellowish color inner circular (Ic) and longitudinal muscle (Im) bundles and fibro muscular connective tissue septa with collagen fiber bundles beneath the epithelial lining (red arrow headed) dividing glandular lobules of the pubertal bull. Vangieson's stain 10x

Table 4.10 Microscopic measurement (μm) of various component of prostate gland (Mean $\pm$ SE) in indigenous bull of different age group (n=28)

Sl no.	Parameters (μm)	Pre pubertal	Pubertal	Post pubertal
1	Interlobular CT thickness	150.00 ^a $\pm$ 6.56	107.00 ^b $\pm$ 3.67	80.00 ^c $\pm$ 4.64
2	Secretary end pieces			
	a) Minimum diameter of luminated acini	63.75 ^b $\pm$ 4.99	135.00 ^a $\pm$ 5.68	135.00 ^{aa} $\pm$ 6.83
	b) Maximum diameter of luminated acini	150.00 ^a $\pm$ 9.28	202.50 ^b $\pm$ 7.52	260.00 ^a $\pm$ 8.04
	c) Epithelial height	11.01 ^b $\pm$ 0.92	14.56 ^a $\pm$ 0.63	16.62 ^{aa} $\pm$ 0.59
3	Duct			
	a) Diameter of duct	350.00 ^c $\pm$ 7.09	545.00 ^b $\pm$ 8.68	650.00 ^a $\pm$ 7.09
	b) Epithelial height	10.93 ^b $\pm$ 0.67	13.82 ^a $\pm$ 0.61	13.93 ^{aa} $\pm$ 0.78

Means with different superscripts within each row for each parameter differ significantly

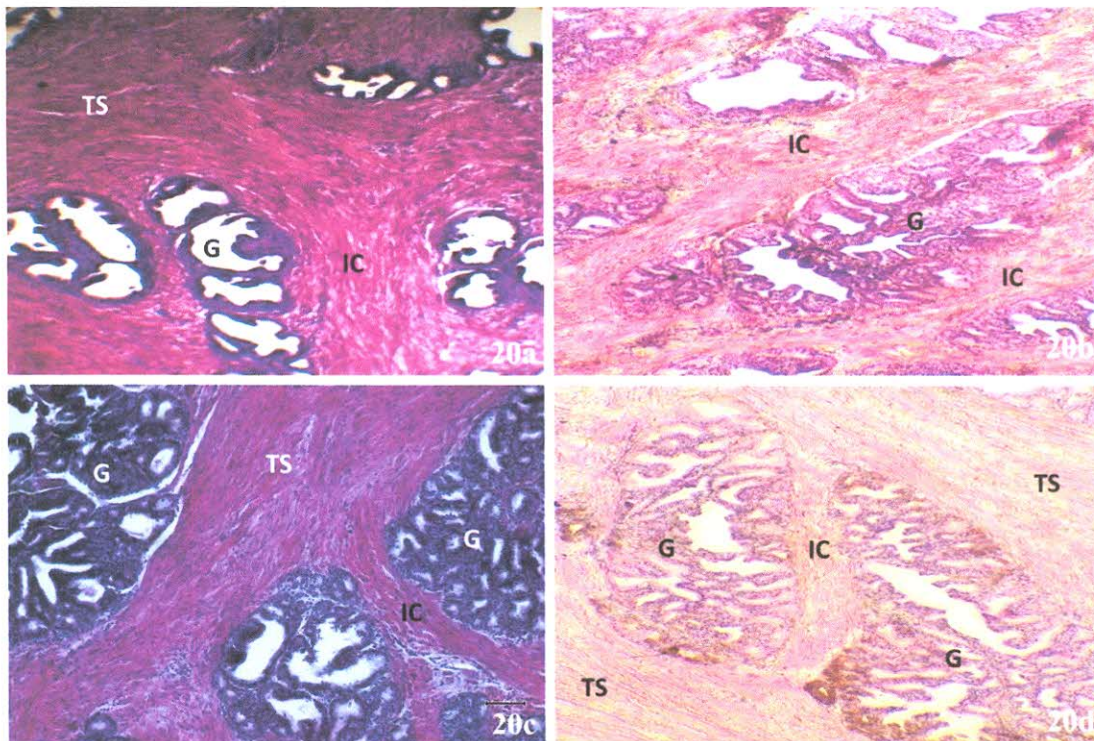


Figure 20. Showing trabeculae septa (TS) and interlobular connective tissue septa(IC) and the glandular unit. [Prepubertal (20a), pubertal(20b) and adult (20c, 20d). HE 10X]

The capsule composed of collagenous tissue fibers and abundant smooth muscles arranged circularly (**Figure 19**). The average interlobular septal thickness was represented in **Table 4.10**. The observation was interlobular connective tissue thickness decreased (**Figure 20**) significantly ($p < 0.01$) along with the advancement of age. The collagen fibers were observed beneath the mucosal lining of the glandular lobules. Several autonomic ganglia, encapsulated nerve endings were observed in the connective tissue.

4. 8. 1 Secretory glandular end pieces

The secretory end pieces were luminated and non luminated distributed within the wall of the pelvic urethra and in the corpus portion of the prostate. In pre-pubertal bull, the mean minimum and maximum diameters of luminated secretory end pieces were $63.75 \pm 4.99 \mu\text{m}$ and $150.00 \pm 9.28 \mu\text{m}$, respectively (**Table 4.10**). In pubertal and adult bull, the average minimum and maximum diameters of luminated acini were $135.00 \pm 5.68 \mu\text{m}$ and $202.50 \pm 7.52 \mu\text{m}$, $135.00 \pm 6.83 \mu\text{m}$ and $260.00 \pm 8.04 \mu\text{m}$, respectively.

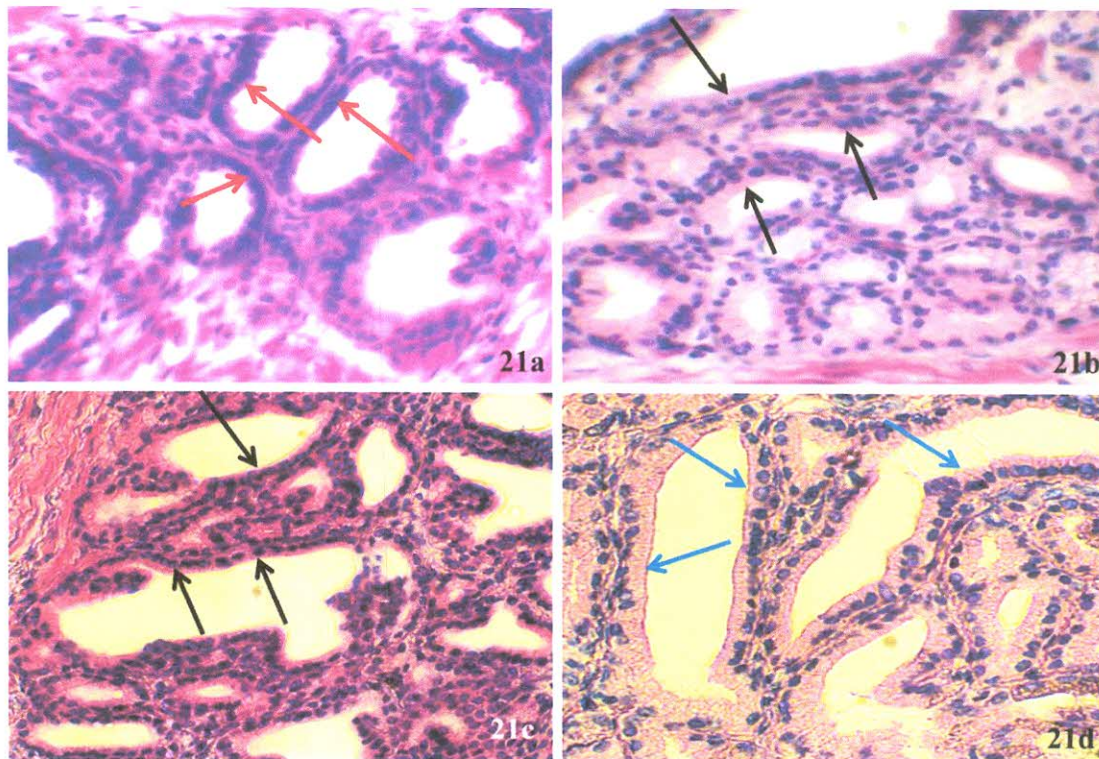


Figure 21. The secretory acini are lined by the simple low cuboidal epithelium (red arrow head) with rounded nucleus, simple columnar epithelium (black arrow head) with large rounded nucleus, simple high columnar epithelium (blue arrow head) with oval or flattened basally placed nucleus in the lobules of prepubertal (21a), pubertal (21b, 21c) and adult (21d) prostate gland bull, HE 40X

This observation was indicated the diameter increases gradually and significantly ($p < 0.01$). In prepubertal bull, the secretory end pieces were lined by simple low cuboidal epithelium with the rounded nucleus (**Figure 21a**). In the pubertal bull, the secretory end pieces were lined by simple columnar epithelium with the rounded to oval nucleus (**Figure 21b, 21c**). In the adult bull, the secretory end pieces were lined by simple high columnar epithelium with the oval basally placed nucleus (**Figure 21d**). Cytoplasm of the lining epithelium don't have secretory granules in prepubertal that appeared in pubertal and highly loaded in adult bulls. Due to the increased secretory granules, the nucleus gradually moved towards the basement membrane and turned from round to oval. Secretory granules in the cells as well as secretion in the lumen increased along with the advancement of age. The height of the lining epithelium increased (**Table 4.10**) with the advancement of age significantly ($p < 0.01$). Although the all three groups of bull showed both luminated and non luminated secretory end

pieces. With the advancement of age, the number of luminated secretory end pieces increased over the non luminated secretory end pieces. The glandular alveoli were mainly mucous type, only a few serous alveoli.

4. 8. 2 Ducts

The ducts of the corpus prostate opened into numerous openings in the pelvic urethra. The lobules of the pars disseminata contained ducts which opened into the pelvic urethra. Interlobular ducts or collecting sinuses had irregular lumen lined by pseudostratified columnar epithelium or simple columnar epithelium in prepubertal bulls (**Figure 22a**). The average diameters of duct and epithelial height were represented in **Table 4.10**. The duct of prepubertal bulls were lined by multilayered stratified cuboidal at the developing internal enfolding in the lining epithelium. The ducts of the pubertal bulls contained folded mucosa lined by simple columnar, pseudo stratified columnar or sometimes stratified cuboidal epithelium with large rounded dark nucleus in the centre of the cells (**Figure 22b**).

The ducts of the adult bulls contained folded mucosa were lined by simple columnar, pseudo stratified columnar or sometimes stratified cuboidal epithelium with comparatively smaller dark rounded or oval shaped nuclei located basally of the cells (**Figure 22c, 22d**). The typical enfolding observed from prepubertal to adult bulls and height and number of the enfolding increased with age (**Figure 22**). The average diameters of the ducts and the height of the epithelium increased significantly ($p < 0.01$) with the advancement of age. The ducts of the disseminata prostate and the ducts of the corpus prostate had similar histological feature with histometric exception. The ducts were opened into the lumen of the urethra having transitional epithelium nearer at the opening. The lumen of the duct of prepubertal bulls showed clear, lumen of pubertal and adult bulls showed secretion indicating the functional state of the gland.

