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Seminal Glands of Buffalo Bulls (*Bos bubalis L.*) During Prepubertal and Pubertal Periods: Histological and Histochemical

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Abstract

The current investigation was carried out on the seminal glands of six apparently healthy mature male Buffalo bulls and six immature. The specimens were collected and examined anatomically and histologically after being fixed in 10% buffered neutral formalin. The specimens were subjected to processing till paraffin sections were obtained and stained. The anatomical and histological findings of the seminal glands are varying with age. Anatomically, the immature seminal gland was small sized, flaccid in texture, white in color and measured about 0.80-0.95 gm in weight, 1.95-2.50 cm in length, 1.00-1.15 cm in width and about 0.46-0.60 cm in thickness. Meanwhile, in mature one became larger in size, firm in texture, pale yellow in color, multi-lobulated where had a very large lobules on its outer surface giving, cluster of grapes liked appearance. It measured about 7-8 gm in weight, 5-6 cm in length, 2-2.5 cm in width and about 80-1.3 cm in thickness. Microscopically, the seminal gland was observed consisting of a stroma of connective tissue and a parenchyma of differentiating and undifferentiating acini in immature animal. Meanwhile, in mature one, the parenchyma was consisted of secretory end pieces and well-developed duct system. With advancement of age, the thickness of the capsule and trabeculae increased, also the number of secretory end pieces per unite area increased and the amount of inter-glandular connective tissue stroma reduced.

Keywords: Seminal gland; Stroma; Parenchyma; Tubulo-alveolar; Secretory units; Buffalo bulls (*Bos bubalis L.*); Histological; Histochemical

Introduction

The water buffaloes among the large animals play an important role in agriculture and have great economic and productive importance. They are considered the main milk, meat and hide producing animals. They have the ability to convert the poor quality roughages into rich human foods, which is attributed to the features of its digestive system. Such productivity depends on the management strategy which promotes the maximum reproductive efficiency [1].

The seminal gland is one of the most important male accessory genital glands in the body. It play an important role in semen quality as it contains fructose and citric acid and spermatozoa- activating substances such as proteins, enzymes, mucus, vitamin C, flavin, riboflavin, sorbitol, inositol, ascorbic acid, prostaglandin, phosphoryle choline, ergothionin which are the vital components of the semen [2-4]. The high fructose concentration provides nutrient and energy for the sperm motility [5,6].

The seminal gland appears as a lateral evagination of the vas deferens [6]. They are a pair of compact, lobulated gland with an excretory duct. The duct of this gland open with ductus deferens forming ejaculatory duct which open in colliculus seminalis by ostia ejaculator on dorsal aspect of pelvic urethra near the neck of urinary bladder as in horse and ruminant Or open separately on the dorsal aspect of pelvic urethra as in pig [7,8]. Colliculus seminalis is cavernous tissue present at the neck of urinary bladder filled with blood during ejaculation to close the opening of the urinary bladder to prevent mix between semen and urine during ejaculation or entrance of semen to inside the urinary bladder [8].

The seminal glands of domestic animals consist of two parts. The first part is elongated, multi-lobulated, compact secretory unit; glandular tissue like the cluster of grapes. The second one is the excretory duct which joins with ductus deferens [6,8].

In one day Gaddi goat *(Copra hircus),* the seminal gland was small white cord like structure at the point of union of ampulla ductus deferens with the urethra, while at 12 month of age, it become 'S' shaped and is easily identified due to its clusters appearance [9]. Its appearance looked like cluster of grapes and so called vesicular gland in bulls [10]. The seminal glands were a tubular organ with swollen ends in the neonatal and elongated with lobulation in the pubertal buffaloes [11].

The seminal gland is flaccid in the bull which had one year old or less. The firmness of glands increased with advancement of age where the bull which had two years of age, the seminal glands acquire a very firmness in consistency and become tense in texture [10]. The size of the seminal glands is increased with advancement of age from 5 to 29 months in Friesian bulls [12].

The length, breadth, thickness and weight of the vesicular gland varied among the different indigenous bulls and also in the right and left vesicular gland of the same bull. The right vesicular gland was more in anatomical measurements than those of the left one. Where, the vesicular glands measured 9.44 cm in length, 2.78 cm in breadth, 1.45 cm in thickness and 19.90 gm in weight of right vesicular gland, whereas the left one was measured 8.58 cm in length, 2.41 cm in breadth, 1.30 cm in thickness and 18.46 gm in weight [4].

The weight of seminal gland, increased with the advancement of age in Friesian bulls from 5 to 29 months where, the weight of gland are increased from 6.37 gm in age group one year to 9.16 gm in age

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group two years [10]. The weight of gland grew at a very fast rate in the pre-pubertal goats and only slowly in the pubertal and post pubertal animals [13,14].

In one day Gaddi goat, the color of gland is white, but with advancement of age, the color of gland is slightly converted to whitish yellow [9].

In one day Gaddi goat *(Copra hircus)*, the seminal glands are non-lobulated with smooth surface. While, in mature Gaddi goat the seminal glands are more lobulated like cluster of grapes and have a rough surface. With advancement of age the lobulation of the gland increased and become more obvious and clear [9,10,15-18].

The excretory duct of the seminal gland joins with ductus deferens to form an ejaculatory duct. The latter, opens in colliculus seminalis by ostia ejaculator on the dorsal aspect of pelvic urethra near the neck of urinary bladder. However, in pig the excretory duct of seminal gland opens separately on the dorsal aspect of pelvic urethra [8]. While in man, the excretory duct of seminal vesicle opens into ductus deferens as it enters prostate gland [3].

The bull seminal glands are compound, lobulated gland [18,19]. Each gland is divided into stroma and parenchyma. The stroma, consist of capsule and trabeculae. The capsule of this gland consists of two coats; the outer coat is dense irregular fibrous connective tissue with elastic fibers while the inner one is rich in bundles of smooth muscle fibers [10,11,16,20,21]. The abundant smooth muscle bundles produce strong peristaltic contraction that participates in the expulsion of the seminal gland secretion [8].

Thick well developed fibro muscular septa are derived from thick fibro muscular capsule, dividing the gland into lobes. The inter-lobular septa are mainly formed from muscular elements [22]. In ram, the thicker the septa, the more muscle fibers extending into it [15].

The parenchyma consists of large number of secretory end pieces and system of ramified secretory tubules. The secretory units are collected together in groups, each being surrounded by thin bundles of smooth muscle fibers which are intermingled with fibro architectural design comprised of circularly arranged collagenous bundles with reticular fiber [9,10,16]. In Gaddi goat, with the advancement of age, the number and size of secretory end pieces per unit area increased and the-inter glandular connective tissue stroma decrease [9].

The epithelium of the secretory end pieces of the seminal glands is pseudo-stratified columnar type being consists of tall columnar secretory cells, short round basal cells and also there is a third type of few narrow cells with darkly stained non-granular cytoplasm [10].

