Bulgarian Journal of Veterinary Medicine, 2014, **17**, No 1, 9–17
ISSN 1311-1477; online at http://tru.uni-sz.bg/bjvm/bjvm.htm

Original article

MORPHOLOGICAL STUDIES ON THE SEMINAL GLANDS OF MATURE BUFFALO BULLS (*BOS BUBALIS* L.)

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Summary

Ghonimi, W., A. Sharaf, M. Hafez Bareedy, A. Balah & A. Awad Abuel-Atta, 2014. Morphological studies on the seminal glands of mature buffalo bulls (*Bos bubalis* L.). *Bulg. J. Vet. Med.*, **17**, No 1, 9–17.

The current work was carried out on the seminal glands of 20 healthy male buffalo bulls. The specimens were collected and examined anatomically and histologically after being fixed in 10% buffered neutral formalin. Paraffin sections were obtained and stained. Grossly, the vesicular glands were a pair of elongated glands located on the dorsal aspect of pelvic urethra near the neck of the urinary bladder and found attaching lateral to the terminal parts of ductus deferens. Microscopically, the vesicular gland was covered with thick well-developed fibro-muscular capsule, formed of smooth muscle bundles intermingled with fibrous connective tissue. The gland was divided into lobes with thick well-developed fibromuscular connective tissue septae. The gland parenchyma consisted of numerous secretory units of tubular and tubulo-alveolar types and ducts were lined by pseudostratified columnar epithelium consisting of two cell types: columnar cells and basal cells.

Key words: gross anatomy, histomorphology, mature buffalo bull, vesicular gland

INTRODUCTION

Buffaloes are of a great economic and productive importance as well as of a considerable biologic interest in the tropical and subtropical areas. In Egypt, tremendous demands are focused on buffaloes for their strength and resistance against different diseases and ecological conditions (Rahman *et al.*, 2010).

The vesicular gland is the largest accessory sex gland of the bull genital tract (Egli, 1956). It is considered the main source of fructose and citric acid in most domestic ruminants where the fructose provides energy and nutrition for spermatozoa, keeping the sperm live, active and motile. The fructose concentration of ram and bull semen is particularly high (Hafez, 1987). Several authors have studied the micromorphological and histochemical structure of the seminal vesicle of different animals, especially laboratory ones: Wong (1983) in guinea pigs, Mukerjee & Rajan (2006) in rats, Chapman & Chapman (1980) in fellow deer as well as

in humans (Brewster, 1985). Meanwhile, in buffalo bulls, the seminal glands have received little attention.

The current work comes as a response to the national strategy plan for increasing and improving the productivity and reproductivity of buffaloes in Egypt and aimed to throw more light on the morphological structure of seminal gland in buffalo bulls.

MATERIALS AND METHODS

Seminal glands of 20 apparently healthy mature buffalo bulls were collected from Zagazig slaughterhouse, Sharkia province for anatomical and histological studies. The vesicular glands were immediately fixed in 10% buffered neutral formalin. Thin paraffin sections (7–10 μm) were obtained and stained with Harris's haematoxylin and eosin (H&E) for routine histological studies, Van Gieson's stain for demonstration of collagen fibres and muscle cells, Bromophenol blue (BPB) for detection of general proteins, Periodic Acid Schiff technique (PAS) for detection of neutral mucopolysaccharides, Alcian blue at pH 2.5 for detection of acidic mucopolysaccharides and toludine blue for detection of metachromatic mast cell granules. The pictures were taken with Olympus BX 21 light microscope.

RESULTS

Gross anatomy

The seminal gland was laterally attached to the terminal part of ductus deferens, where the excretory ducts of seminal glands 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 (Fig. 1A). The seminal gland was elongated, straight, tube-liked 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 (Fig. 1B).

Microscopic anatomy

The mature seminal gland was characterised by a stroma of well-developed fibromuscular capsule and thick trabeculae