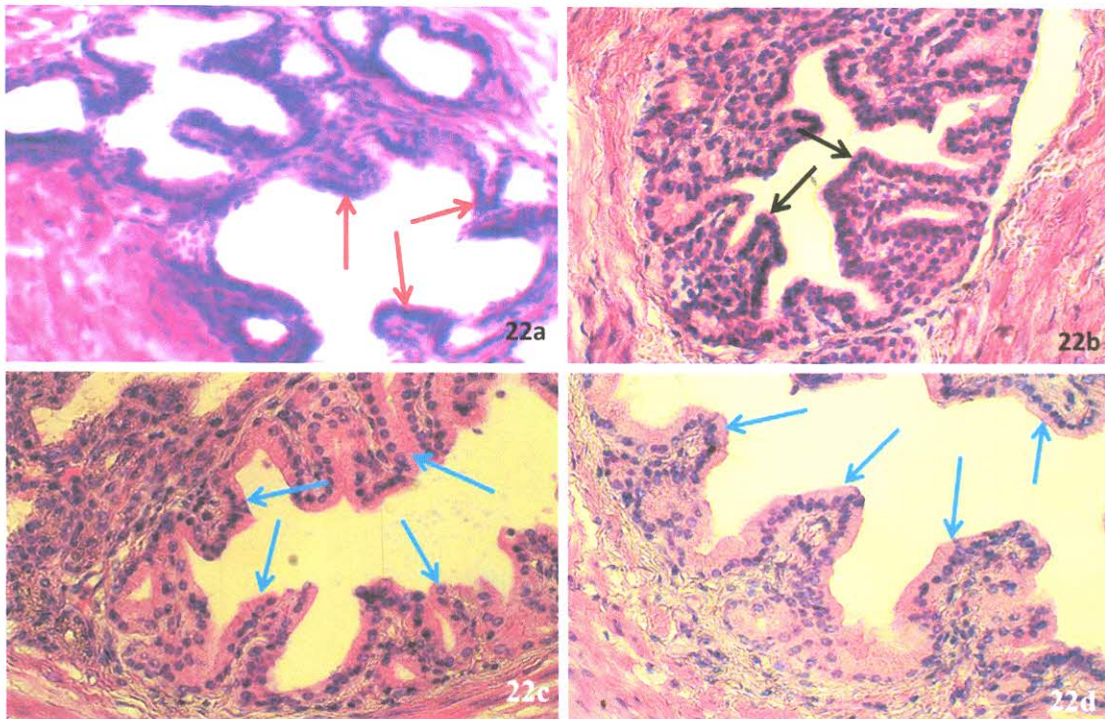


Figure 22. Prostate gland showing ducts of the glandular lobules developing with typical internal mucosal folding lined by mostly simple columnar or pseudo stratified columnar epithelium (arrow head) containing rounded or oval dark nuclei of the pre pubertal (22a), pubertal (22b) and adult (22c, 22d) bull. HE x40

4. 9 Gross parameters of bulbourethral glands

The bulbourethral glands of the bulls were paired, rounded or ovoid in shape located either sides of the pelvic urethra cranial to the ichiatic arch (**Figure 23-24**). They are covered by a thick fibrous capsule and the thick striated bulbocavernous muscles. Gross morphological parameters of bulbourethral gland (Mean $\pm$ SE) of indigenous bull from pre pubertal to adult stage presented in **Table 4.11**. The table reveals that the left bulbourethral gland is larger and heavier than the right one in all parameters (length, width and thickness and weight) in all three groups. The changes of gross parameters were increased significantly ($p<0.01$) with age and reached the maximum at post-pubertal or adult stage indicates the full function of the gland may attain at the adult stage.

Table 4.11 Gross morphological parameters of bulbourethral gland (Mean $\pm$ SE) in indigenous bull from pre pubertal to adult stage (n=28)

Parameters		Pre pubertal		Pubertal		Adult	
		Left	Right	Left	Right	Left	Right
1	Weight (gm)	0.93 $\pm$ 0.07 **	0.86 $\pm$ 0.07 **	2.65 $\pm$ 0.21 **	2.30 $\pm$ 0.24 **	3.73 $\pm$ 0.16 **	3.27 $\pm$ 0.14 **
2	Length (cm)	1.47 $\pm$ 0.11 **	1.37 $\pm$ 0.09 **	1.63 $\pm$ 0.05 **	1.55 $\pm$ 0.04 **	2.08 $\pm$ 0.08 **	1.98 $\pm$ 0.08 **
3	Width (cm)	1.08 $\pm$ 0.10 **	0.99 $\pm$ 0.10 **	1.50 $\pm$ 0.06 **	1.44 $\pm$ 0.05 **	1.99 $\pm$ 0.09 **	1.91 $\pm$ 0.10 **
4	Thickness (cm)	0.80 $\pm$ 0.04 **	0.75 $\pm$ 0.03 **	1.17 $\pm$ 0.06 **	1.13 $\pm$ 0.06 **	1.50 $\pm$ 0.03 **	1.41 $\pm$ 0.03 **

Asterisk (*) indicates significant difference (*p<0.05, **p<0.01 and NS= non significant)



Figure 23. The figure showing the bulbourethral glands of prepubertal indigenous bull (arrow head) were paired, rounded shaped attached at the terminal end of the pelvic urethra

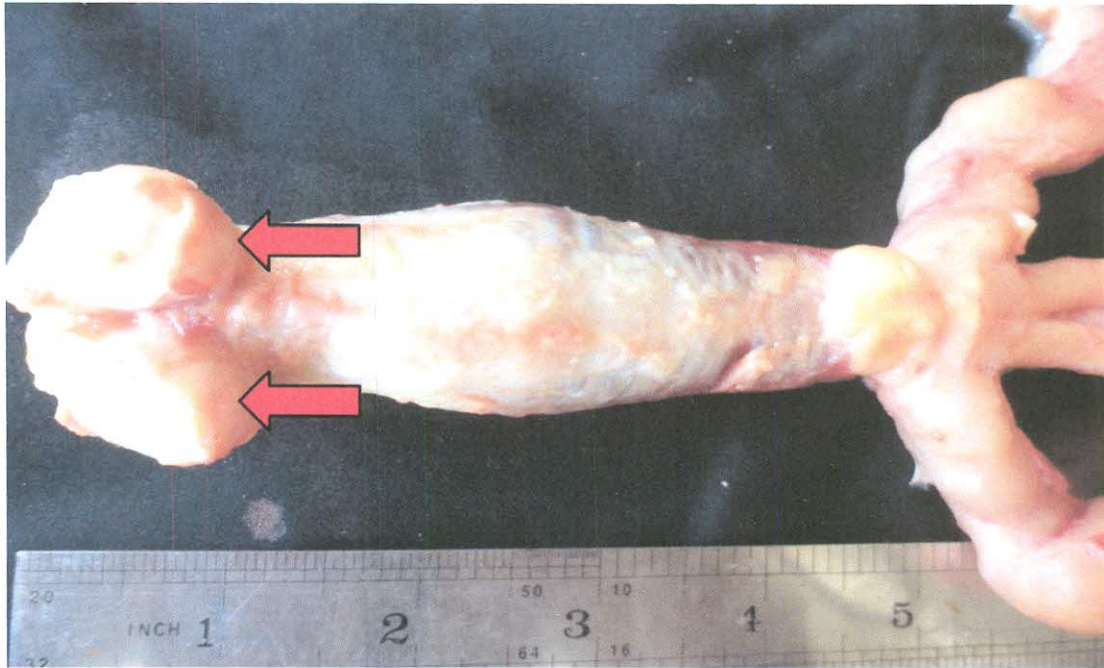


Figure 24. The figure showing the bulbourethral glands of adult indigenous bull (arrow head) were paired, comparatively oval shaped attached at the terminal end of the pelvic urethra

4. 10 Histological parameters of bulbourethral glands

4.10.1 Capsule

Bulbourethral gland was covered by collagenous fibrous connective tissue fibers. The average thickness of connective tissue capsule in prepubertal bull was $85.63 \pm 3.60 \mu\text{m}$. It gave off interlobular connective tissue septae in the gland and the divided the gland into lobes and lobules. The intralobular connective tissues were dispersed and surrounded the glandular end pieces or secretory unit of the glandular lobules. The average thickness of interlobular connective tissue was $56.87 \pm 2.33 \mu\text{m}$ in the prepubertal bull. The capsule was rich in collagen fibers, whereas interlobular and intralobular connective tissue had composed of mild reflection. The loose connective tissue contained some fibroblasts cell, blood vessels and nerves.

Table 4.12 Microscopic measurement of various component of bulbourethral gland (Mean $\pm$ SE) in indigenous bull of different age group (n=28)

Parameters (μ m)	Pre pubertal	Pubertal	Adult
1. Thicknesses of capsule	85.63 ^c $\pm$ 3.60	169.38 ^b $\pm$ 2.20	333.75 ^a $\pm$ 3.44
2. Thicknesses of interlobular tissue	56.87 ^a $\pm$ 2.30	33.75 ^b $\pm$ 1.57	24.37 ^c $\pm$ 1.47
3. Glandular end pieces			
a) Minimum diameter of luminated acini	53.12 ^c $\pm$ 1.88	118.75 ^b $\pm$ 2.46	133.75 ^a $\pm$ 3.63
b) Maximum diameter of luminated acini	94.37 ^c $\pm$ 2.40	145.00 ^b $\pm$ 3.67	220.62 ^a $\pm$ 4.68
c) Epithelial height	17.75 ^b $\pm$ 1.33	22.12 ^a $\pm$ 2.79	23.87 ^{aa} $\pm$ 0.69
4. Duct			
a) Diameter of duct	143.12 ^c $\pm$ 7.15	166.25 ^b $\pm$ 5.97	240.00 ^a $\pm$ 4.64
b) Epithelial height	14.75 ^b $\pm$ 0.88	19.87 ^a $\pm$ 1.26	21.87 ^{aa} $\pm$ 0.58

Means with different superscripts within each row for each parameter differ significantly

4. 10. 2 Glandular end pieces

The bulbourethral gland of bull was compound tubulo-alveolar in appearance. The duct of the glandular lobules was usually surrounded by luminated and non luminated glandular unit (**Figure 26c, 26d**). According to the nature of secretion it was a mixed gland containing two types of glandular end pieces mucous and serous. Mucous acini were more in number than the serous acini. The serous acini of the glandular lobule were arranged in group. The lining epithelium was pyramidal or columnar in type with basally placed round, oval or sometimes flattened nuclei. The mucous acini of the gland were lined by simple columnar or pyramidal type cells with distinct cell membrane (**Figure 26e, 26f**). The cytoplasm was clear with the basally placed nucleus having darkly stained round, oval, angular or sometimes flattened in shape according to age. Gradual changes along with the advancement of age were clearly visible at mucous acini.

The luminal space observed larger in prepubertal bull. The luminal space of glandular acini usually observed smaller with the advancements of age. In prepubertal bull, the glandular acini observed rounded or oval in shape (**Figure 26**). In pubertal bull, the glandular acini were observed oval, elongated in shape. Whereas, in adult bull, the glandular acini were oval and elongated with larger in size from the preceding group of bull. Mucous acini were more abundant in number than the serous acini (**Figure 26**).

In the prepubertal bulls, the lining epithelium of mucous acini are short columnar with basally placed irregularly rounded nuclei of the cell and having distinct cell boundary observed. Thus, providing larger lumen, cytoplasm having less zymogenic granules and nuclei were rounded indicating least/nonfunctioning state of the gland. Comparatively larger and rounded nuclei observed in the prepubertal bull (**Figure 26a, 26b**). In the pubertal bulls, lining epithelium became taller (tall columnar) or pyramidal type epithelium with basally placed nuclei comparatively smaller rounded or oval or sometimes flattened in shape living narrow acinar lumen (**Figure 26c, 26d**).