The tall columnar cells have small lipid droplet and rich in glycogen, which give +ve alkaline reaction. Some columnar cells possess light, bleb-like apical projections. These cells extend from basement membrane to the luminal surface of the gland [18]. Moreover, the secretory end pieces of the seminal glands in Gaddi goat exhibited apocrine mode of secretion [9]. The cytoplasm of the secretory epithelium is vacuolated in sections prepared by ordinary methods. Cytoplasmic vacuoles occur mainly in animals in mature age [10]. The high, diameter and activity of this cell depend up on testosterone level in blood [3,9]. The nucleus is situated in the basal third of the cell. It is round, ovoid or slightly irregular in shape and contains coarse chromatin together with one or two prominent nucleoli [9].

The basal cells have large lipid droplets occupying most of the cytoplasm often in an infra-nuclear position. Approximately 50% of the lipid material is cholesterol, 20% is triglyceride and 10% is phospho-lipid. These cells rest on a basement membrane and not reach to luminal surface of the secretory units and embeded in between tall columnar cells [22].

The secretory ducts are lined with cuboidal or columnar cells according to the activity of the glandular epithelium, or by stratified columnar epithelium in the horse. The intra-lobular secretory ducts drain the slightly coiled tubular portions of the tubulo-alveolar gland, and then pour into the main secretory duct [18].

Archana et al [9], Banks [17], Wrobel KH and Bergmann [18] and Samuelson [19] clarified that the seminal gland histologically is consisted of 4 layers; tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia. Tunica mucosa is highly folded. The folds appear as fingers like projections that embedded inside the gland lumen. The mucosal folds unit together, forming a reticulated surface. The latter, is making the lumen irregular and recessed. It gives a honey comb-shaped appearance for the gland lumen. Also, the lumen is large to store the secretion between ejaculations [9,18].

The lining epithelium of the seminal gland is pseudo-stratified columnar epith consisted of tall columnar secretory cells and short, round, basal cuboidal cells [9,18,19,22]. In human, this epithelium is rich in secretory granules, having ultrastructural characteristics similar to those found in protein-synthesizing cell [3].

The propia sub mucosa consists of highly vascularized loose connective tissue that becomes dense beside the connective tissue septa [18-20].

Tunica muscularis consists of thick layer of interwoven smooth muscle fibers that surround each lobule of the gland, these smooth muscle fibers are arranged in two layers; an inner circular and outer longitudinal layers. These smooth muscle fibers vary in its amount, width, thickness, arrangement and orientation around the gland which has sympathetic innervations. Contraction of this muscle layer leads to evacuate the gland secretion during ejaculation [18,19]. With advancement of age, the thickness of this layer increased in Gaddi goat [9].

Tunica adventatia is composed of loosely arranged mesh work of collagen, reticular and elastic fiber. Many blood vessels, lymph vessels, nerves and autonomic ganglia are seen surrounding the tunica muscularis [18,23]. With advancement of age, the collagen and reticular fiber become coarser and the elastic fiber become irregular in distribution. Moreover, the thickness of the adventitia increases as tunica muscularis in Gaddi goat [9].

Several authors had engaged themselves in studying the micromorphological and histochemical structure of the seminal vesicle of different animals specially laboratory ones as in guinea pigs [24], in rat [25], in Fellow Deer [26] and in human [27]. Meanwhile, in buffalo bull, the seminal gland received a little attention. So, the designed current work comes to through more light on the anatomical and microscopic cyto-architecture features of the seminal glands in the immature and mature buffalo bull.

Materials and Methods

Seminal glands of twelve apparently healthy Buffalo bulls (six mature and six immature) were collected from Zagazig slaughter house in Sharkia province for anatomical, histological and histochemical studies. The seminal glands were immediately collected after slaughter, where the length (anteroposterior), width (lateromedial), thickness (dorsoventral) were measured using vernier calipers and non-stretchable nylon thread. The glands were weighed to the nearest milligram in fresh

state. Then the weight was recorded using electronic digital weighing machine. Very thin slices of the vesicular gland were immediately fixed in 10% buffered neutral formalin. The fixed specimens were dehydrated in ascending grades of ethanol, cleared in benzene and embeded in paraffin. 7-10 µm thick sections were obtained and stained with:

- Harris's hematoxylin and Eosin (H & E) for routine histological studies.
- Van Gieson's stain for demonstration of collagen fibers and muscle cells
- Periodic acid Schiff technique (PAS) for detection of neutral mucopolysaccharides.
- Combined Van Gieson's with Alcian blue at PH (2.5) for demonstration of collagen fibers and acidic mucopolysaccharides.
- Silver impregnation technique for demonstration of the reticular fibers.
- Toludine blue stain for detection of metachromatic granules of mast cells and general proteins.
- Alcian blue at PH (2.5) for detection of acidic mucopolysaccharides.
- Weigert's elastic for demonstration of elastic fibers

The methods of processing and staining were quoted from Bancroft and Gamble [28]. The microphotography were taken using a digital Dsc-W 130 super steadycyper shot camera connected to an Olympus BX 21 light microscope.

Results

Anatomically, the seminal gland of buffalo bull was located in the pelvic cavity. It's anatomical findings; shape, color, size, consistency, lobulation, situation and relations as well as the measurements; length, width, thickness and weight were recorded and figured out. They were relatively differed in immature than those in mature seminal glands of buffalo bull.

The immature seminal glands were a pair of glands, present on the dorsal aspect of the pelvic urethra near the neck of the urinary bladder (Figure 1). The seminal gland appeared small sized, comma- shaped, flaccid, soft in texture, white in color and growing small lobules with smooth surface were characterizing the developing vesicular gland where, some small out growth-like buds were scattered on the outer surface of vesicular gland (Figures 1 and 2). The immature seminal gland measured 1.95-2.50 cm in length, 1.00-1.15 cm in width, 0.46-0.60 cm in thickness and 0.80-0.95 gm in weight.

The mature seminal glands appeared elongated with lobulated surface, present on dorsal aspect of pelvic urethra near the neck of the urinary bladder (Figures 1 and 2). The seminal gland was latterally attached to the terminal parts of ductus deferens, where the excretory ducts of the seminal gland opened with the end of ductus deferens forming an ejaculatory duct. The later, opened on the dorsal aspect of pelvic urethra near the neck of urinary bladder (Figure 1). The mature seminal gland was elongated, straight, tube-liked structure, large in size, hard, tough and firm in texture, pale-yellow in color, multilobulated; large lobules on it's outer surface giving the cluster of grapes-like appearance of the gland (Figures 1 and 2). The mature seminal gland measured 6 - 7 cm in length, 2.5 - 3 cm in width, 1– 1.3 cm in thickness and 9 – 9.5 gm in weight.