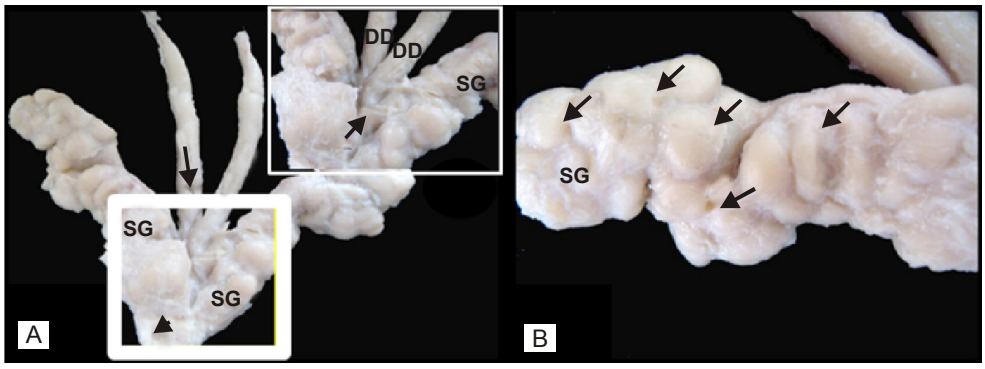

Fig. 1. Gross anatomy of mature seminal gland. **A.** Mature seminal gland (SG) with ductus deferens (arrow) and prostate gland (arrowhead). The higher magnification images of boxed area in the right upper corner shows the point of junction between seminal gland and ductus deferens (DD) (arrow). **B.** The mature seminal gland (SG) is a multi-lobulated gland similar to punch of grape (arrows).