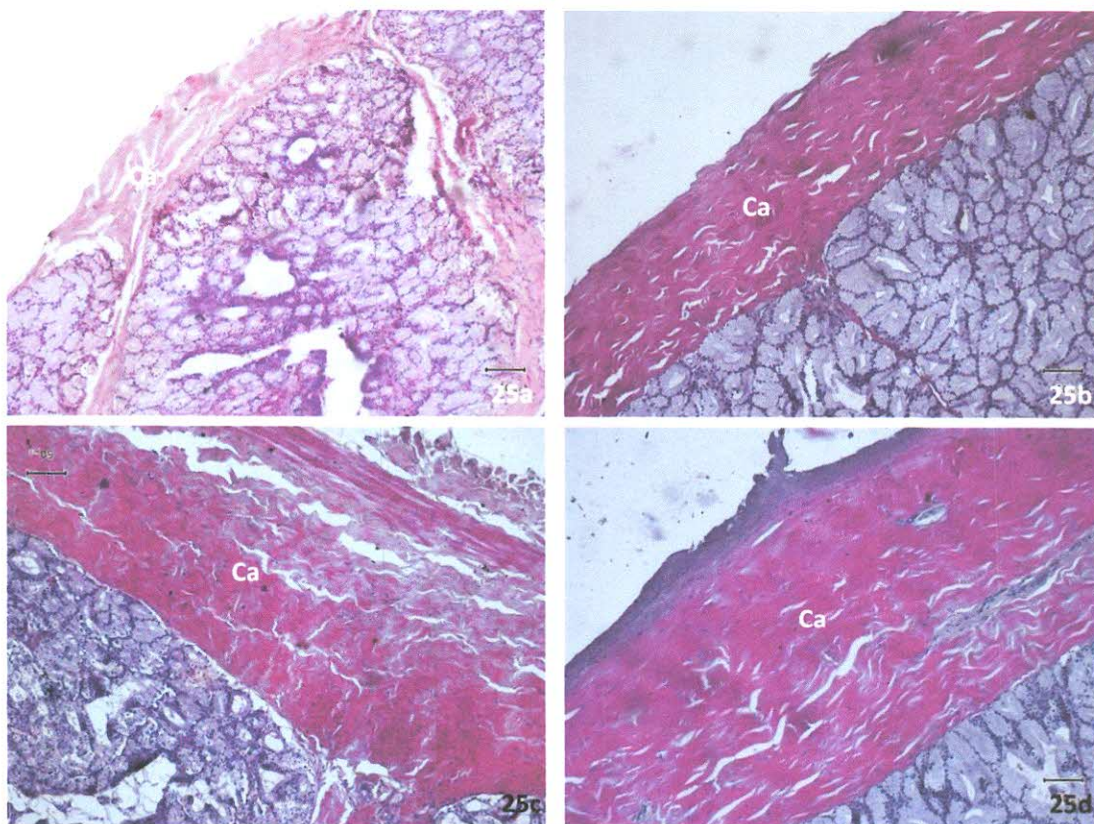


Figure 25. Bulbourethral gland showing thickness of capsule(Ca) consisting of circularly arranged collagenous connective tissue of prepubertal (25a), pubertal (25b) and adult bull (25c, 25d). HE 10x

In the pubertal bull, the average thicknesses of capsule and interlobular connective tissue septa were $169.38 \pm 2.20 \mu\text{m}$ and $33.75 \pm 1.57 \mu\text{m}$, respectively. Whereas, in adult bull the average thicknesses of capsule and interlobular connective tissue septa were $333.75 \pm 17.46 \mu\text{m}$ and $24.37 \pm 1.47 \mu\text{m}$, respectively. The result showed that the thicknesses of capsule increased significantly ($p < 0.01$) with age and adult bulls attained with highest thickness (**Figure 25**). On the other hand, the interlobular septae decreased significantly ($p < 0.01$) with the advancement of age providing space for glandular tissue (**Table 4.12**).

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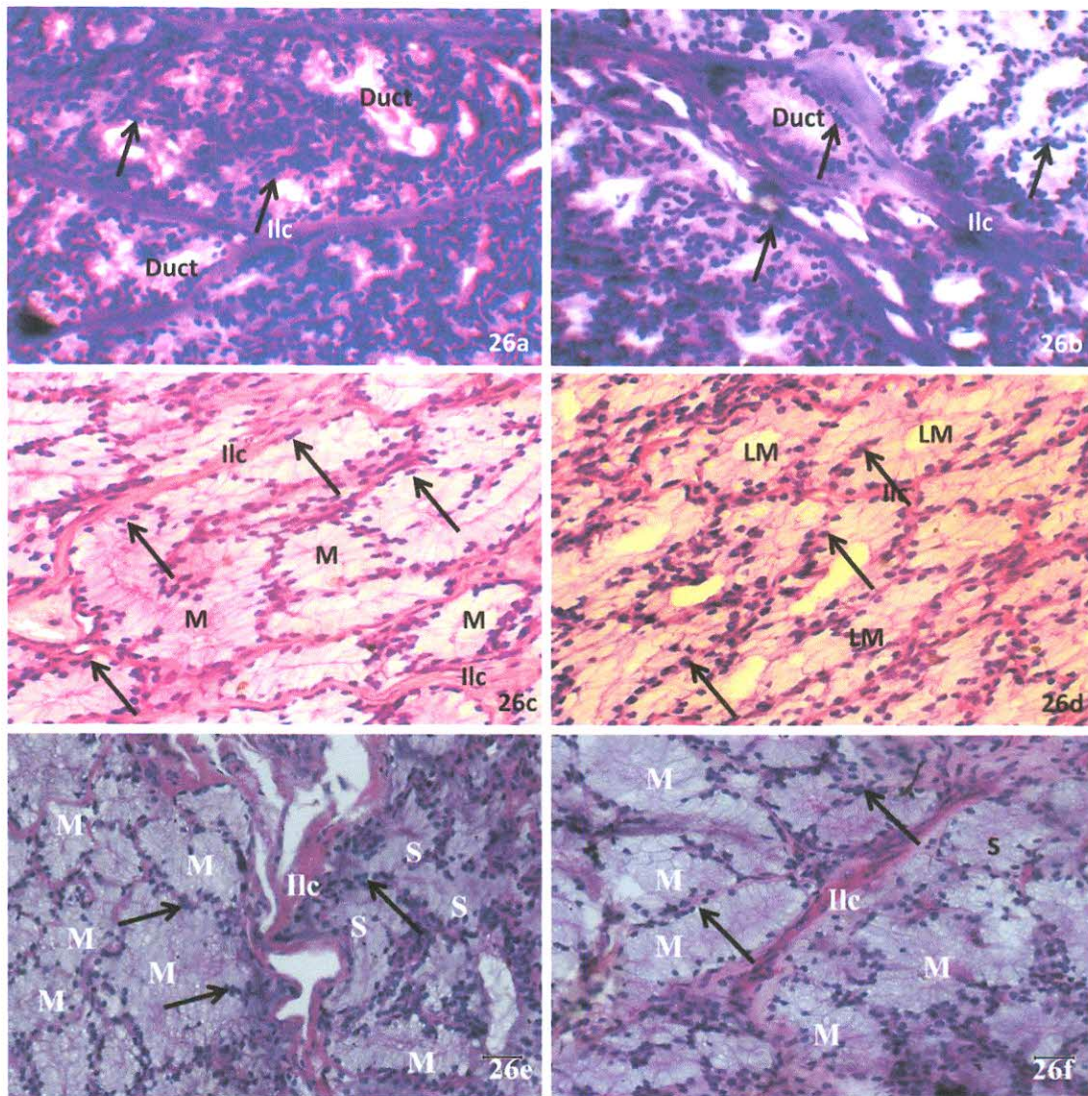


Figure 26. Bulbourethral gland showing interlobular connective tissue septa (Ilc), serous secretory acini (S), mucus acini (M) lined by tall columnar or pyramidal epithelium (arrow headed), low columnar with rounded large nuclei in prepubertal(26a, 26b), high columnar with comparatively smaller rounded or flattened nuclei in pubertal (26c, 26d), high columnar or large pyramidal with smallest rounded or flattened nuclei in adult (26e, 26f) bulls. HE 40X

Cytoplasm's have distinct zymogenic granules, indicating the gland having more secretory function. The nuclei pressed toward the basement membrane by the pressure of secretion and become flattened in appearance (**Figure 26c, 26d**). The epithelial cells were rested on the thin connective tissue layer of the alveoli. The linings of cell boundaries of pubertal and adult bulls were distinct.

In adult bulls, the lining epithelium of the mucous acini became tallest columnar or pyramidal with basally placed nuclei comparatively smaller rounded or oval or sometimes flattened in shape. Two types of glandular pieces were seen in bulbourethral gland, mucous and serous units (**Figure 26e, 26f, 27a, 27b**). The mucus and serous acini were observed rounded, oval and elongated tube like with tiny luminal space remaining almost no acinar lumen. Cytoplasm's have distinct zymogenic granules, indicating the gland having more secretory function. The nuclei pressed toward the basement membrane by the pressure of secretion and become flattened in appearance. Cytoplasm were full of zymogenic granules, flattened nuclei are rested on basement membrane (**Figure 26e, 26f**).

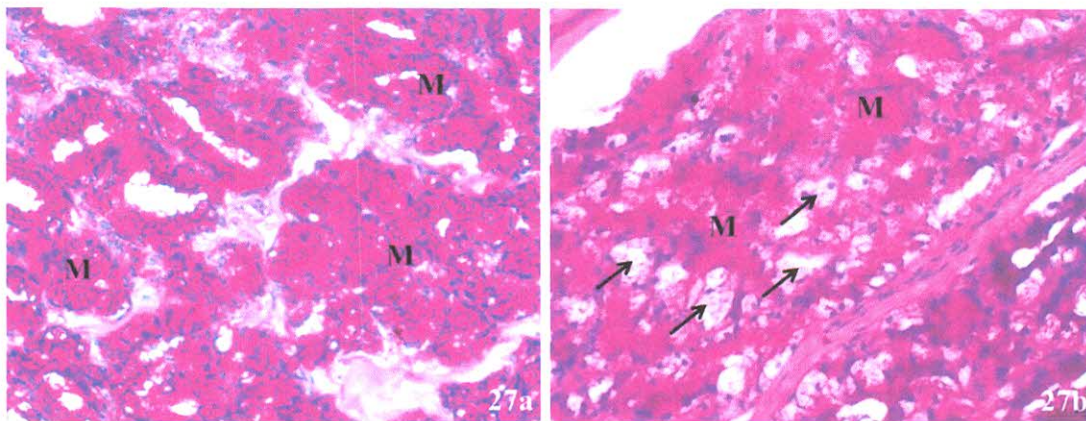


Figure 27. Bulbourethral gland showing vacuolated serous secretory acini (arrow headed), mucus acini (M) strongly PAS positive of pubertal (5a) and adult bull (5b). PAS 40X

The glandular acini were serous and mucus type clearly observed in adult bull (**Figure 26e, 26f, 27**), but in prepubertal bulls were not clear the type of the acini. The almost mucus acini were observed in the pubertal and adult bulls. The serous acini were usually observed in a glandular lobule surrounded by connective tissue septae or sometimes arranged in a group within the mucus acini in a glandular lobule of adult bull.

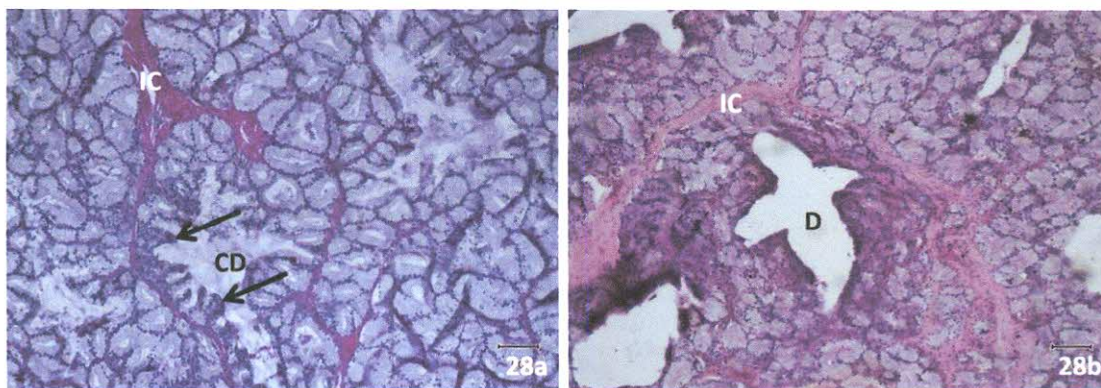


Figure 28. Bulbourethral gland showing intra glandular star shape duct (D), collecting duct (CD) with internal folded mucosa (arrow headed) and interlobular septa (IC) of adult bull. HE x10

Histometric parameters revealed that capsular thickness increased along with the age whereas inter lobular connective tissue thickness decreased along with the development of age which provides more space for developing glandular tissue. Diameters of acini and their epithelial height increased significantly along with the advancement of age. Almost same findings observed in duct system of the gland (**Table 4.12**). The interlobular duct of the bulbourethral glands were sometimes oval or star shaped (**Figure 28b**) and the central collecting sinuses of the bulbourethral glands showed mucosal enfolding with the pseudostratified epithelium (**Figure 28, 29**). The main excretory ducts of the glands were lined by pseudostratified columnar epithelium with elongated nucleus and lining became folded into the lumen (**Figure 30**). The gross, histological and histometric parameter clearly revealed that bulbourethral gland start functioning well at pubertal age and reached maximum functional stage at adult age.