Histologically, the immature seminal gland was consisted of a stroma and a parenchyma. The stroma was formed of capsule, trabeculae and inter- acinar connective tissue (Figure 3). The capsule was formed mainly of smooth muscle bundles (Figure 3), irregular collagenous C.T. (Figure 4) together with few elastic fibers (Figure 5). Externally, the capsule was enveloped with loose C.T. enclosing B.Vs (Figure 3). Indistinct trabeculae was extended from the capsule, divided the gland into small lobes (Figure 3). The gland parenchyma was formed of central cistern, less differentiated and undifferentiated acini in the surrounding connective tissue that arranged in cluster in each lobule (Figure 3). The vertical sagittal section along the whole length of the gland; from free gland end towards it's attachement with the ductus deferens, showed

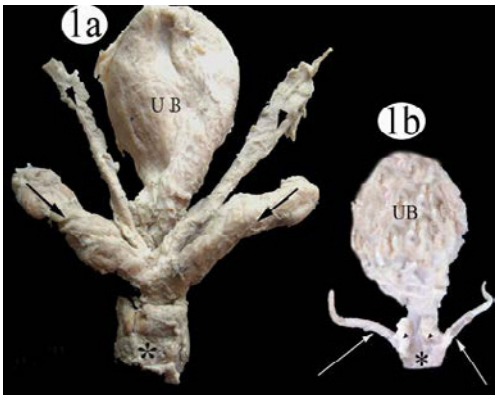

Figure 1: A) photograph showing mature seminal gland (arrow), ductus deferens (arrow head), urinary bladder (UB) and urethra (star). B) showing immature seminal gland (arrow head), ductus deferens(arrow), urinary bladder (UB) and urethra (star)

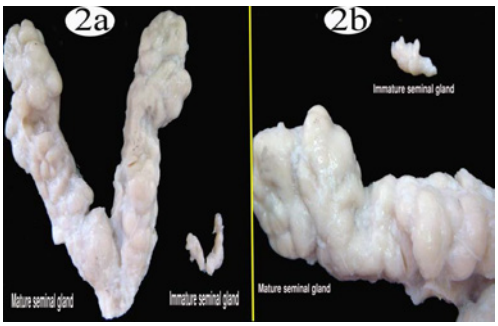

Figure 2: A) A photograph showing mature seminal gland (right side) and immature seminal gland (left side). B) high magnification showing mature seminal gland (down side) and immature seminal gland (upside).

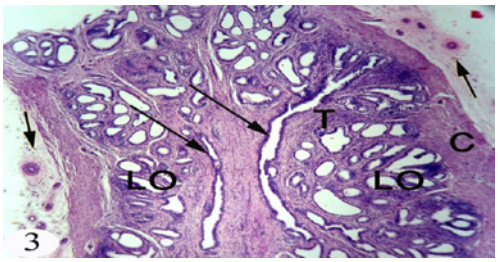

Figure 3: A photomicrograph of the longitudinal section of the immature seminal gland showing highly vascularized loose connective tissue enveloped the gland (short arrow), capsule (C), trabeculae (T), small lobules (LO) and central cistern (long arrow). Stain: H& E Obj.x4: Oc.x10

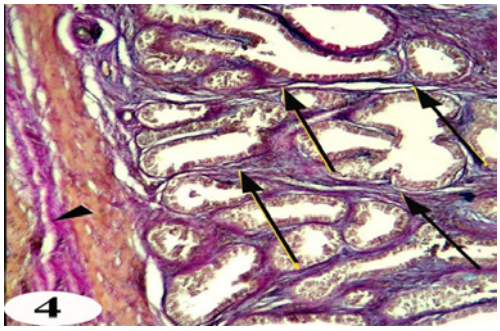

Figure 4: A photomicrograph of the immature seminal gland showing the distribution of collagenous fibers in capsule (arrow head) and the distribution of collagenous fibers in intra lobular stroma (arrow). Stain : Alcian blue (2.5) & Van Gieson's Obj. x10 : Oc.x10

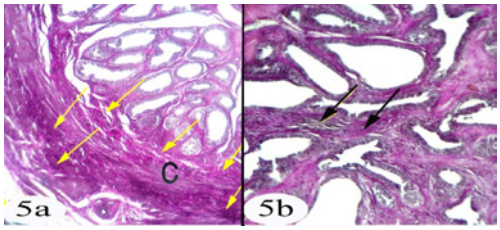

Figure 5: A) A photomicrograph of the immature seminal gland showing the distribution of the elastic fibers in the capsule (arrow), capsule (C). B) showing the distribution of elastic fibers in the inter-acinar connective tissue stroma (arrow). Stain : a, b) Weigert's elastic a) Obj. x4: Oc.x10 b) Obj. x10: Oc.x10

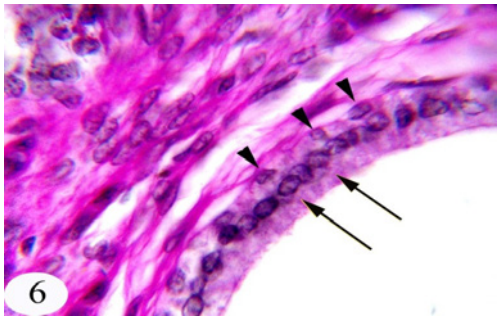

Figure 6: A photomicrograph of the immature seminal gland showing the linning epithelium of the acini; tall columnar cells (arrow) and basal cells (arrow head) Stain : PAS Obj.x100 : Oc.x10

the distribution of the parenchymal components into dorsal, lateral and ventral portions, in between them, there was a thickest central stromal structure in the gland (Figure 3).

The linning epithelium of the parenchymatus components was tall columnar cells; its height was 2-3 folds of width. Nuclei were rounded and sub-central. On the basement membrane and among the bases of columnar cells, one row of individual oval basal cells with flattened nuclei was present (Figure 6). The differentiated parenchymatus components of the seminal glands were surrounded by highly cellular connective tissue (Figure 6), collagenic fibers mainly (Figure 4). The collagenic fibers intermingled with few elastic fibers (Figure 5).

Resembling the immature seminal gland, the mature one was formed of two parts; stroma and parenchyma. The stroma was composed of capsule, trabeculae and inter-acinar connective tissue stroma. The parenchyma was composed of secretory end pieces and secretory duct.

UN like the immature seminal gland, the mature one was characterized by a stroma of well-developed fibro-muscular capsule and thick trabeculae (Figure 7). The capsule was mainly formed of smooth muscle bundles (Figure 7) with inter-muscular irregular collagenous connective tissue (Figure 8). From the capsule, thick well-developed fibro-muscular trabeculae were extended internally, dividing the gland into lobes of varying shapes, number and sizes (Figure 7). A separate triangular lobe was completely surrounded by fibro-muscular stroma from all sides; where, it covered from up by capsule and from the other two sides by septa (Figure 9). The septa had the same structure of the capsule but with less fibrous and more muscular. It was formed mainly of smooth muscle bundles (Figures 7 and 9). The thicker the septa, the more musculatures extending into it (Figures 7 and 9). In comparison to the immature gland, the thickness of the capsule and the trabeculae were increased with advancement of age.

The parenchyma was formed of numerous secretory end pieces and ducts. The secretory end pieces were compound tubular and tubulo-alveolar type, they were irregular in shape and different in size (Figure 10).

The secretory end pieces were lined by secretory epithelium of simple columnar type with individual small basal cells arranged on the basement membrane among the attached basal parts of the columnar cells (Figures 11-14).