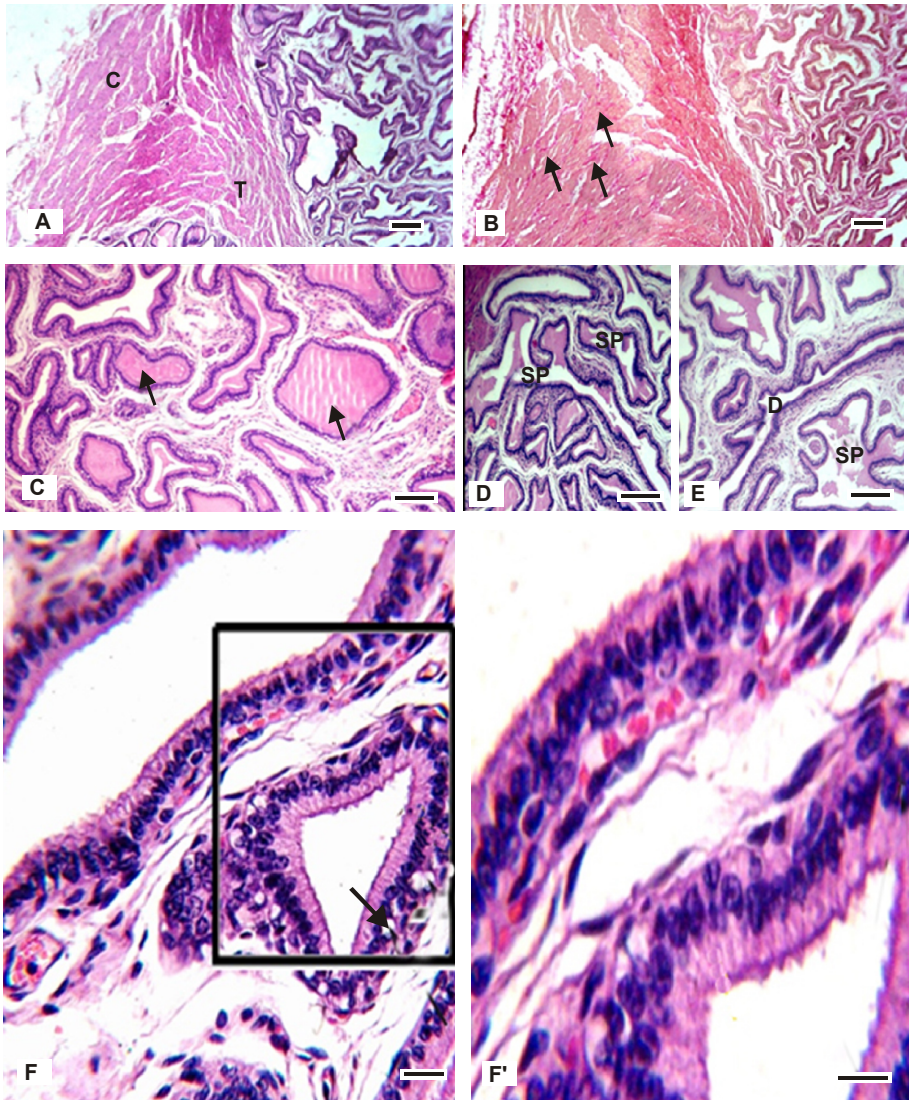

Fig. 2. Histological structure of the seminal gland. **A.** Fibro-muscular capsule (C) and trabeculae (T) of mature seminal gland in 20-month-old buffalo bull. H&E, bar = 200 µm **B.** Distribution of collagenous fibres in the capsule (arrow). Van Gieson's stain, bar = 200 µm. **C.** Acinar glands filled with eosinophilic secretion (arrow) and highly vascularised interacinar connective tissue. H&E, bar = 200 µm. **D.** Glandular lobules are occupied with tubular and tubulo-alveolar secretory end pieces (SP) and ducts (D) in **E.** H&E, bar = 200 µm. **F.** Vesicular tubular and tubulo-alvealar end pieces lined with tall columnar cells and presence of vacuoles in the basal cells (arrow). **F′**. Higher magnification to the boxed area in **F.** showing tall columnar epithelium, H&E, bar = 50 µm.

(Fig. 2A). The capsule was mainly formed of smooth muscle bundles with inter-muscular irregular collagenous connective tissue (Fig. 2B). Thick well-developed fibro-muscular 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 loose connective tissue (Fig. 2C). The parenchyma was formed of numerous secretory end pieces and secretory ducts. The secretory end pieces were of compound tubular and tubulo-alveolar type, with irregular shape and different size (Fig. 2D, E). Secretory end pieces were collected together in clusters 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 (Fig. 2F, F´). Highly eosinophilic vacuolated secretion was found to fill the 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 in the basal cells of mature seminal gland epithelium (Fig. 2F, F´).

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 cyto-

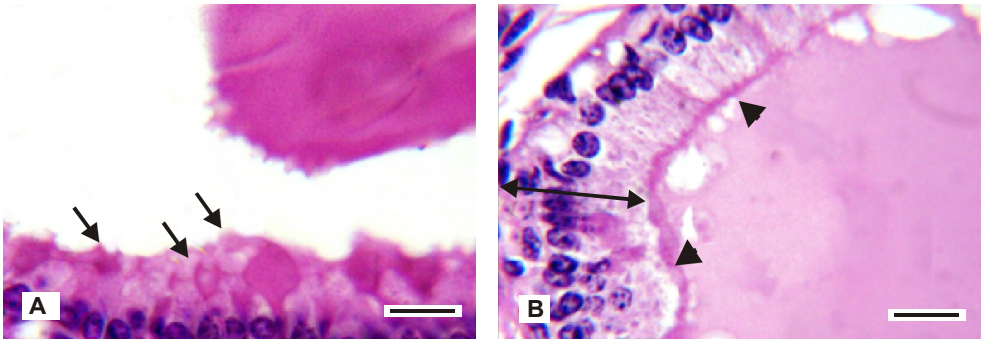

Fig. 3. A. Apical blebs (arrows) in the lining epithelium of glandular end pieces. **B.** Acidophilic apical brush border (arrowheads) of glandular end pieces. H&E, bar = 50 µm.

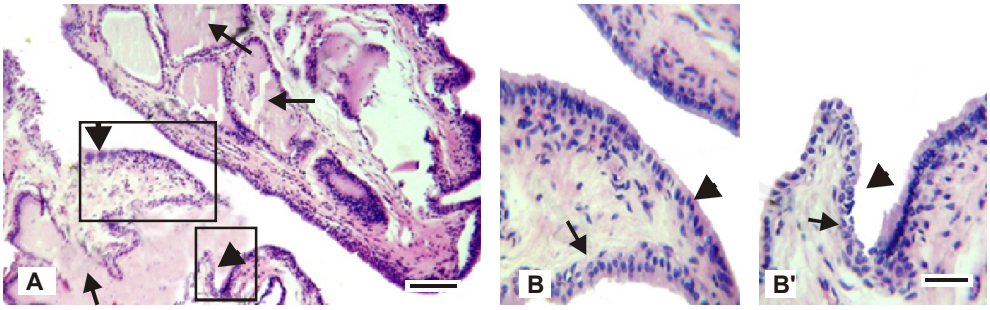

Fig. 4. A. The duct lumens are folded (black arrow), secretory units with eosinophilic secretion (arrowheads); H&E, bar=200 µm; **B, B´:** Higher magnification to the boxed areas in **A**: simple columnar lining epithelium (arrow) and stratified epithelium (arrowhead). H&E, bar=50 µm.