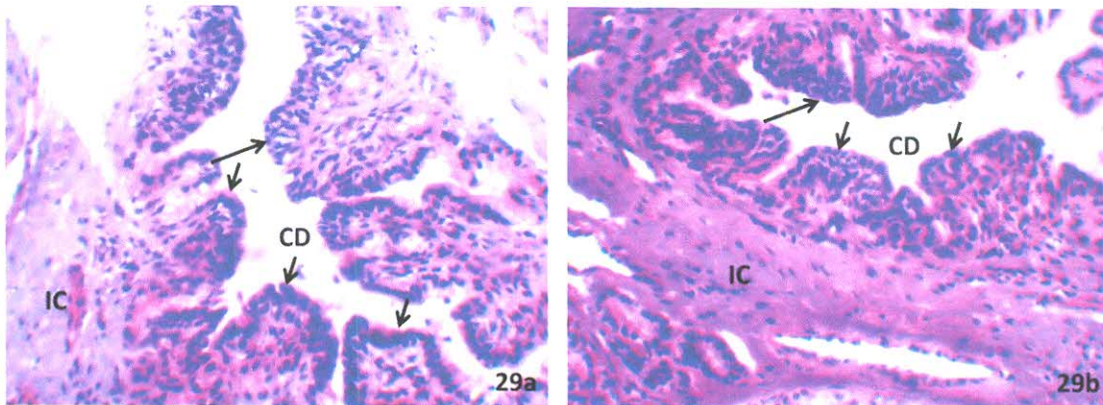


Figure 29. Bulbourethral gland showing interlobular duct or collecting duct (CD) with internal folded mucosa (arrow headed) and interlobular septa (IC) of prepubertal bull. HE x40

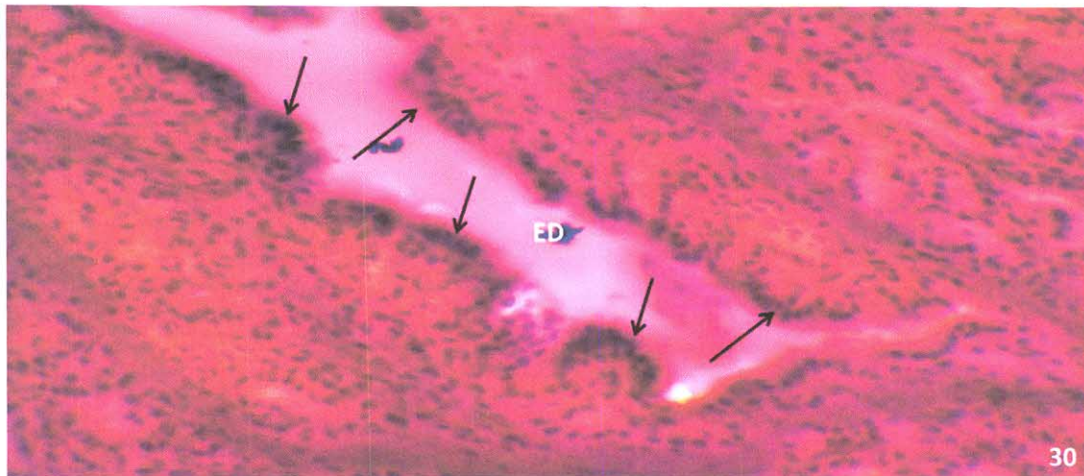


Figure 30. Bulbourethral gland showing larger main excretory duct (ED) with internal folded mucosa (arrow headed) lined by pseudo stratified columnar epithelium of prepubertal bull. HE x40

CHAPTER V
DISCUSSION

CHAPTER V

DISCUSSION

5.1. Body weight and scrotal circumference

Age related variations in body weight of bulls were observed in this study. The body weights of prepubertal, pubertal and adult bulls were 82.39 ± 5.72 kg, 205.34 ± 11.27 kg and 319.22 ± 9.75 kg respectively. The body weight of bulls were higher in adult group than prepubertal and pubertal group which indicated that the body weight of bulls increases with the advancement of age. This observation was supported by the observation of Islam (2003), Shamsuddin (1996) and Gofur *et al.*, (2007). They stated that the age related variations in weight observed in their study. This observation suggested that the body weight of bulls increases with the advancement of age.

The average scrotal circumferences of bulls of prepubertal, pubertal adult bulls were 19.04 ± 0.90 cm, 30.65 ± 0.97 cm and 35.00 ± 0.49 cm respectively. The scrotal circumferences changes significantly with the age and body weight. This finding was in agreement with the results of Evans *et al.* (1995), Islam (2003), Shamsuddin (1996) and Gofur *et al.* (2007). This finding conferred that the enlargement of scrotal circumference was correlated with the age and body weight.

The testes were suspended in a vertical direction by spermatic cord and were housed in a long, ovoid cutaneous diverticulum at the prepubic region termed as scrotum. This study was similar with that of Getty (1975), Hafez (2000) and Gofur *et al.* (2007) in ruminants.

5.2 Gross parameters of testes

The average circumference of the left testis and right testis of prepubertal bulls were 8.78 ± 0.44 cm and 7.78 ± 0.52 cm, respectively. In pubertal bulls, average circumference of the left testis and right testis were 12.30 ± 0.39 cm and 11.66 ± 0.33 cm, respectively. In adult bulls, the average circumference of the left testis and right testis were 14.01 ± 0.29 cm and 13.13 ± 0.31 cm, respectively. This finding indicates that the left testis is slightly larger than the right in each animal and the circumference increased

with the advancement of age. The left testicular circumferences of indigenous bulls always higher in percentage of the scrotal circumferences than the right. Whereas, the percentage of skin and connective tissue thickness increases with advancement of age. Similar data was not available in literature.

The shape of the testes of bulls was elongated and had an oval outline. Each testis represented two surfaces (medial and lateral), two borders (free borders or ventral, attached borders or dorsal) and two rounded extremities head and tail. The lateral surface was more convex than the medial one which was somewhat flattened because of the contact with the well defined scrotal septum. The left testicular size of indigenous bulls was slightly larger than the right in all three groups. The size of the testes varied in different age groups and even in between left and right testes of the same age group. At present study revealed that with the advancement of age that the size of the both left and right testis increased. This observation was similar with that of Getty (1975) in ruminants. The testis was oval in shape, convex laterally and relatively flattened along the medial surface which was similar to the observations of Archana *et al.* (2014) in Gaddi goats.

The average left and right testicular weights in prepubertal bulls were 18.57 ± 1.40 gm and 16.10 ± 1.64 gm (without epididymis), respectively. In pubertal bulls, the left and right testicular weights in prepubertal bulls were 129.22 ± 19.89 gm and 123.86 ± 19.53 gm (without epididymis), respectively. In adult bulls, the left and right testicular weights were 204.36 ± 14.10 gm and 188.41 ± 13.59 gm (without epididymis), respectively. The weights of the left and right testes of bulls were unequal and the left testis was significantly ($p < 0.01$) heavier than the right. The left and right testicular weights (without epididymis) were 0.022% and 0.019% of the body weight of the prepubertal indigenous bulls. In the pubertal bulls, the left and right testicular weights (without epididymis) were 0.062% and 0.060% and in adult bulls, were 0.064% and 0.059% of the body weight increased with the advancement of age. Similar data was not found in the literature. In present study the testicular weight varied in different age groups and even in the right and left testes of the same animal. After separation of epididymis, the weight of left testis was higher than those of right. The findings of the study were in agreement with the reports of Getty (1975) and Gofur *et al.* (2007). The

weight of testes was higher in the adult bulls than those of the previous groups of bulls indicating that weight of testes increase with advancement of age, body weight and scrotal circumference of Bull. These findings were similar to that of Islam (2001) and Gofur *et al.*, (2007).

The average lengths of the left and right testis (without epididymis) of prepubertal bulls were 4.97 ± 0.12 cm and 4.85 ± 0.10 cm respectively. In pubertal bulls, the average lengths of the left and right testis were 10.03 ± 0.64 cm and 9.67 ± 0.59 cm respectively. In adult bulls, the average lengths of the left and right testis were 11.93 ± 0.31 cm and 11.76 ± 0.40 cm respectively. In all three groups of bulls, the length of testes whether epididymis present or not, it was slightly higher in the left testis than the right, which increased with the advancement of age. The findings were similar with those of Getty (1975), Islam (2001), and Gofur *et al.* (2007).

The average breadths of the left and right testis (without epididymis) of prepubertal bulls were 2.77 ± 0.11 cm and 2.58 ± 0.12 cm, respectively. In pubertal bulls, the average breadths of the left and right testis were 4.23 ± 0.25 cm and 4.20 ± 0.28 cm respectively. In adult bulls, the average breadths of the left and right testis were 6.05 ± 0.48 cm and 5.53 ± 0.43 cm respectively. In all three groups of bulls, the breadths of testes were increased with the advancement of age and left testis slightly higher than the right, which. Similar data was not found in the available literature.

The average volumes of the left and right testis (without epididymis) of prepubertal bulls were 27.92 ± 1.05 mm³ and 25.31 ± 1.06 mm³ respectively. In pubertal bulls, the average volumes of the left and right testis were 114.75 ± 28.24 mm³ and 107.87 ± 27.21 mm³ respectively. In adult bulls, the average volumes of the left and right testis were 211.16 ± 8.77 mm³ and 194.50 ± 11.25 mm³ respectively. In all three groups of bulls, the volumes of testes were slightly higher in the left testis than the right, which increased significantly ($p < 0.01$) with the advancement of age. Volume was always higher in left testis than the right; this may be due to the usual developmental process. Similar data was not found in the available literature.

In the present study, the body weight was not significantly correlated with all testicular parameters in all three groups of bulls. In prepubertal bulls, the body weight was

significantly correlated with testicular weight with ($r=0.95$, $p<0.05$) and without ($r=0.97$, $p<0.01$) epididymis. In pubertal bulls, the body weight was significantly correlated with testicular circumference in the left testis ($r=0.95$, $p<0.05$). In adult bulls, the body weight was not significantly correlated with testicular parameters. Similar data was not found in the available literature.

The scrotal circumference was not significantly correlated with all testicular parameters in all three groups of bulls. In prepubertal bulls, the scrotal circumference was significantly correlated with testicular circumference with ($r=0.98$, $p<0.01$) right testis. In pubertal bulls, the scrotal circumference was significantly correlated with testicular circumference in the right ($r=0.96$, $p<0.01$) testis and in the left ($r=0.98$, $p<0.01$) testis. The testicular volume was also correlated with scrotal circumference in the left ($r=0.96$, $p<0.05$). In adult bulls, the scrotal circumference was significantly correlated with testicular circumference with ($r=0.95$, $p<0.05$) left testis. Similar data was not found in the available literature.

5.3 Histological parameters of the testes

The visceral layer of the tunica vaginalis consisted of mesothelium and connective tissue layer. The tunica albuginea contains mainly the collagen fibers with a few elastic and reticular fibers. The tunica vasculosa is the inner layer of the covering contains mainly blood vessels which lie adjacent to the parenchyma to the testis. Connective tissue septa radiate from the mediastinum towards the capsule and divided the parenchyma of the testis into a number of incomplete pyramidal lobules. The average thicknesses of prepubertal, pubertal and post pubertal bull's testes were 150.00 ± 3.76 μm , 332.50 ± 30.19 μm and 573.75 ± 29.66 μm , respectively. The thicknesses were increases with the advancement of age. Similar observation was found in indigenous pubertal bull stated by Gofur *et al.* (2007) and in Gaddi goats by Archana *et al.* (2014). Archana *et al.* stated that at about 12 months of age, the tunica albuginea of goat showed three distinct layer as outer loose connective tissue layer having collagenous fibers, middle vascular layer containing blood vessels and smooth muscles fibers and the inner layer consists of dense regularly arranged collagenous and reticular fibers. No such lamination was found in the prepubertal and pubertal bulls, but in adult bulls the loose connective tissue layers looks divided in sections.

The testes of indigenous prepubertal bulls encapsulated by tunica albuginea which was reported earlier in goats (Baishya *et al.*, 1986; Islam *et al.*, 2001; Karmore *et al.*, 2003) and in other domestic animals (Trautmann and Fiebiger, 1957). In goats (Gupta, 1989) the fibro muscular capsule of the testes resembling the tunica albuginea which measured 105µm at birth and 125µm at 6 months of Beetal goats. Whereas, in Nagpuri goat (Karmore *et al.*, 2003) layer was very thin (33.02 ± 2.34 µm) and in Gaddi goats (Archana *et al.*, 2014) it was 116 µm at birth, 416 µm at prepubertal, 616 µm at pubertal and 697 µm at post pubertal animals. In buffalo (Goyal and Dhingra, 1973) the thickness increases progressively from zero to 52 weeks of age. Another study in pubertal indigenous bull (Adhikary *et al.* 2014) observed that thickness of tunica albuginea increased with the advancement of age. The tunica albuginea contains mainly the collagen fibers. The presence of smooth muscles in tunica albuginea (Gupta, 1989) was dissimilar to the present study of indigenous prepubertal bull. The study supported by earlier report in goat (Islam *et al.*, 2001, Karmore *et al.*, 2003), in Murrah buffalo (Chandrapal, 1976) and in bulls (Trotter, 1959; Dellmann and Wrobel, 1976). The tunica vasculosa is the inner layer of the covering contains mainly blood vessels. Tunica intima of the blood vessels composed of elastic fibers. The tunica albuginea was thicker in the left testis than the right one also reported before in indigenous bull (Gofur *et al.*, 2008).