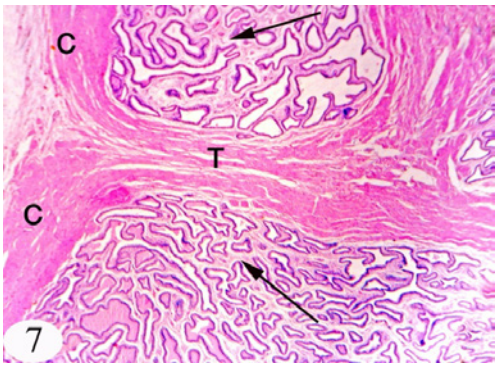

Figure 7: A photomicrograph of the mature seminal gland showing the fibro-muscular capsule (C), trabeculae (T) and 2 large lobes (arrow) Stain : H& E Obj. x4 : Oc.x10

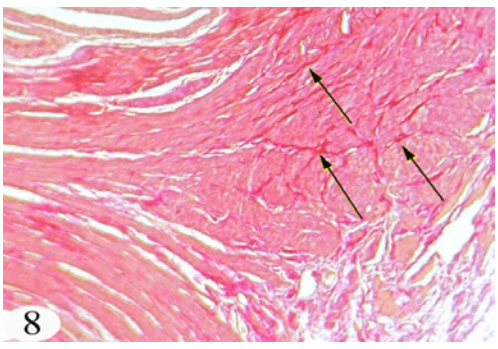

Figure 8: A photomicrograph of the mature seminal gland showing the distribution of collagenous fibers in capsule (arrow). Stain : Van Gieson's Obj. x4 : Oc.x10

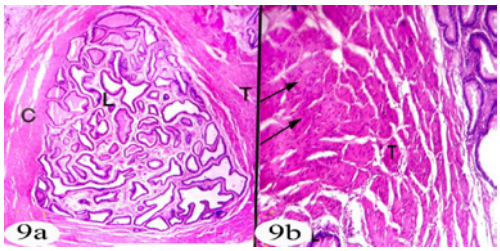

Figure 9: A) A photomicrograph showing separate lobe (L), capsule (C), trabeculae (T). B) Showing the arrangement of smooth muscle bundles in trabeculae (arrow), trabeculae (T). Stain: a, b) H&E a, b) Obj.x4: Oc.x10

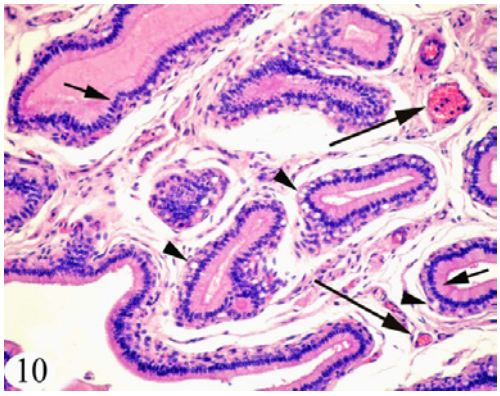

Figure 10: A photomicrograph of the mature seminal gland showing highly vascularized loose connective tissue in between the secretory acini (long arrow), tall columnar cells (short arrow) and vacuoles in basal cells (arrow head) Stain: H&E Obj.x40: Oc.x10

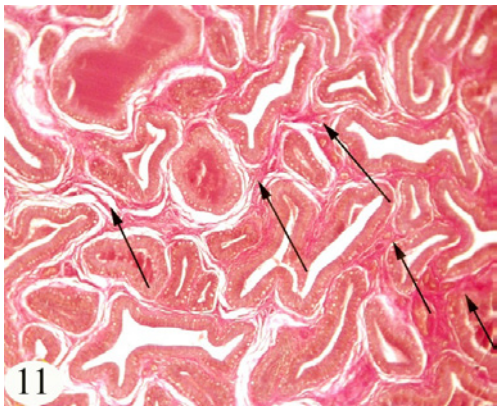

Figure 11: A photomicrograph of the mature seminal gland showing the distribution of collagenous fibers in between acini (arrow) Stain : Van Gieson's Obj.x10 : Oc.x10

The columnar cells were secretory, chief, principle cells. They were tall cells with variable height, measuring about 24-27 micro-meter in height. They rest smoothly on the basement membrane then extending from the later till reach to the luminal surface (Figures 15-18). The free surface of these cells was slightly 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, and might appear free in the lumen of the secretory tubules and alveoli (Figure 19).

The cytoplasm of columnar cells showed to be acidophilic (Figure 18) and vacuolated. The later were small in size and were located in the basal part of cytoplasm. These vacuoles were observed in cytoplasm of columnar cells and basal cells but more in the basal cells in mature gland (Figure 10), while in immature one not observed. There were acidophilic supra nuclearly secretory granules in the cytoplasm of secretory cells

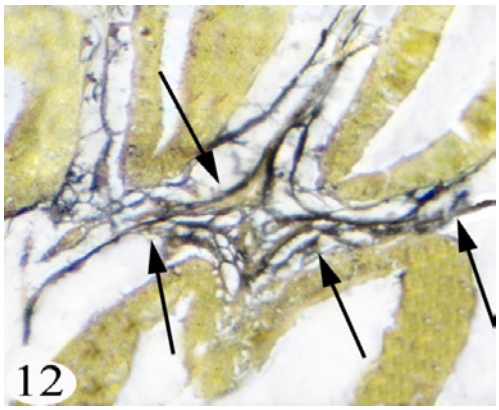

Figure 12: A photomicrograph of the mature seminal gland showing the distribution of reticular fibers in between acini (arrow) Stain : Silver stain Obj. x40 : Oc.x10

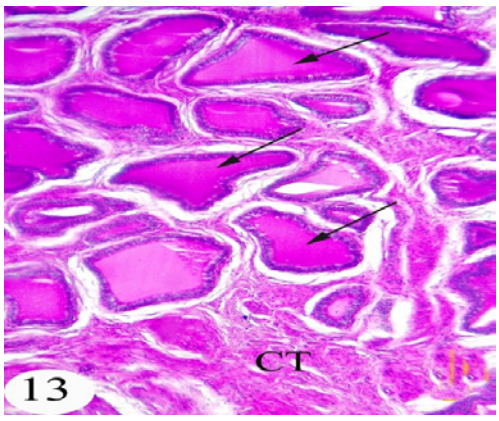

Figure 13: A photomicrograph of the mature seminal gland showing PAS positive reaction of the secretion (arrow) and positive reaction of the inter acinar connective tissue (CT) Stain: PAS Obj.x10:Oc.x10