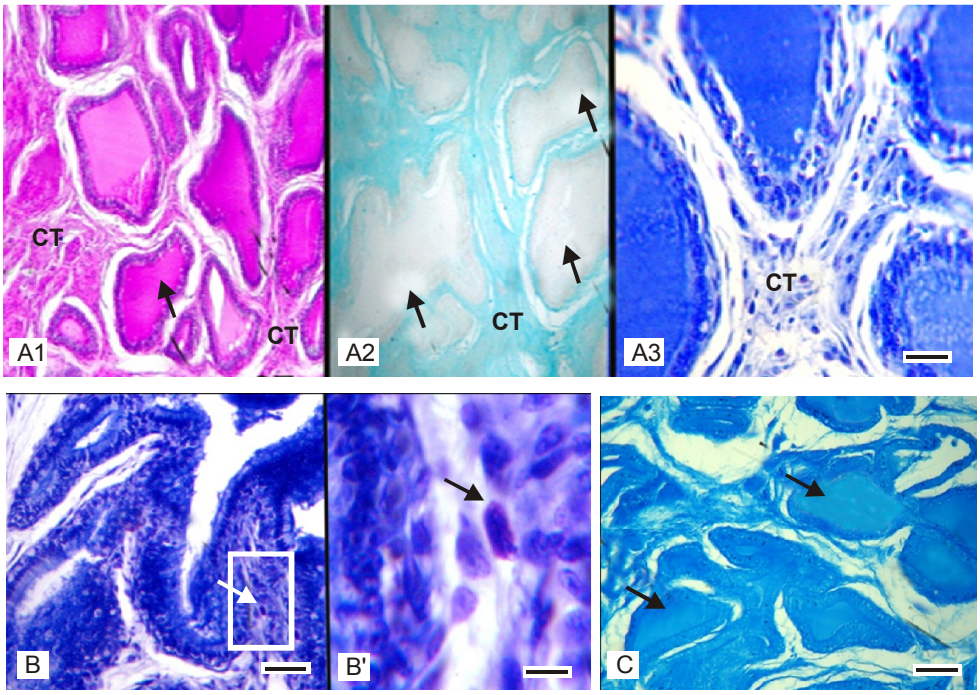

Fig. 5. Histochemical staining of mature seminal gland. **A.** Glandular secretory units (arrow) are PAS positive (A1) and are AB negative (A2). However, intralobular connective tissue (CT) stroma is Alcian blue positive (A2). Toludine blue positive reaction of both glandular secretion and peri-acinar connective tissue (CT) stroma (A3). PAS (A1) and Alcian blue (A2, A3), bar = 200 µm. **B.** Mast cell in intralobular connective tissue stroma (arrow). **B´**: higher magnification to the boxed area showing toluidine blue positive mast cell. Toluidine blue, bar = 200 µm (B) and 50 µm (B´) **C.** Bromophenol blue positive glandular acinar secretion (arrows). Bromo phenol blue, bar = 200 µm.

plasmic projection. Some of these cells had light, bleb-like apical projections. These projections were acidophilic had inverted pear shape-like structures (Fig. 3A). The cytoplasm of columnar cells were acidophilic (Fig. 3A, B) 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. So, they didn't form a continuous layer. 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.

The lumen of intralobular ducts appeared highly evaginated and invaginated forming folds like structure similar to tongue papillae in shape (Fig. 4A) and lined by tall columnar cells with individual basal cell and acidophilic cytoplasm. The nuclei were spherical, central, lightly stained, which lined with simple columnar cells with individual basal cells (Fig. 4B, B´).

Histochemically, both PAS (Fig. 5A1) and toludine blue (Fig. 5A3) strongly positive staining was observed in the gland secretory end pieces. However the glandular secretion reacted negatively with alcian blue (Fig. 5A2). Furthermore, the peri-acinar stroma surrounding the secretory end piece reacted positively with PAS (Fig. 5A1) and toludine blue (Fig. 5A3). In the peri-acinar connective tissue stroma, few individual elongated mast cells were observed in toludine blue stained section (Fig. 5B). In addition the stromal connective tissue was alcian blue positive (Fig. 5B). The glandular secretion reacted positively with bromophenol blue (Fig. 5C).

DISCUSSION

The current work revealed that the seminal glands were elongated, straight tube-like structures, compact multi-lobulated glands similar to bunch of grapes as described by Fahmy & Osman (1972) in water buffalo bulls. The consistency of mature seminal glands was firmer and harder than in immature ones. The results of our investigation are in agreement with Fahmy & Osman (1972) who reported that seminal glands were flaccid in texture in the bulls one year of age or less. The firmness of seminal gland increased with advancement of age; the 2-year-old bulls being with denser seminal glands.