These testicular septula divide the testicular parenchyma into a varying number of pyramidal or cone shaped testicular lobe and lobules. It was composed of collagen fibers and continuous with the mediastinum testis. Similar observation was found in indigenous bull by Gofur *et al.* (2008) and in goats by Archana *et al.* (2014).

The intertubular or inter-sex cord tissue was fibro cellular consisted of fibroblast, mesenchymal cells and differentiated Leydig cells with varying number. The fibrous component of the interstitial tissue consisted of mainly collagenous fibers. Usually, Leydig cells were found in a large group (> 50 cells) in the present study, but sometimes observed few cells in small groups (5-15) in prepubertal indigenous bulls. The Leydig cells were rounded, ovoid or roughly triangular with round or ovoid nuclei generally present eccentrically towards the broader end of the cell. The observations were dissimilar to the reports in one day old kid (Archana *et al.*, 2014,). It was noted

that the Leydig cells were occurred singly or 2-3 in a group within the fibroblast cells. In goats (Gupta, 1989), the Leydig cells were definite at 6 month of age. In calves (Santamarina and Reece 1957), the Leydig cells were well differentiated in 80 days old calves, in buffalo calves (Chandrapal (1976), 9-10 months of age the cells were differentiated. But another study of indigenous pubertal bull (Adhikary *et al.*, 2014) recognized that the Leydig cells were occurred in a small group at 2-4 teeth (permanent incisor) bearing bulls and occurred singly or dispersed small group in post pubertal or 6 teeth (permanent incisor) bearing bulls. There is no available literature regarding Leydig cells in prepubertal bulls.

In pubertal bulls, the interstitial tissues consisted of loose connective tissue materials, blood and lymph vessels, fibrocytes, free mononuclear cells and especially the interstitial endocrine cells of Leydig present in the collagen fibrous network in between the seminiferous tubules. The Leydig cells were large than the prepubertal bulls polymorphs, ovoid or polygonal in shape occurred in a small groups or more than one with spherical nuclei with granular cytoplasm.

In adult bulls, the cells of Leydig occurred in the interstitial tissues as a dispersed form within the connective tissue materials beside the blood capillaries as a large, polymorph cells. The cells were usually single or sometimes in a small group.

The cells of Leydig varied in number and arrangement at the different stages of indigenous bulls. The number of Leydig cells apparently decreases with the advancement of age and arrangement of Leydig cells usually large groups in prepubertal, small groups or few cells in pubertal and singly dispersed form in adult indigenous bulls. These observations were similar in human noted earlier that the decline in Leydig cell numbers stated by Kaler and Neaves (1978) and in rats slightly changeable observed by Kaler and Neaves (1980) with the advancement of age. But in the stallion, Johnson and Neaves (1980) stated that the Leydig cells increases in numbers with the advancing of age. Not only the total Leydig cell volume per testis increased fivefold and the total number of Leydig cells per testis increased more than three fold.

The seminiferous tubules having no lumen generally called sex cord. In the present study, the testicular parenchyma of prepubertal bulls consisted of solid, non-luminated, tubular structures arranged as coiled and straight appearance. The solid tubular seminiferous tubules were surrounded by fine collagenous connective tissue fibers. This observation was similar to the findings in goats (Kamal and Devi, 2012). The intertubular tissues were fibro cellular. The sex cord having basement membrane and the cords were filled up by the centrally placed large primordial germ cells (PGC) designated as gonocytes and small indifferent basal cells. The basal cells were spherical in shape laid along the periphery of the sex cord; their numbers were more than the PGC of the cord. In goats (Kamal and Devi, 2012), the solid sex cord contained peripheral primitive Sertoli cells and large centrally placed germ cells located in an eosinophilic materials. The PGC had large spherical shape with distinct cell boundary contain rounded large nucleus, located in between small basal cells. The cytoplasm was slightly acidophilic. The typical spermatogenic cells and Sertoli cells could not identified in the present study of the prepubertal bulls. It was thought that, after degeneration of PGC the luminization started and few sex cords showed initiation of lumen. The large PGC showed cytoplasmic degeneration and nuclear fragmentation. The centrally grouped PGC sometimes distributed all over the cord in the apical portions of the basal cells. The gonocytes or PGC numbers varied from cord to cord. The PGC or gonocytes migrated centrifugally then the cells called prespermatogonia of the cord. Several cords were totally free from gonocytes. However, no such reports were found in the literature to compare the present findings. These prespermatogonia were also observed in buffalo calves (Singh 1996) and in other domestic animals (Dellmann and Wrobell, 1987). Another study in domestic animal (Lee and Burger 1983), it was reported that central gonocytes migrated towards the periphery of the sex cords with the advancement of age and entered three proliferative phases by a series of mitotic divisions and prespermatogonia were formed which gave rise to future generations of spermatogonia. In Japanese black bulls, the gonocytes were replaced by prespermatogonia at the age of 3 months (Hamano *et al.*, 2001).

The luminization of seminiferous tubules, typical spermatogenic cells and Sertoli cells could not identified in the present study of the prepubertal bulls. It was thought that, after degeneration of PGC the luminization started and few sex cords showed some

initiation of lumen. The large PGC showed cytoplasmic degeneration and nuclear fragmentation. In goats (Kamal and Devi, 2012), noted that the variable degree of degeneration in the form of vacuolated cytoplasm and different stages of karyolysis present in seminiferous tubules. Luminization of the cords completed by 6 months of age in Assam goats and it was showed distinct lumen at 4 months of age (Kamal and Devi 2012) in ram (Kishore *et al.*, 2006). The age of luminization of these sex cords was observed in domestic animals being at 20-24 weeks of postnatal age of cattle (Abdel-Raouf 1960), 3 months of age in buffaloes (Ramaya *et al.*, 2007). In present study, the indigenous prepubertal bull showed solid sex cord mostly non-luminated, few of the solid sex cord was initiating lumen through degeneration of PGC at 9 to <12 months of age.

Prepubertal bulls become pubertal and the solid sex cord became luminized at the advancement of bulls, true seminiferous tubules. The testicular parenchyma mainly consisted of seminiferous tubules containing three components, basement membrane somatic cells and spermatogenic cells. The tubules were two ended loops, round and oblong in outline varying in appearance due to the complex coiling of the tubules at the different angles and levels. The numbers of the seminiferous tubules were increased with the advancement of age. The size of the seminiferous tubules increased with the age of the animals. A distinct network of reticular fibers interspersed with the collagenous fibers was marked in the basement membranes supporting the germinal epithelium of the tubules. The intertubular space decreased with the advancement of age whereas the tubular convolution increased with the advancement of age. This observation was similar to agreement in indigenous pubertal bulls stated by Gofur *et al.* (2008).

The average cross sectional length and breadth of seminiferous tubules were $566.50 \pm 26.52 \mu\text{m}$ and $144.50 \pm 26.52 \mu\text{m}$ in prepubertal bulls, respectively. In pubertal bulls, the average cross sectional lengths and breadths were $637.90 \pm 18.75 \mu\text{m}$ and $165.25 \pm 18.75 \mu\text{m}$, respectively. In adult bulls, the average cross sectional lengths and breadths were $728.50 \pm 18.75 \mu\text{m}$ and $159.50 \pm 18.75 \mu\text{m}$, respectively. The parameters showed the cross sectional lengths and breadths increased with the advancement of age. This observation was similar to that of indigenous bulls stated by Gofur *et al.* (2008).

The testicular parenchyma was developed with the differentiation of spermatogenic cells. The lining complex stratified epithelium was composed of spermatogenic cells forming chronological lineage and sustentacular or Sertoli cells in the tubules. The spermatogenic cells were observed different stages of growth, proliferation and differentiation in the seminiferous tubules.

The spermatogenic cells were located in between the Sertoli cells in an orderly manner with four to eight layers occupying the space between the basal lumina and the lumen, while there successive descendants were progressively closer to the lumina of the seminiferous tubules, called spermatogenic lineage. The orderly type growing stratification showed 5-8 layers of cells in the pubertal bulls, whereas post pubertal or adult bulls showed 3-5 layers of spermatogenic cells. The spermatogenic cells were four categories, the primitive germ cells or spermatogonia, the primary spermatocytes, the secondary spermatocytes, spermatids or spermatozoa. In another study of indigenous bulls, Gofur et al. stated similar findings. They stated that the stratification of growing spermatocytes was more in the tubules of bulls of 2PI (2 permanent incisor teeth) age group 5-8 layers than the 4pi age group 3-6 layers. In other observations of bovine and caprine revealed that the seminiferous epithelium comprised of spermatogonia (3 types), primary spermatocytes in different stages of meiosis, secondary spermatocytes, spermatids (2 types) and spermatozoa located between the Sertoli cells and arranged in many layers (3-7), reported by Santamarina and Reece (1957), Abdel-Raouf (1960), Chandrapal (1976), Bordoloi (1979), Deshpande *et al.* (1985) and Gupta (1989). All four types of cells were found within the fully luminated seminiferous tubules at the 18 month of early pubertal bulls. In most cases, the primary spermatocytes, secondary spermatocytes and spermatids were almost developed within the 12-18 months period of time. Whereas, Archana et al. reported completed spermatogenesis appeared at 12 months old testis of Gaddi goats. At present observation in indigenous bulls showed initiation of luminization of sex cord started just before 12 months of bulls. However, Islam *et al.*, (2001) noted that occurrence of spermatids in 180 days and spermatozoa in 720 days old black Bengal goats. Primary spermatocytes and round and oval spermatids appeared first time at 330 and 450 and 510 days of postnatal life in buffalo (Deshpande *et al.* 1985). On the other hand, some

reports on Jersey bulls and Holstein bulls it was 9 months, 10 months respectively (Fossland, 1954). Curtis and Amann (1981) reported 32 weeks age in Holstein bulls.

The primitive germ cells or spermatogonium was the smaller cells adherent to the basement membrane. The cells were fixed, spherical or cuboidal in shape between the Sertoli cells (supporting cells). The primary spermatocytes were large spherical or oval shaped cells with large round or spherical centrally placed nucleus just next to the spermatogonia on their inner side. They were biggest cells with large rounded or oval nuclei than the other spermatogenic cells. The cells showed different stages of proliferation. Secondary spermatocytes were located internally and smaller than the primary spermatocytes, but larger than the round spermatids. They were rounded in shape and contained eosinophilic granular cytoplasm and the nuclei were spherical, centrally placed with distinct nuclear membrane. The numbers of secondary spermatocytes were negligible numbers in the present study than the much greater primary spermatocytes and round spermatids at all three stages of indigenous bulls. This indication was second maturation division of secondary spermatocytes was speed up fasted to spermatids. Their population especially receded in the adult bulls. These observations were aggrement with Archana *et al* (2014) in Gaddi goats and Chandrapal (1976) in buffaloes.

Spermatids were smaller cells located adjoined to the lumen of the seminiferous tubules. But in the transformational stages of the spermatids were seen usually as an early or cap spermatids or round spermatids and late or flagellated or elongated spermatids. The round spermatids were the smallest cells of the spermatogenic series. They possessed spherical nuclei in their cytoplasm. The elongated or flagellated spermatids observed elongated nuclei with condensed nuclear materials stained deeply and remain attached to the apical part of the Sertoli cells, migrated centripetally towards the lumina of the seminiferous tubular lumen. These observations reported in goats by Archana *et al.*, (2014) noted that the spermatids were three types, rounded, elongating and elongated were indentified in the testes of >12 months of age. Roy *et al.* (1985) also reported them to be round than elongated and finally rod shaped.

Spermatozoa were the final form of spermatids usually found attached to the apical part of the Sertoli cells at their luminal border and sometimes found in the tubular lumen.

Spermatozoa also observed within the spermatogenic lineage just nearer to the apex of the Sertoli cells. The head was elongated or long oval in shape with flagellated tail occurred pubertal and adult bulls, but the early pubertal bulls showed smaller numbers of spermatozoa than the late pubertal and adult bulls. The first appeared spermatozoa at after 18 month of age in the present study. It had head, neck, body and tail parts which were described earlier by Trautmann and Fiebiger (1957) and Dellmann and Wrobel (1976) head, neck, middle piece, principal piece and end piece of the spermatozoa in domestic animals.