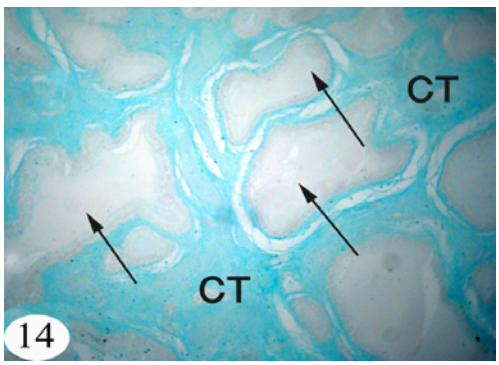

Figure 14: A photomicrograph of the mature seminal gland showing Alcian blue negative reaction of the secretion (arrow) and alcian blue positive reaction of inter acinar connective tissue (CT). Stain: Alcian blue (2.5) Obj. x40: Oc.x10

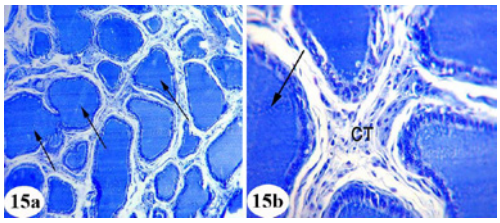

Figure 15: A) A photomicrograph of the mature seminal gland showing Toludine blue positive reaction of the secretion (arrow) and peri acinar connective tissue stroma. B) showed the same. Stain : Toludine blue a) Obj. x10 : Oc.x10. b) Obj. x40 : Oc.x10

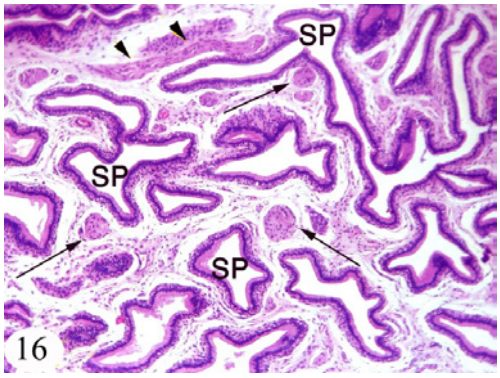

Figure 16: A photomicrograph showing the secretory end pieces (SP), circular smooth muscle bundles (arrow) and longitudinal smooth muscle bundles (arrow head). Stain: H&E Obj.x10: Oc.x10

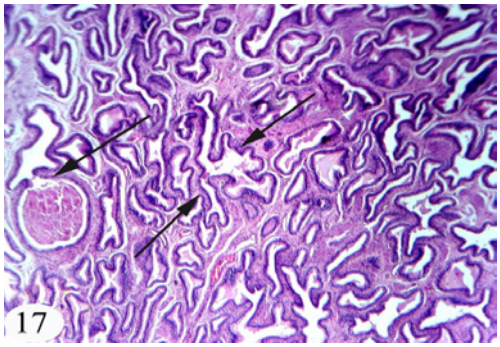

Figure 17: A photomicrograph showing the branched tubular and tubule-alveolar acini (arrow). Stain: H& E Obj. x4: Oc.x10

(Figure 18). These supra nuclearly secretory granules were positively reacted with PAS (Figure 20). The nuclei of the columnar cells were usually slightly irregular in shape; round, oval and elongated. It located in the basal third of the cell, containing coarse chromatin together with two or three prominent nucleoli (Figures 18-20).

The basal cells were flat, trigonal, ovoid, or Lense- shaped cells, with ill-defined cell boundaries. These cells rested on the basement membrane and arranged individually, it scattered in between secretory columnar cells, distributed irregularly, and not reach luminal surface. So, they didn't form a continuous layer. These cells were short, measuring about 5- 7 micro-meter in height and for about 9- 11 micro-meter in width (Figures 18,20). The cytoplasm of the basal cells was lightly acidophilic, with many vacuoles of variable size that occupied most of the cytoplasm often in infra-nuclear position (Figure 10). However, the cytoplasm was free from any secretory granules (Figures 18 and 20). Nuclei were spherical, angular or elongated, where found to be arranged parallel to the basement membrane (Figure 18).

Highly eosinophilic vaculated secretion found to be filled the acinar lumen giving foamy appearance of the luminal secretions (Figure 18). The secretion was reacted positively with PAS (Figures 13 and 20), and with toluidine blue (Figure 15). Meanwhile, with alcian blue the secretion was negatively reacted (Figure 14). Within the lobes, large irregular collecting sinuses were observed. The secretory end pieces

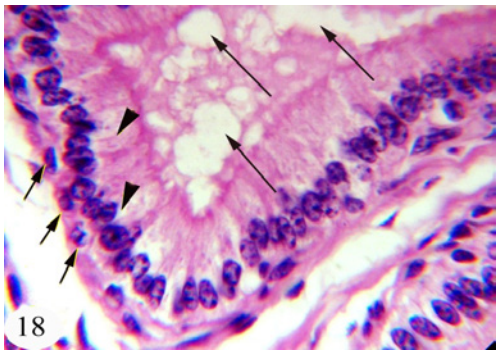

Figure 18: A photomicrograph showing the lining epithelium of the acini; tall columnar (arrow head) and basal cells (short arrow) and vacuolated intra-luminal secretion (arrow). Stain: H&E Obj.x100: Oc.x10

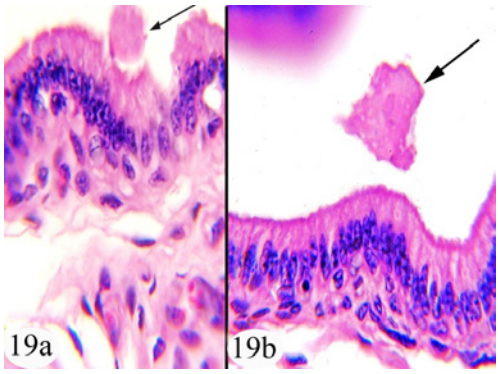

Figure 19: A photomicrograph showing detached apical blebs (arrow). Stain: a, b) H&E a, b) Obj.x100: Oc.x10

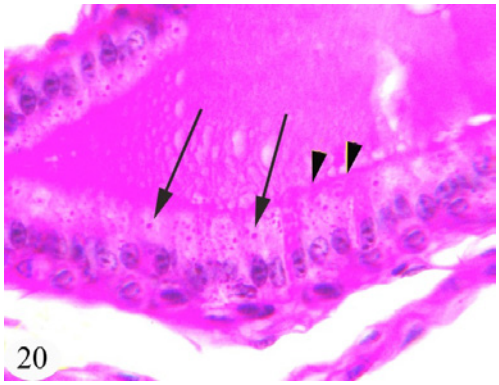

Figure 20: A photomicrograph showing PAS positive reaction of apical brush border (arrow head) and PAS positive reaction of secretory granules (arrow). Stain: PAS Obj.x100: Oc.x10

within the lobes opened into these sinuses either directly or through a connecting duct-like structure that drains the secretion from the secretory units (Figure 21). The intra-lobular ducts were lined by tall columnar cells with individual basal cell. The columnar cells appeared had acidophilic cytoplasm with tiny apical secretory blebs. The nuclei were spherical, central, lightly stained and had 2 or 3 prominent nucleoli (Figure 22).