The seminal gland is composed of stroma and parenchyma. The stroma is formed of capsule, trabeculae and inter-acinar stromal connective tissue. Meanwhile, the secretory end pieces were the main parenchymal components. This finding is in agreement with Fahmy & Osman (1972) in water buffalo bulls, Yao & Eaton (1954) in goat,s Abbas (1976) in rams and Bacha & Bacha (2000) in ruminants but not corresponding to data of Chandrapal (1976), Sudhakar *et al.* (1986) in bulls, Banks (1992), Eurell & Frappier (2006) and Samuelson(2007) in bovines, Archana *et al.* (2009) in goats who found that the seminal gland consisted of four layers – tunica mucosa, tunica propia – submucosa, tunica musculosa and tunica adventitia.

The mature seminal glands were surrounded by a thick well-developed fibromuscular capsule, formed of smooth muscle bundles intermingled with fibrous connective tissue. Such result is given by Fahmy & Osman (1972) in water buffalo bulls. Yao & Eaton (1954) observed that seminal gland showed many lobulated sacs enclosed in a sheath of fibromuscular tissue in goats.

From the capsule, thick well-developed fibromuscular trabeculae were extended internally, dividing the seminal glands into lobes of varying shapes, numbers and sizes, as also shown by Osman & Zaki (1965) in bull, Abbas (1976) in ram and Mosaliam (1981) in camel prostate. Our data are also comparable those of Yao & Eaton (1954) who described the vesicular gland of the goat as many lobulated sacs embedded in a sheath of fibromuscular tissue, each sac being divided into several compartments by a thick layer of fibromuscular trabeculae.

The intra-lobular stroma was formed of highly vascularised connective tissue, which is similar to reports of Abbas (1976) in rams. Also, this observation partially agrees with Eurell & Frappier (2006), Samuelson (2007) in bovines who reported that the tunica propia submucosa consists of highly vascularised loose connective tissue which becomes dense connective tissue at septa dividing the gland into lobes and lobules.

The gland parenchyma was formed of numerous secretory end pieces connected each with the other forming a system of secretory units. The observation is in agreement with Osman & Zaki (1965) in bulls, Abbas (1976) in rams, and also Yao & Eaton (1954) in goats, who reported that the gland parenchyma 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 fibres which intermingled. The fibroarchitectural design comprised of circularly arranged collagenous fibrous bundles with reticular fibres.

The secretory units were lined with secretory simple columnar epithelium with some individual basal cells scattered among columnar cells. This finding goes hand to hand with data of Abdel-Raouf (1960), Fahmy & Osman (1972), Amselgruber & Feder (1986) in bulls, Aumuller & Seitz (1990) in bovines, Abbas (1976) and Skinner *et al.* (1968) in rams, Wrobel (1970) in goats, Veneziale *et al.* (1974) in guinea pigs. In humans, Riva & Aumuller (1994) reported that the secretory end pieces of seminal gland were lined with two types of cells – columnar and low basal cells. Three types of cells including A, B and C cells have been identified by Yao & Eaton (1954), Gupta (1989) in goats; Ploen (1980), Singh *et al.* (1980) in rams, Chandrapal (1976) and Sudhakar *et al.* (1986) in buffaloes and Aumüller & Seitz (1990) in cattle. Moreover, four types of cells (columnar, basal, dense and clear cells) were identified in boars (Badia *et al.* 2006).

The basal cells were flat, trigonal, ovoid, or lense-shaped cells including vacuoles with variable size. Also, these cells were short, measuring about 5–7 μm in height and for about 9–11 μm in width similar to what was communicated by Aumüller & Seitz (1990) in bovines. According to those authors, the B-type was basal, reserve and fat storing cuboidal cella. These cells were short, measuring about 4– 6 μm in height and 9–11 μm in width. The intralobular ducts were lined with simple columnar cells with individual basal cells. This lining epithelium was slightly changed to a bi-layer of cuboidal cells. Also, some focal areas were lined with stratified epithelium. This investigation agrees with Dellmann & Eurell (1998) in bulls and horses, stating that the secretory ducts were lined by a cuboidal or columnar according to the activity of the gland epithelium in bulls or by stratified columnar epithelium in the horses.

In conclusion, the seminal glands are essential reproductive accessory genital glands in buffalo bulls. Anatomically, the mature SG has firm texture, pale yellow colour and is multilobulated similar to a punch of grapes. However the histological structure of SG is similar to the ordinary structure of any compact organ including the stroma and the parenchyma. Moreover, many changes in the histological structure have been observed in the advanced age including thickness of capsule and trabeculae, increase number of secretory unites and decrease of interglandular C.T stroma.

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Paper received 31.12.2012; accepted for publication 18.03.2013

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