The cells of the spermatogenic lineage especially spermatogonium, primary and secondary spermatocytes had more staining affinity than the spermatids and spermatozoa in the routine staining technique in the present observation. This observation was similar to that Gofur *et al.* (2008) in the indigenous bulls. The distribution and populations of spermatogenic cells in different seminiferous tubules observed variable. In the Gaddi goats, Archana *et al.* (2014) reported that, the spermatogenic cell population of seminiferous tubular lumen of prepubertal animals were recorded 35%, 26%, 4%, 26% and 9% population of spermatogonium, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa, respectively and these were 34%, 20%, 3%, 23% and 20% in pubertal goats. But population of primary and secondary spermatocytes was 18% and 2% respectively in the post pubertal goats. They also noted that fasted rate of spermatogenesis in pubertal animals. The process of spermatogenesis was slow in the post pubertal animals. At present observations could not be compared due to non availability of literature in this context.

Sertoli cells were tall, irregularly columnar cells that broad basal portion laid on the basal lamina and extended to the lumen of the seminiferous tubules. The cells were looking transparent other than the ovoid nucleus in the routine staining methods in the tubules of pubertal and adult bulls. The nuclei were spherical, oval or elongated in shape, located near the basement membrane. Sertoli cells were located singly. The clear cell boundaries were not distinct. There was no available literature regarding Sertoli cells to compare the present study.

5.4 Gross parameters of vesicular gland

The vesicular glands of indigenous bull were located on the dorso-lateral aspect of the neck of the urinary bladder. It was lateral to the ampulla of vas deferens and the beginning part of the pelvic urethra. The glands were paired, compact and lobular. Its surface covered by a thick capsule of fibrous connective tissue. The lobulations of vesicular gland were distinct. These observations of the vesicular gland similar with the findings of bull (Getty 1986, Nunez 1993 and Neves *et al.* 2013). At the junction of left and right vesicular gland with the union of ampulla and urethra a small area of fibrous cord present for connecting the gland. The right and left glands of each bull were unsymmetrical, multilobulated in shape in bulls (Getty, 1975). From the pre pubertal stage to adult or post pubertal the sizes become larger and lobulations become very much distinct. It was to the similar observations of Asdell (1955), in bovines. Chandrapal (1976) reported that vesicular gland was tubular shaped with swollen ends in the neonatal and elongated and lobulated in the pubertal buffaloes. This may be due to the species variation.

The gross morphological parameters of the vesicular gland of indigenous bull revealed that the left and right vesicular gland significantly different in weight and length in the present study. Left vesicular glands were slightly higher than the right in all parameters. This may be due to the usual variation, but no such available report in the literatures. The rate of growth is higher during prepubertal to pubertal stage than pubertal to adult stage old bull development. The weight, length, width and thickness of left and right vesicular gland of pubertal and post pubertal group were significantly increased in the present study. These observations were similar to the results of Kundu (1980) and Gupta *et al.* (1993).

The length of the left and right vesicular gland were 7.55 ± 0.36 cm and 7.23 ± 0.38 cm in pubertal indigenous bull, 9.18 ± 0.70 cm and 8.86 ± 0.60 cm in the post pubertal or adult bull. This observation was dissimilar from the statement of Getty (1975) in bull, stated that the length of the vesicular gland was 10-12cm and breadth 3cm. The present study was observed smaller and it may be breed variation. The length and breadth of vesicular gland were 8.58cm and 2.41cm in indigenous adult bull of Bangladesh, were mostly similar reported by (Rahman *et al.*, 2010).

The left and right vesicular weights were 0.0046% and 0.0045% of the body weight of the prepubertal indigenous bulls, 0.0067% and 0.0061% in pubertal bull and it was 0.0063% and 0.0058% of the body weight of the adult bulls respectively. In the present study, the vesicular weight percentage found followed by adult and prepubertal bull. The body weights gaining's were simultaneously increased more than that adult vesicular gland. Percentage was always slightly higher in the left than the right in all groups. Such types of data were not available in the literature for comparison of the present study.

The left and right vesicular glands weights were increased in pubertal bull about 357% and 340% respectively of the prepubertal vesicular weight. Whereas, the left and right vesicular glands weights were increased in adult about 526% and 500% respectively of the prepubertal vesicular weight. The left and right vesicular glands lengths were increased in pubertal bull about 176% and 180% respectively of the prepubertal vesicular length. Whereas, the left and right vesicular glands lengths were increased in adult about 214% and 220% respectively of the prepubertal vesicular lengths. Such types of data were not available in the literature for comparison of the present study.

5.5 Histological parameters of vesicular gland

Histologically, the vesicular gland of indigenous bull was a multi lobulated compound tubulo-alveolar gland enclosed by capsule of smooth muscle bundles with inter-muscular irregular collagenous connective tissue. This observation was similar to the reported in bull (Marettova and Legath, 2010). In buffalo bull, Ghomini *et al.* (2014) stated that thick well-developed fibro-muscular trabeculae divided the vesicular glands into lobes of varying shapes, numbers and sizes as stated my present study. Marettova and Legath (2010) stated that thick tunica muscularis gave off interlobular septa into the gland proper was predominantly muscular. Groups of smooth muscle cells were observed to arise from muscle layer and enter the mucosa layer. The present study revealed that smooth muscle cells form incomplete layer.

The tunica adventitia composed collagenous connective tissue consisting of collagen, reticular and elastic fibers as described in goat (Gupta, 1989) and Buffalo (Sudhakar *et al.* 1986). The many blood vessels, nerve fibers and autonomic ganglions were observed

in the tunica adventitia as in man (Glenister, 1976). The histometric data of tunica adventitia showed that the continuous and progressive and significantly increased from prepubertal to adult stage of bull in the present study. Tunica muscularis has similar trend except the interstitial tissue changes were seen more prominent from the prepubertal to pubertal stage than the pubertal to adult stage of development. The notable changes were seen from the prepubertal to pubertal stage than between pubertal to adult stage. It was help to conclude that tunica adventitia and muscularis grew. In the growth of the capsule, interlobular septa and in the interstitial tissue of the vesicular gland were similar trend as reported Beetal goats (Gupta, 1989) and ram (Singh *et al.*, 1980).

The vesicular gland of indigenous bull was a lobulated compound tubulo-alveolar gland as luminated and non luminated. The luminated and non luminated secretory lobules were located separately by the interlobular fibromuscular septa. It was described as Murrah bull (Sudhakar *et al.*, 1986) and goats (Kundu). Each lobule consisted of tunica mucosa lamina epithelialis and lamina propria, tunica muscularis and tunica adventitia. All layers were similar to the description in different domestic animals (Trautmann and Fiebiebiger, 1957; Dellmann and Wrobel, 1976; Sudhakar *et al.*, 1986). The lobule of the gland showed folded mucosa, lined by the pseudo stratified columnar epithelium. The numbers of glandular units were more in per unit area in the pre pubertal bull than the pubertal and adult. In the prepubertal bull, minimum and maximum diameters of glandular end pieces were $72.50 \pm 4.54 \mu\text{m}$ and $200.00 \pm 5.99 \mu\text{m}$, respectively in the luminated acini. In the pubertal bull, minimum and maximum diameters of glandular end pieces were $110.00 \pm 4.64 \mu\text{m}$ and $256.25 \pm 4.61 \mu\text{m}$, respectively in the luminated acini. In the post pubertal or adult bull, minimum and maximum diameters of glandular end pieces were $155.00 \pm 3.28 \mu\text{m}$ and $340.00 \pm 4.64 \mu\text{m}$, respectively in the luminated acini. These observations indicated that the maximum and minimum diameters of luminated glandular end pieces increased gradually and significantly with the advancement of age. The glandular size was increased with the advancement of age with significantly changed from pre pubertal stage and maximum at adult stage. These findings were accordance with the reported in goat (Archana *et al.* 2009). They noted also that the diameters of luminated and non-luminated glandular end pieces were significantly increased with the age.

Secretory end pieces or alveoli were arranged together in a group where the alveoli of the gland apex were large in size. In the prepubertal bull, the small and large rounded and/or oval secretory alveoli were observed in a same lobule. In the pubertal bull, the small and large rounded, oval and multigonal secretory alveoli were observed in a same lobule. In the adult bull, the shape of the small and large rounded, oval and multigonal secretory alveoli were grouped in a same lobule. The alveoli of each lobule of the gland showed gradually folded mucosa in pubertal and adult bull, whereas, in prepubertal bull, almost no mucosal enfolding observed. There was no available literature regarding the secretory alveoli and their mucosal enfolding to compare the present study.

The alveoli usually lined by cuboidal to columnar or pseudostratified columnar epithelium. The collecting sinus of each lobule had similar pseudo stratified epithelium into which open the glandular tubes (Dellmann and Eurell, 1998). In the alveoli and collecting sinus lined by three type of cells were identified in the pseudo stratified epithelium containing A, B and C. It was also described earlier in goat (Yao and Eaton, 1954; Wrobel, 1970; Gupta and Singh, 1982; Ploen, 1980) sheep (Skinner *et al.*, 1968; Singh *et al.*, 1980) and buffalo (Sudhakar *et al.*, 1986).

Type A cells were tall columnar cells having distinct cell boundaries with the oval, round or elongated nucleus. The cytoplasm of the cells was finely granular and faintly eosinophilic, but the apical border was slightly basophilic in nature in the present study. Type B cells were located in the basal lamina in between the basal lamina and type A cells having round or oval nucleus with indistinct cell boundaries. In goat (Archana *et al.*, 2009) the Type B cells were located in the basal lamina in between the type A cells were contrary observation with other similar characteristics. The shape of the cell was spherical and their cytoplasm was clear as described in goat (Archana *et al.*, 2009). Type C cells were narrow columnar cells with darkly stained cytoplasm with hyperchromic nuclei interspersed between A cells. In the present study, some cells of epithelia containing hyperchromic nuclei observed as type C cells with similar size and shape of type A cells. They were fewer than the type A and B cells. The observation of type C cells was numerous in the pre pubertal bull, but rarely existed in pubertal and adult bull. The similar findings were observed in goat (Archana *et al.*, 2009) and

buffalo (Sudhakar *et al.*, 1986). They noted that the type C cells were more in young and very less number in adult and concluded it might be intermediate stage in the cycle undergone by the epithelial cells. Hib (2003) reported in men, the glandular mucosa has a pseudo stratified epithelium with high non-ciliated columnar cells, basal cells and oval cells.

The lining epithelium of end pieces of vesicular gland were pseudo stratified columnar epithelium. In the pre pubertal bull, the epithelium showed simple cuboidal or simple columnar type. Pseudo stratification was clear at the pubertal and adult bull in the vesicular gland in the present study. The glandular alveoli, tubules and ducts were lined with epithelium similar to tunica mucosa. The lining cells were also A, B and C type like mucosa with similar cytological appearances. The epithelial height of glandular acini and duct were $10.00 \pm 0.64 \mu\text{m}$ and $11.00 \pm 0.40 \mu\text{m}$ in the prepubertal bull, $21.10 \pm 0.93 \mu\text{m}$ and $20.80 \pm 0.68 \mu\text{m}$ in the pubertal bull and $21.50 \pm 0.88 \mu\text{m}$ and $21.30 \pm 0.59 \mu\text{m}$ in the adult bull, respectively. The present study indicated that epithelial height of the duct and alveoli increases with the advancement of bull, but no significantly difference among them. It was remarked that chief secretary cells A were did not grow structurally than the functionally attains at puberty or adult. Gupta and Singh (1982) stated in goats almost similar observation. They reported the lining pseudo stratified columnar epithelium was $22.5 \pm 0.6 \mu\text{m}$ in mean height. The tall columnar cells occupied the whole thickness of the epithelium. The present study indicated that epithelial height of the duct and alveoli increases with the advancement of bull, but no significantly difference among them. It was remarked that chief secretary cells A were did not grow structurally than the functionally attains at puberty or adult. These observations were accordance with the Beetal goat (Gupta, 1989). Although, the increasing of the alveoli and decreasing in the epithelial height of the lumen of the vesicular gland of 7-8 month old Madras red ram lambs also observed (Naidu and Pattabhiraman, 2001).