The secretory end pieces and ducts were surrounded with a highly vascularized loose connective tissue (Figure 10). The loose connective tissue was formed mainly of fine collagenic fibers (Figure 11) intermingled with reticular fibers (Figure 12). Bundles of longitudinal and circular smooth muscle fibers were observed among the secretory end pieces (Figure 16). Moreover, the inter-acinar connective tissue was reacted positively with PAS (Figure 13), alcian blue (Figure 14) and toluidine blue (Figure 15).

With advancement of age the number of secretory end pieces per unite area increased and the amount of inter-acinar connective tissue stroma was reduced (Figure 17).

The secretory units and ducts in all parts of the seminal gland stored the increased amount of the secretion inside it's lumen but did not store spermatozoa except at the part of the gland that close to the point of union between the seminal gland and the ductus deferens where, few peripheral acini with narrow lumen near large ducts had sperms and other acini had eosinophilic secretions with sperms inside it's lumen (Figure 23).

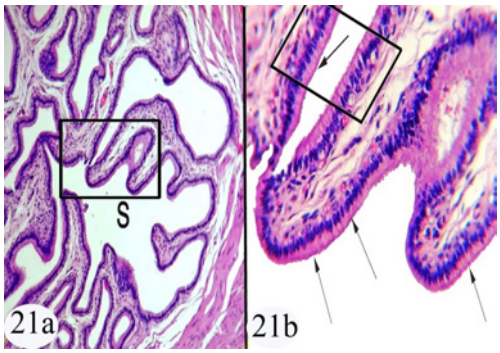

Figure 21: A) A photomicrograph showing collecting sinus (S) and B) higher magnification to the square area illustrated in (Figure 21a) showing the linning epith of sinus (arrow). Stain: a, b) H&E a)Obj.x10: Oc.x10 b) Obj.x40 : Oc.x10

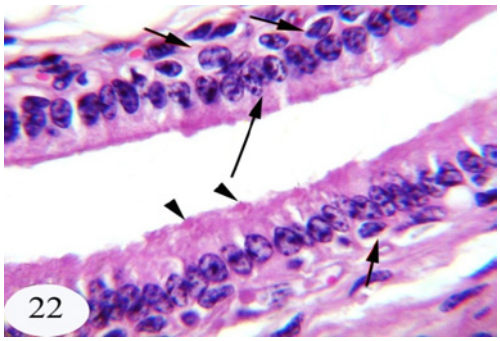

Figure 22: Higher magnification of (Figure 21b) showing the columnar cells linned the duct (long arrow) , basal cells (short arrow) and tiny apical blebs (arrow head). Stain: H&E Obj.x100: Oc.x10

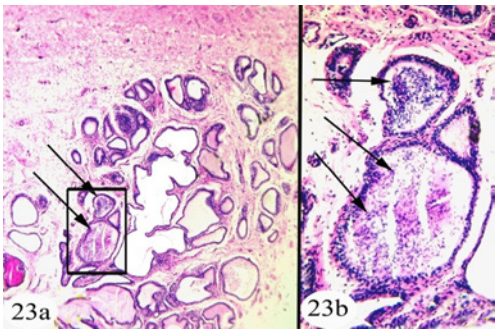

Figure 23: A) A photomicrograph showing sperms inside the acinar lumina (arrow) and B) higher magnification to the square area illustrated in (Figure 23a) showing the same. Stain: a, b) H&E a) Obj. x4: Oc.x10 b) Obj. x10: Oc.x10

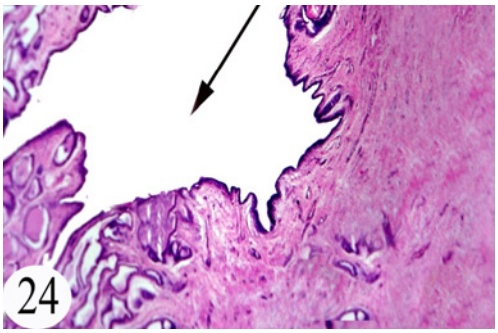

Figure 24: A photomicrograph showing the main excretory duct (arrow) Stain: H&E Obj. x4: Oc.x10

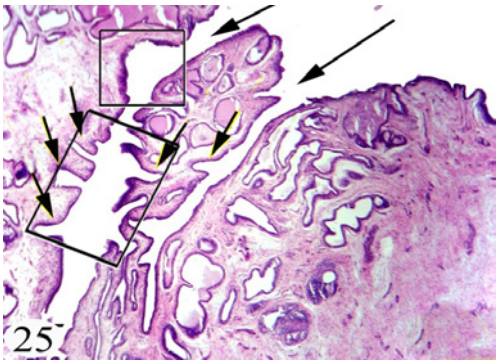

Figure 25: A photomicrograph showing the connecting ducts (short arrow), collecting ducts (long arrow).
Stain: H&E Obj. x4: Oc.x10

Loose connective tissue enclosed several secretory acini, some filled with secretion and other devoid of secretion. These acini surrounding large, irregular and wide cisternal-liked channels. These channels appeared consisting of large numbers of small, short connecting ducts. The later connecting with each other forming larger, longer and wider collecting ducts (Figure 24).

These collecting ducts lined with simple columnar cells with individual basal cells. Some focal areas linned with stratified epithelium (Figures 25 and 26). The collecting ducts connecting with each other forming larger, wider and main excretory duct; labyrinth-liked structures which considered the main drainage pathway of the gland (Figure 27).

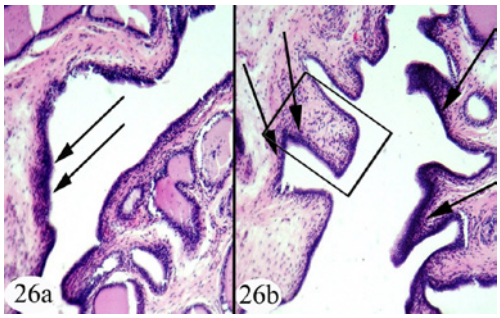

Figure 26: A) High magnification to the squares area illustrated in (Figure 25) B) Showing thickening in the duct linning epithelium (arrow). Stain: a, b) H&E a, b) Obj. x10: Oc.x10

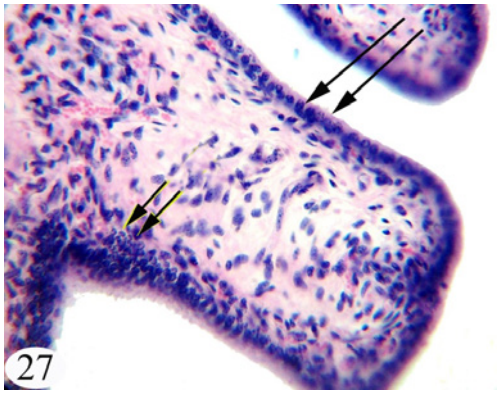

Figure 27: High magnification to the squares area illustrated in (Figure 26b) showing the simple linning epithelium of the duct "long arrow, and the conversion of the linning epithelium to stratified linning epithelium (short arrow). Stain: H&E Obj. x40: Oc.x10.

Discussion

The current work revealed that the seminal glands were a pair of glands. This finding is in agreement with Samuelson [19] in bull & boar in goat [9] and in boar [29] who reported that, the vesicular glands were a pair of lobular accessory sex glands.

The immature seminal gland situated at the point of union of ampulla with the pelvic urethra close to the urinary bladder. This finding is in agreement with [9] in one day Gaddi goat who reported that, the seminal gland was located at the origin of the urethra from the urinary bladder. Meanwhile, in the mature buffalo bull, the seminal gland was attached lateral to the terminal parts of ductus deferenss where the excretory ducts of seminal gland open with the end of the ductus deferens forming ejaculatory duct, the latter open on the dorsal aspect of pelvic urethra near the neck of urinary bladder. This finding is in agreement with [6,8] in bull**,** and with [9] in 12 months -old age goat, who reported that the seminal glands were situated on the dorso-lateral aspect of the neck of the urinary bladder and the initial part of the pelvic urethra lateral to the ampulla of the vas deferens.

The immature seminal glands were comma-shaped**,** appeared as small buds at the point of union of the ampulla with the pelvic urethra, and have very small lobules on its outer surface. Meanwhile, the mature seminal glands were elongated straight tube- like structure, compact, multi-lobulated glands like cluster of grapes and so called vesicular gland, this investigation is similar to Fahmy and Osman [10] in water buffalo bull, in goat [14,30], also this investigation goes hand in hand with Chandrapal [11] in buffaloes who reported that the seminal glands were tubular shaped with swollen ends in the neonatal and elongated with lobulation in the pubertal buffaloes. However, these result were in partial agreement with Archana et al. [9] in goat who reported that, in one day Gaddi goat *(Copra hircus),* the seminal gland was small white cord like structure appeared at the point of union of ampulla with the urethra, while at 12 month- old age become 'S' shaped**.** In boar, Dellmann and Eurell [22] reported that, the seminal gland was large bag like structure which be much larger and less compact than that in bull, while in stallion, Eurell and Frappier [18] and Dellmann and Eurell [22] reported that, the seminal glands were elongated sac like, pear shaped gland and true vesicles.

The consistency of immature seminal glands was flaccid in texure. Meanwhile, the mature seminal glands acquired a very firmness and became hard and firm. Our investigation is in agreement with Fahmy and Osman [10] in bull who reported that, the seminal glands were flaccid in texture in the bull which had one year old or less. The firmness of glands increased with advancement of age where the bull which had two years old age, the seminal glands acquire a very firmness in consistency and become tense in texture.

The immature seminal glands were small in size. Meanwhile, the mature one become larger where with the advancement of age, the size of seminal gland increased. This investigation goes hand in hand with Fahmy and Osman [10] and Osman Zaki [12] in bulls who reported that, the size of seminal glands and body of the prostate increase with progressing of age from 5 to 29 months. Also, This finding is coinciding with Archana et al. [9] in goat who reported that, the size of gland in Gaddi goat increased from birth to prepubertal then to pubertal although in the post pubertal does not grow very significantly.

The color of the seminal glands was changed from white in immature buffalo bull to yellow in mature one. This investigation is in parallilism with Fahmy and Osman [10] in water buffalo bull who added that, the color of the glands depended on their secretory activity as, the color of gland take the color of its secretion. In immature bull the gland was non secretory, so it was white in color meanwhile, in mature one, the gland secrete watery faint straw-yellow coloured secretion, so the gland color become yellow. The color finding goes hand in hand with Archana et al. [9] in Gaddi goat who reported that, in one day Gaddi goat *(Copra hircus)* the color of gland was white while, with advancement of age the color of gland slightly converted to yellow (whitish yellow).

The immature seminal gland measured about 1.95-2.50 cm in length, 1-1.15 cm in width and about 0.46-0.60 cm in thickness. Meanwhile, in mature one, it measured about 6-7 cm in length, 2.5-3 cm in width and about 1-1.3 cm in thickness. Such result indicate that, the anatomical measurements of buffalo bull seminal glands increased with advancement of age, This investigation goes hand in hand with Al-Guedawy [31] in water buffalo bull who reported that, the weight, length, breadth, thickness of seminal gland in buffalo bulls at 2 month old were 0.95 gm, 2.72 cm, 1.15 cm, 0.60 cm. while, at 17 month old were 9.45 gm, 5.69 cm, 2.49 cm, 1.04 cm. Moreover, goes hand in hand with [4] in mature Indigenous bull who reported that, the vesicular gland measured 9.44 cm in length, 2.78 cm in breadth, 1.45 cm in thickness. Furthermore, our findings were in partially agreement with Osman [32] in Egyptian buffalo bulls who reported that, the weight, length, breadth and thickness of the seminal glands of 9 months, 1.5 years and 2.5 years aged animals were found to be 5.22 gm, 3.7 cm, 2 cm, 0.6 cm and 7.87 gm, 5.6 cm, 2.5 cm, 1 cm and 8.62 gm, 6.1 cm, 2.6 cm, 1.1 cm respectively.

Our findings revealed that, the seminal gland was composed of two main parts; stroma and parenchyma. The stroma was formed of capsule, trabeculae and inter-acinar connective tissue. Meanwhile, the secretory end pieces were the main parenchymal components. This finding is in agreement with Fahmy and Osman [10], Ghonimi et al. [33] in water buffalo bull, Yao and Eaton [21] in goat, Abbas [15] in ram and Bacha WJ and Bacha LM [20] in ruminant but not in coincidence with Chandrapal [11] and Sudhakar [23] in bull, Banks [17], Eurell J and Frappier [18], Samuelson [19] in bovines, Archana et al. [9] in goat who reported that the seminal gland histologically was consisted of 4 layers; tunica mucosa, tunica propia-submucosa, tunica musculosa and tunica adventitia.

The immature seminal glands were surrounded with thin layer of fibro-muscular capsule. Meanwhile, the mature one surrounded by thick well-developed fibro-muscular capsule. The capsule was formed of smooth muscle bundles inter-mingled with fibrous connective tissue. Such result is similar to Fahmy and Osman [10] in water buffalo bull who reported that, the seminal gland was surrounded by a fibro-muscular connective tissue capsule, and with Yao and Eaton [21] in goat who reported that, the seminal glands in section showed many lobulated sacs enclosed in a sheath of fibro-muscular tissue. And also is in agreement with Abbas [15] in ram who reported that, the seminal gland was covered with a well-developed fibro-muscular capsule that was formed of smooth muscle bundles with fibro-elastic connective tissue.

The seminal glands were invested with a peri-capsular loose connective tissue adventitia. It was a thick layer, including many blood vessels of considerable size, lymph vessels, fat cells and nerve fibers. The fibrous connective tissue condensed to form a thin fibro-elastic layer that joined the highly vascularized loose connective tissue adventitia with the fibro-muscular capsule. This investigation goes hand in hand with Abbas [15] in ram who reported that, outside the capsule, the fibrous connective tissue condensed to form a thin fibro-elastic layer that connected the loose connective tissue adventitia with capsule, this loose connective tissue layer had collagen, reticular and elastic fibers in between them blood vessels of considerable size, lymph vessels, fat cells and nerve gangilia were present. Moreover, this result goes hand in hand with Archana et al. [9], Gupta [34] in goat and Sudhakar [23] in bull who reported that, the tunica adventitia were comprised of loosely arranged meshwork of collagen, reticulin and elastic fibers, also many blood vessels, nerve fibers and autonomic ganglia were seen in tunica adventitia.