The lumen of the intralobular ducts or collecting sinus appeared highly evaginated and invaginated forming primary mucosal folds like structure elongated in shape especially in pubertal and post pubertal bulls and lined by tall columnar or pseudo stratified columnar cells. In prepubertal bulls, the luminal surface of the duct less invagination

with simple columnar epithelium. In buffalo bull (Ghomini *et al.* 2014) reported similar findings, the lumen of the interlobular ducts appeared highly evaginated and invaginated forming folds like structure similar to tongue in shape and lined by tall columnar cells. The diameters of the duct and epithelial height increase with the advancement of age.

5.6 Gross parameters of prostate gland

The prostate glands of bull consisted of two portions, external portion or corpus prostate and internal or pars disseminata portion. The two portions were distinguished, compact external portion or corpus prostate was observed dorsal of the pelvic urethra at the level of the colliculus seminalis. This observation was similar to the observation in bovine (Dellmann and Wrobel, 1976) and other domestic animals (Arthur 1975, Dellman and Brown 1987; Dyce *et al.* 2009). Other observations stated that it is unpaired gland about the size of a chestnut located on the outside of the urethra just posterior to the excretory ducts of the vesicular gland (Frandsen and Spurgeon 1992, Dyce *et al.* 1987, and Nickel *et al.* 1973/17). The corpus prostate was a white colored band of tissue observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. The corpus prostate was found as a band in all three groups of bulls. In prepubertal to adult bulls, the corpus prostate was observed as a distinct white color band below the cord joining the two vesicular glands. In adult animals, it was observed approximately higher in raised area of the gland with dorsal depressing line, whereas in prepubertal bull dorsal surface was convex.

5.7 Histological parameters of prostate gland

The corpus portion and disseminate portion of the prostate gland has similar histological structures except the histometric parameters. The corpus prostate was well demarcated with the pars disseminate prostate by the fibro muscular connective tissue septa. The corpus capsule enclosed by the connective tissue composed of collagenous fibers along with smooth muscle cells. The fibro muscular capsule was surrounded by the skeletal muscle (M. urethralis). This observation was similar to that of buffalo bull (Sudhakar *et al.*, 1985). In bull (Kainer *et al.* 1969) and mature bovine bull (Trotter, 1959) the capsule and urethralis muscle was also found in the pars disseminate portion.

The capsule composed of collagenous connective tissue fibers and predominately smooth muscles especially in the corpus prostate. This was similar to the observations of bull (Dellmann, 1992). The smooth muscle fibers were observed predominantly in the capsule, trabeculae and interstitial connective tissue septa than the fibrous components. The capsules gives off trabeculae and the trabeculae gives off interlobular septa and the glandular parenchyma divided into lobe and lobules. The thickness of capsule and trabeculae were varies with the location of the gland. Usually, capsular thickness was higher than the trabecular and finally interlobular septa lesser than the trabecular. This observation also similar to the findings in goats (Archana *et al.*, 2011). Thickness became lesser from capsule to trabeculae, the trabeculae to interlobular septae. With the advancement of ages of bull the thickness of interlobular septae became decreases. The observation was similar to buffalo bulls (Chandrapal and Bharadwaj, 1984). They also noted that the capsule of prepubertal bulls relatively thicker and had numerous coarse collagenous and reticular fibers with few elastic fibers and post pubertal buffalo bulls contained abundant smooth muscle fibers. The connective tissue trabeculae extending from the capsule entered into the parenchyma of gland forming interlobular fibro muscular septae and divided the gland into lobes and lobules as stated in goats (Archana *et al.*, 2011) and buffalo (Sudhakar *et al.*, 1985).

The secretary end pieces were luminated and non luminated in the corpus portion of the prostate or body of the corpus prostate which is similar to the findings in buffalo (Sudhakar *et al.*, 1985). In pre-pubertal bull, the mean minimum and maximum diameters of luminated secretary end pieces were $63.75 \pm 4.99 \mu\text{m}$ and $150.00 \pm 9.28 \mu\text{m}$, respectively. In pubertal and adult bull, the average minimum and maximum diameters of luminated acini were $135.00 \pm 5.68 \mu\text{m}$ and $202.50 \pm 7.52 \mu\text{m}$, $135.00 \pm 6.83 \mu\text{m}$ and $260.00 \pm 8.04 \mu\text{m}$, respectively. This observation was indicated the diameter increases gradually and significantly (($p < 0.01$)). There was no such literature to compare the present study in different stages of bull.

In prepubertal bull, the secretary end pieces were lined by simple low cuboidal epithelium with the rounded nucleus. In the pubertal bull, the secretary end pieces were lined by simple columnar epithelium with the rounded to oval nucleus. In the adult bull, the secretary end pieces were lined by simple high columnar epithelium with the oval

basally placed nucleus. Cytoplasms of the lining epithelium don't have secretory granules in prepubertal that appeared in pubertal and highly loaded in adult bulls. Due to the increased secretory granules, the nucleus gradually moved towards the basement membrane and turned from round to oval. Secretory granules in the cells as well as secretion in the lumen increased along with the advancement of age. The height of the lining epithelium increased with the advancement of age significantly ($p < 0.01$). There was no such literature regarding the present study in different stages of bull. The glandular alveoli were mainly mucous type with few serous alveoli which was dissimilar to the adult buffalo majority of the alveoli were serous only a few mucous alveoli (Chandramouly, 1971). But in another observation in buffalo (Sudhakar *et al.*, 1985) noted that the characteristic serous and mucous components of the gland became evident at 4 years of age.

The lining epithelium of the duct was simple columnar or pseudostratified epithelium. The lumen of the prepubertal prostatic duct were very clear indicated no secretion or the gland not reaching in secretory stage. But in the lumen of the pubertal and adult prostatic duct showed secretion indicated that the gland became secretory stage. The lining epithelium of the duct became enfolded from prepubertal to adult stage. The number and height of the enfolding increased with the advancement of age. It may be due to the functional development of the ductal epithelium. On the other hand, the luminal diameters were observed decreasing with the increasing mucosal enfolding with an age. The parameters were not compared to present study as because of lacking of available literature in bulls. In buffalo the ducts of the pars disseminate prostate had wider lumen having lined by stratified cuboidal epithelium with indistinct cell membranes (Sudhakar *et al.*, 1985). The ducts were opened into the lumen of the urethra having transitional epithelium nearer the opening in the present study of all three groups of bull. This observation was similar to that of in buffaloes (Chandramouly 1971) and bull (Kainer *et al.*, 1969; and Dellmann and Wrobel 1976).

5.8 Gross parameters of bulbourethral gland

The bulbourethral glands of the bulls were paired, rounded or ovoid in shape located either sides of the pelvic urethra cranial to the ichiatic arch. They are covered by a thick fibrous capsule and the thick striated bulbocavernosus muscles in indigenous bull. This observation is similar to that of in goat (Archana *et al.*, 2011), buffalo (Chandrapal, 1976; Sudhakar, 1982) and other domestic animals (Eurell and Frappier, 2007). In present study the gross morphological findings of the bulbourethral glands indicated that the length, width and thickness and weight of the left glands were slightly higher than the right in all three age groups. Whereas in goat (Archana *et al.*, 2011) the right and left bulbourethral gland were almost symmetrical in all measurement. Observation in goat (Gupta, 1989) revealed a continuous growth of the gland with age but with a faster growth took place during the first 12 months of post-natal life. The present observation indicated that the glandular growth and development from the prepubertal to pubertal stage faster than the pubertal to adult. The left bulbourethral gland increased 185% during prepubertal to pubertal stage of bulls growth and 40% during pubertal to adult stage of bulls growth. The right bulbourethral gland increased 167% during prepubertal to pubertal stage of bulls growth and 42% during pubertal to adult stage of bulls growth. The glandular weight increased 300% during prepubertal to pubertal age of bull growth and 100% during pubertal to adult stage of development (Stallcup, 1969). This variation may come from the age difference of the bull sample. Also noted in ox (Stallcup, 1969) correlated the weight of bovine testes with the weight of the bulbourethral gland up to 12 months of age but not in adult.

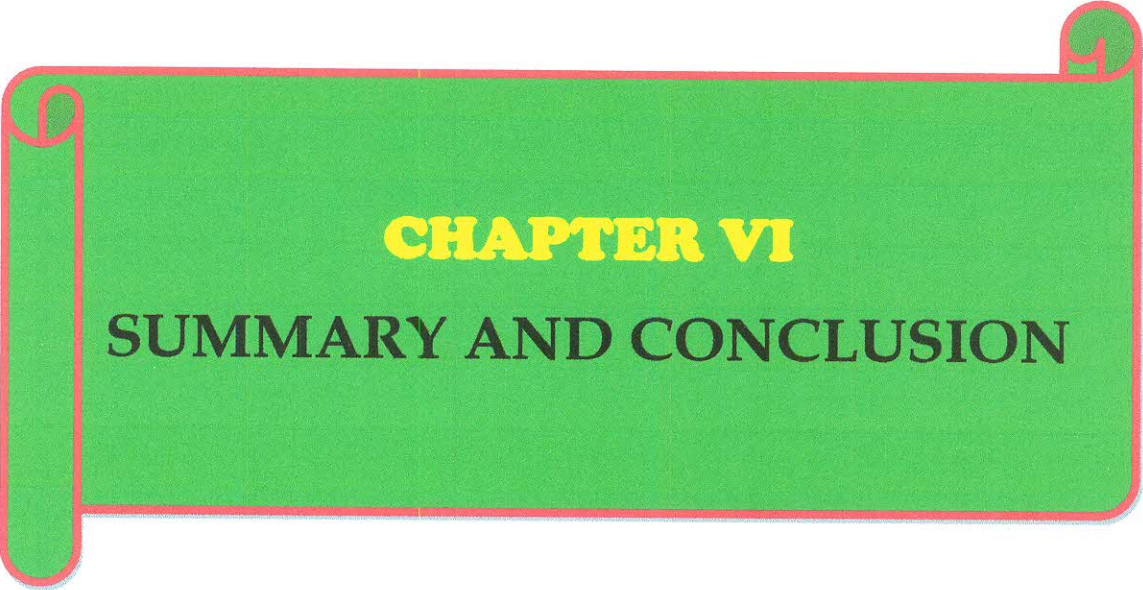
5.9 Histological parameters of bulbourethral gland

Bulbourethral gland was covered by collagenous connective tissue fibers. This observation was similar by the previous report in goat (Archana *et al.*, 2011). It was noted in goat (Kundu, 1980), the nature of the capsule was fibroelastic, whereas the interlobular septae comprised of irregular connective tissue with few muscle fibers. This study showed that the thicknesses of capsule increased significantly ($p < 0.01$) along with the advancement of age and highest thickness was observed in adult bulls. The interlobular and intralobular connective tissue became thinner with the advancement of age and thicknesses decreased significantly ($p < 0.01$). These may be

due to the increase growth and development of glandular tissue. The increasing growth pattern of capsule, reducing thicknesses of interlobular septae and intralobular connective tissue were in accordance with the goat (Archana *et al.*, 2011).

The bulbourethral gland of bull was compound tubulo-alveolar in appearance which was in accordance with the previous study in goat (Archana *et al.* 2011, Eurell and Frappier, 2007). The histometric parameters of all three groups of bull indicated the diameters (Minimum and maximum) of glandular end pieces increased significantly ($p<0.01$) with the advancement of age. The diameters of the duct of the glandular lobule was also increased significantly ($p<0.01$) with the advancement of age. Epithelial height of the glandular end pieces and lining epithelium of the duct increased significantly ($p<0.01$) with the advancement of age. The lining epithelium of the duct cuboidal to columnar. Two types of glandular end pieces were seen in bulbourethral gland, mucous and serous unit. Mucous acini were more in number than the serous acini. The serous acini of the glandular lobule were arranged in group. In the prepubertal bulls, the lining epithelium of mucous acini are short columnar thus providing larger lumen, cytoplasm having less zymogenic granules and nuclei were rounded indicating lest/non functioning state of the gland. In the pubertal bulls, lining epithelium became taller (tall columnar) living narrow acinar lumen. Cytoplasm have distinct zymogenic granules, indicating the gland having more secretory function. The nuclei pressed toward the basement membrane by the pressure of secretion and become flattened in appearance. In adult bulls, the lining epithelium of the mucous acini became tallest remaining almost no acinar lumen. Cytoplasm were full of zymogenic granules, flattened nuclei are rested on basement membrane. Histometric parameters revealed that capsular thickness increased along with the age whereas inter lobular and intralobular connective tissue thickness decreased along with the development of age which provides more space for developing glandular tissue. Diameter of acini and their epithelial height increased significantly along with the advancement of age. Same findings observed in duct system of the gland. There was no available literature in bull in this regard. In goat it was observed that the glandular secretion was predominantly mucous in nature. The lining epithelium was pyramidal or columnar in type with basally placed round, oval or sometimes flattened nuclei (Archana *et al.*, 2011). The mucous acini of the gland lined by tall simple columnar cells with distinct cell

membrane that was reported in male goats (Yao and Eaton, 1954; Wrobel, 1970). The present observation was almost supported by the previous study in goat (Kundu, 1980; Gupta, 1989; and Yao and Eaton, 1954).



CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSIONS

The body weight of bulls were higher in adult group than in prepubertal and pubertal groups which indicated that the body weight of bulls increases with the advancement of age. The scrotal circumferences increased significantly with the advancement of age body weight. The left testis is slightly larger than the right in each group bull. The left testicular circumferences of bulls always higher in than the right. The shape of the testes was elongated and had an oval outline with their long axis oriented vertically. With the advancement of age as they become oval and convex laterally and relatively flattened along the medial surface. The size and weights of the left and right testes of bulls were unequal and the left testis was significantly ($p < 0.01$) heavier than the right. The left and right testicular weights of prepubertal bulls (without epididymis) were 0.022% and 0.019%: in pubertal of 0.062% and 0.060% of the body weight and in adult conditions, it was 0.064% and 0.059% of the body weights. In all three groups of bulls, the length, breadth and volume of testes increased significantly ($p < 0.01$) with the advancement of ages where left testis always larger than the right one.

The thicknesses of tunica albuginea were increases with the advancement of ages. Connective tissue septa radiate from the mediastinum towards the capsule and divided the parenchyma of the testis into a number of incomplete pyramidal lobules. It was composed of collagen fibers and continuous with the mediastinum testis.

The intertubular or inter-sex cord tissue was fibro cellular consisting of fibroblast, mesenchymal cells and differentiated Leydig cells with varying numbers. The intertubular space decreased with the advancement of age whereas the tubular convolution increased with the advancement of age. In pubertal bulls, the interstitial tissues consisted of loose connective tissue materials, blood and lymph vessels, fibrocytes, free mononuclear cells and especially the interstitial endocrine cells of Leydig present in the collagen fibrous network in between the seminiferous tubules. The cells of Leydig varied in number and arrangement with the different stages of indigenous bulls. Usually, Leydig cells were observed in a large group in prepubertal bulls. The Leydig cells were rounded, ovoid or roughly triangular shape with round or

ovoid nuclei. The number of Leydig cells apparently decreases with the advancement of age and arrangement of Leydig cells. Usually, large groups in prepubertal, relatively smaller groups in pubertal and the smallest dispersed form in adult indigenous bulls. The seminiferous tubules having no lumen generally called sex cord. In the present study, the testicular parenchyma of prepubertal bulls consisted of solid, non-luminated, tubular structures arranged as coiled or straight appearance. The sex cord having basement membrane and were filled up by the centrally placed large primordial germ cells (PGC) designated as gonocytes and small basal cells. The PGC were large spherical in shape with distinct cell boundary and rounded large nucleus. The number of gonocytes or PGC numbers varied from cord to cord. At the advancement of ages, prepubertal bulls become pubertal and the solid sex cord became luminized to form true seminiferous tubules. The tubules varying in appearance like two ended loops, round and oblong due to the complex coiling of the tubules at the different angles and levels. The size and numbers of the seminiferous tubules increased with the advancement of ages. The cross sectional diameters increased with the advancement of age.

The seminiferous epithelium composed of spermatogenic cells forming chronological lineage and sustentacular or Sertoli cells in the tubules. The spermatogenic cells showed different stages of growth, proliferation and differentiation in the seminiferous tubules. The spermatogenic cells were located in between the Sertoli cells in an orderly manner with 4-8 layers occupying the space between the basal lumina and the lumen, while there successive descendants were progressively closer to the lumina of the seminiferous tubules, called spermatogenic lineage. The orderly type growing stratification showed 5-8 layers of cells in the pubertal bulls, whereas adult bulls showed 3-5 layers of spermatogenic cells. The spermatogenic cells were four categories, the primitive germ cells or spermatogonia, the primary spermatocytes, the secondary spermatocytes, spermatids or spermatozoa. All four types of cells were found within the fully luminated seminiferous tubules at the 12-18 month of early pubertal bulls. The primary spermatocytes were large spherical or oval shaped cells with large round or spherical centrally placed nucleus just next to the spermatogonia. They were the biggest among all four types of cells. Secondary spermatocytes were located internally and smaller than the primary spermatocytes, but larger than the round spermatids. They were rounded in shape and contained eosinophilic granular

cytoplasm. The nuclei were spherical, placed centrally. Spermatids were the smallest cells among the spermatogenic cells and located innermost layer of the spermatogenic lineage. But in the transformational stages of the spermatids were seen usually as an early cap spermatids or round spermatids and late flagellated or elongated spermatids containing deeply stained elongated nuclei and remain attached to the apical part of the Sertoli cells.

Spermatozoa were the final form of spermatids usually found attached to the apical part of the Sertoli cells at their luminal border and sometimes found in the tubular lumen. The head was elongated or long oval in shape with flagellated tail occurred pubertal and adult bulls. The spermatozoa first appeared at or after 18 month of age in the present study.

Sertoli cells were tall, irregularly columnar cells that broad basal portion laid on the basal lamina and extended to the lumen of the seminiferous tubules. They were lightly stained cells at routine staining methods in the tubules of pubertal and adult bulls. The nuclei were spherical, oval, elongated in shape, located near the basement membrane. Sertoli cells were located singly. The cell boundaries were not distinct.

The vesicular glands of indigenous bull were located on the dorso-lateral aspect of the neck of the urinary bladder. The glands were paired, compact and multi lobular. Its surface covered by a thick capsule of fibrous connective tissue. The right and left glands of each bull were unsymmetrical, multilobulated. From the pre pubertal stage to adult, the sizes become larger and lobulation become more distinct. The excretory duct opens at the seminal colliculus just lateral to the ductus deferens. Left vesicular glands were slightly higher than the right in all parameters. The weight, length, width and thickness of the pre pubertal vesicular gland grew first during prepubertal to pubertal stage than the pubertal to adult. The all parameters of the vesicular gland of pubertal and post pubertal group were significantly increased. The vesicular gland of indigenous bull was a multi lobulated compound tubulo-alveolar gland. The glandular parenchyma divided into lobules of varying shape and size. The left and right vesicular gland had similar histological inspection.

The capsule of the vesicular gland was mainly composed of smooth muscle bundles with inter-muscular irregular collagenous connective tissue. The secretory end pieces were observed luminated or non-luminated in the same lobules. The luminated or non luminated acini of the glandular end pieces increased gradually and significantly with the advancement of age. The collecting sinus or interlobular duct united to form the excretory ducts of vesicular gland. The lobule of the gland showed folded mucosa, lined by the pseudostratified columnar epithelium. In sections, the mucosa forms thin folds projects into the lumen. The free surface of epithelial cells was projected into the lumen of secretory end pieces. The lining epithelium of end pieces of vesicular gland was generally pseudostratified columnar epithelium. In prepubertal bull, the epithelium showed simple cubiodal or simple columnar type. In pubertal and adult bull the lining epithelium become pseudostratified columnar type. The lining cells were A, B and C type like mucosa with similar cytological appearances. Type A cells was tall columnar cells having distinct cell boundaries with the oval, round or elongated nucleus. Type B cells or basal cells were located in between the basal lamina and the type A cells having round or oval nucleus with indistinct cell boundaries. Type C cells were narrow columnar cells interspersed between A cells with darkly stained cytoplasm with hyperchromic nuclei. Epithelial height of the duct and alveoli increases with the advancement of bull, but no significantly difference among them. The type A cells having chief secretory function in the gland, type B cells were resting or reserve cells and type C cells were intermediate type between A and B cells. Lamina propria consisted of loose connective tissue surrounded the alveoli, tubules and some solid end pieces. With the advancement age the glandular alveoli become larger and inter glandular connective tissue stroma was reduced.

The prostate glands of bull consisted of two portions were distinguished, compact external portion or corpus prostate was observed dorsal of the pelvic urethra at the level of the colliculus seminalis. The pars disseminata portion was occupied along the entire wall of the pelvic urethra. The corpus prostate was a white colored band of tissue observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. In prepubertal, pubertal and adult animals, the corpus prostate was a distinct white color band below the cord joining the two vesicular glands. In adult animals, it was observed approximately higher in raised area of the

gland with dorsal depressing line, whereas in prepubertal bull dorsal surface was convex. The corpus portion and disseminate portion of the prostate gland observed similar in histological structures except the histometric parameters. There was a capsule composed of collagenous fibers along with smooth muscle cells. The smooth muscle fibers were observed predominantly in the capsule, trabeculae and interstitial connective tissue septa than the fibrous components. The capsule gives off trabeculae and the trabeculae give off interlobular septa and the glandular parenchyma divided into lobe and lobules. The thickness of capsule and trabeculae were varies with the location of the gland. Usually, capsular thickness was higher than the trabecular and finally interlobular septa lesser than the trabecular thickness. Interlobular connective tissue thickness decreased significantly ($p < 0.01$) along with the advancement of age.

The secretory end pieces were luminated and non luminated distributed in the corpus portion of the prostate. In prepubertal bull, the both types of secretory end pieces were lined by simple cuboidal epithelium with the rounded nucleus. In the pubertal bull, the glandular unit was lined by simple low columnar epithelium with the large rounded nucleus. In adult, glandular unit lined by simple high columnar epithelium with the oval or flattened basally placed nucleus. Due to the increased secretory granules, the nucleus gradually moved towards the basement membrane and become flattened. The lining epithelial cells of both pubertal and post pubertal glandular acini contained secretory granules, but the secretory granules were more in adult group. Secretory granules in the cells as well as secretion in the lumen increased along with the advancement of age. The height of the lining epithelium increased significantly ($p < 0.01$) with the advancement of age.

Interlobular ducts or collecting sinuses had irregular lumen lined by pseudostratified columnar epithelium or simple columnar epithelium in prepubertal bulls. The ducts of the pubertal and adult bulls contained folded mucosa were lined by simple columnar, pseudo stratified columnar with comparatively smaller dark rounded or oval shaped nuclei located basally of the cells. The typical enfolding observed from prepubertal to adult bulls and height and number of the enfolding increased with advancement of age. The average diameters of the ducts and the height of the epithelium increased significantly ($p < 0.01$) with the advancement of age.

The bulbourethral glands of the bulls were paired, rounded or ovoid in shape located either sides of the pelvic urethra cranial to the ischiatic arch. They were covered by a thick fibrous capsule and the thick striated bulbocavernous muscles. The weight, length, width and thickness increased significantly ($p < 0.01$) with age and left glands were slightly heavier than the right in all three groups. Each bulbourethral gland was covered by fibrous connective tissue capsule composed of circularly arranged collagenous tissue fibers. The thicknesses of capsule increased significantly ($p < 0.01$) with age as a result adult bulls attained with highest thickness. The bulbourethral gland of bull was compound tubuloalveolar in appearance with serous and mucous acini. In prepubertal bull, the glandular alveoli were arranged in small groups which were either luminated and non luminated. Diameters of glandular alveoli and epithelial height increased significantly ($p < 0.01$) with the advancement of age. The lining epitheliums of prepubertal bulls were simple columnar epithelium with irregularly rounded nuclei placed basally and cell boundaries were distinct. In the pubertal and adult bull, the lining epithelium became taller, simple columnar or pyramidal type epithelium with basally placed nuclei comparatively smaller, rounded, and oval or flattened in shape. The mucus and serous acini were rounded, oval or elongated tube like with tiny luminal space. In prepubertal bull, the glandular acini were rounded or oval in shape. In pubertal bull, the glandular acini were observed oval, elongated in shape. Whereas, in adult bull, the glandular acini were oval and elongated with larger in size from the preceding group of bull. Mucous acini were more abundant in number than the serous acini. The serous alveoli were lined by pyramidal type epithelium with cytoplasmic secretary granules. The luminal surface of the duct was irregular and folded internally. The collecting ducts lined by cuboidal to low columnar epithelium. The diameters of glandular ducts and height of the lining epithelium increased significantly ($p < 0.01$) with the advancement of age. The larger excretory ducts of the bulbourethral glands were lined by pseudostratified epithelium.



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