With advancement of age, the thickness of capsule and tunica adventitia was relatively increased. This investigation is in agreement with Archana et al. [9] in goat who reported that, the thickness of the tunica adventitia, capsule and trabeculae increased with aging while the interstitial tissue is shrunk correspondingly.

From the capsule, a thick well-developed fibro-muscular trabeculae were extended internally, dividing the seminal gland into lobes of varying shapes, number and sizes, this investigation is in agreement with Fahmy and Osman [10], Ghonimi et al. [33] in bull**,** Abbas [15] in ram and Mosaliam [35] in camel prostate**,** also similar to Yao and Eaton [21] who described the vesicular gland of the goat as showing many lobulated sacs embedded in a sheath of fibro-muscular tissue and each sac is divided into a number of compartments by a thick layer of fibro-muscular trabeculae.

The septa had the same structure of the capsule but they were less fibrous and more muscular. Such result is very close to those described after Abbas [15] in ram and Mosaliam [35] in camel prostate**.**

The thicker the septa, the more muscle fibers extending inside the septa. Also, this smooth muscle bundles run parallel to the longitudinal axis of the gland. This investigation is in agreement with Abbas [15] in ram**,** Mosaliam [35] in camel prostate who reported that, the more thickening in the septa, the more muscle fibers extending into it. Also similar to Eurell and Frappier [18], Samuelson [19], Bacha WJ and Bacha LM [20] in bovines who reported that, the septa in ruminants were thick due to it contain abundance of smooth muscle, but in stallion and boar the septa consist of predominantly of connective tissue with some smooth muscles.

With advancement of age the thickness of the capsule and trabeculae relatively increased, where the thickness in the immature bull was indistinct and thin while, in the mature one, it became larger, thicker and well-developed. This investigation goes hand in hand with Archana et al. [9] in goat.

The intra lobular stroma was represented as highly vascularized connective tissue, formed mainly of collagenous fibers, reticular fibers inter-mingled with individual smooth muscle bundles that arranged in circular and longitudinal manner, surrounding the secretory units of gland. This investigation is in agreement with Abbas [15] in ram**.** Also**,** this investigation is in partially agreement with Eurell, Frappier [18], Samuelson [19] in bovines who reported that, the tunica propia submucosa consists of highly vascularized loose connective tissue which becomes dense ct.at septa which divided gland into lobes and lobules

Aggregation of circular and longitudinal individual smooth muscle bundles around the secretory units of gland might be involved in the evacuation of the gland secretions. The importance of this aggregation from smooth muscle fibers is explained by Junqueira [3] who stated that, The abundant smooth muscle produces strong peristaltic contraction that participate in the expulsion of the seminal gland secretion.

With advancement of age, the number of secretory end pieces per unit area increased and the amount of inter-glandular connective tissue stroma reduced . This investigation is in agreement with the finding of Archana et al. [9] in goat. Such finding revealed hyper-activity of smooth muscle bundles in the mature buffalo bulls than in the immature one.

The gland parenchyma of the immature seminal glands was formed mainly of central cisterns surrounded with differentiated and un differentiated acini in the surrounding connective tissue that arranged in cluster in each lobe. This investigation is in a similarity with Archana et al. [9] in one day old kids who reported that, the parenchyma showed lobules with central cisterns, branching ducts and differentiated acini in the surrounding connective tissue.

The parenchyma of mature gland was formed of large number of secretory end pieces which connected with each other forming system of secretory unit. Such result is in agreement with Osman and Zaki [12], Osman [32] in bull and Abbas [15] in ram. Also, similar Yao and Eaton [21] in goat who reported that, the gland parenchyma was consisted of large number of secretory end pieces and system of ramified secretory tubules. The secretory units were collected together in groups; each was surrounded by thin bundles of smooth muscle fibers which intermingled with Fibro architectural design comprised of circularly arranged collagenous fibrous bundles with reticular fibers.

The secretory units were lined with secretory epithelium of simple columnar with some individual basal cuboidal cells scattered among columnar cells**.** This finding goes hand in hand with Fahmy and Osman [10], Abdel-Raouf [16], Ghonimi et al. [33], Amselgruber and Feder

[36] in bull**,** Aumüller and Seitz [37] in bovines, Abbas [15], Skinner et al. [38] in ram, Wrobel [39] in goat**,** Brewster [27] in human and Veneziale et al. [40] in guinea pigs who reported that the secretory end pieces of seminal glands were found lined by two types of cells; columnar cells with variable height and low basal cells. While**,** the three types of cells; A, B and C have been identified by Yao and Eaton [21], Gupta [30], Gupta and Singh [41] in goat, Ploen [42], Singh et al. [43] in ram and Chandrapal [11], Sudhakar et al. [23] in buffalo and also Aumüller and Seitz [37] in bovines**.** Moreover, 4 types of cells were identified; columnar, basal, dense and clear cells in boar [29].

Within the lobules, large irregular collecting sinuses were found where, the secretory end pieces within the lobules opened in the sinuses either directly or through a connecting duct which drains the secretion of a number of secretory units. This investigation is completely goes hand in hand with Mosaliam [35] in camel prostate.

The secretory units and ducts stored the increased amount of the secretion inside its lumen but not store spermatozoa except at the point of union between the seminal gland and ductus deferens where, few peripheral acini with narrow lumen near large ducts had sperms. This result indicating that, in addition to the seminal gland was considered one of the main sources of metabolites, that essential for the sperm viability, life ability and keeping the sperm alive, active, motile for very long period. The seminal glands play a partial role in the sperm storage. This investigation is in a partial agreement with Hafez [6], Eurell and Frappier [18] in bovines and ovines, who stated that, the seminal glands stored the increased amount of the secretion inside its lumen but not store spermatozoa. Moreover, Gartner, Hiatt [2] in monkey reported that the seminal glands once were believed to store spermatozoa, where some of which are always present in the lumen of this gland.

Conclusion

We are concluded that the seminal glands are essential reproductive accessory genital glands of buffalo bulls. Its anatomical findings; shape, size, consistency, lobulations, measurements, cyto-archeticture and histological structure are varying with age. With advancement of age, the thickness of the capsule and trabeculae increased, also the number of secretory end pieces per unite area increased and the amount of inter-glandular connective tissue stroma reduced. Moreover, the secretory units were lined with pseudo-stratified columnar epithelium which, is consisted of two type of cells; columnar cells and basal cells. The secretory columnar cells of seminal gland showed morphological diversity, which could be attributed to different stages of the secretory cycles, where some of the lobules were in the secretory stage, other in the synthesizing and storage stage while, other were exhausted of secretion.

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