

Original Research Paper

Histomorphochemical Studies on Accessory Glands of Iraqi Blackbuck (*Caprus Hircus*)

¹Waleed J. A. Al-Kelaby, ²Wafaa Hadi Mousa AL-Hashem,
³Zahraa S. M. Al Kaabi and ^{4,5}Hany Akeel Al-Hussaniy

¹Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Kufa, Iraq

²Department of Basic Science, Faculty of Dentistry, University of Kufa, Iraq

³Department of Laboratory Investigations, Faculty of Science, University of Kufa, Iraq

⁴Faculty of Pharmacy, Bilad Alrafidain University College, Diyala, 32001, Iraq

⁵Dr. Hany Akeel Institute, Iraqi Medical Research Center, Baghdad, Iraq

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Corresponding Author:

Waleed J. A. Al-Kelaby

Department of Anatomy,

Histology and Embryology,

Faculty of Veterinary Medicine,

University of Kufa, Iraq.

Email:

waleedj.abed@uokufa.edu.iq

Abstract: The various species' male accessory genital glands vary and are intimately related to the pelvic urethra. These glands secrete nutritional substances, buffers, and mucous to increase volume, serve as lubricants, reduce the minor acidity of vaginal contents and make the environment more hospitable to motile sperm. This study aims to study the histological and histochemical structure of the male accessory sex glands in caprine Iraqi breeds. The male accessory genital glands of ten adult males of the local Iraqi buck (*Caprus hircus*) weighing 48-65 kg and aged 18-26 months were obtained from the Al-Najaf slaughterhouse. The specimens were prepared and stained with Hematoxylin and Eosin (H and E), Periodic Acid-Schiff Reaction (PAS), and Masson's trichrome stain. In the ampulla, the glands revealed thin, wide tubules bordered by a simple columnar epithelium and pseudostratified columnar epithelium with rounded nuclei. The seminal vesicle, buck's mucous membrane, is folded according to histology. It is a simple columnar epithelium with basal cells in between and nuclei that range from spherical to oval. The gland capsule comprises loose connective tissue (collagen fibers and smooth muscle) that responds favorably to Masson's trichrome stain. The prostate gland, the glandular tissue of the pelvic urethra, is implanted in the wall. The simple columnar epithelium lines these alveoli; however, histological sections reveal that the prostate gland duct-lining epithelium has converted to pseudostratified columnar epithelium. The gland secretion is seromuroid with a positive PAS staining result. In the bulbourethral gland, varied sizes and shapes of secretory units were distinguished. Most secretory units are alveolar in shape, while some are saccular or tubular. The seminal vesicle, bulbourethral glands, and vas deferens ampulla are well-developed and paired. Bucks have scattered prostate glands. The pseudostratified columnar epithelium lines the branching tubular ampulla. Simple columnar epithelium and basal cells cover the mucosa of the seminal vesicle.

Keywords: Histology, Histochemical, Black Buck, Male Accessory Sex Gland

Introduction

Males make up half of the herd, and the male reproductive system is crucial. Between the ductus deferens and the base of the penis, there are a series of accessory sex glands. These glands secrete seminal plasma, which helps transit sperm to the female reproductive canal while providing nutrition and

protection until ultimate maturation. The male reproductive system is crucial in farm animal reproduction because it generates sperm. These are needed for fertilization to increase the farm animal population size, which is economically vital to countries worldwide (Bansal *et al.*, 2021). Male accessory genital glands are closely related to the pelvic urethra and vary by species. They include the prostate glands, seminal

vesicular glands, bulbourethral glands (Cowper's gland), and the ductus (Vas) deferens ampulla. These glands produce mucus, occasionally seromucous, nutritional secretions and buffers. These fluids improve volume while acting as lubricants (Mohammed and Doohi, 2017).

The ampulla is the glandular enlargement of the terminal parts of the ductus deferens near the urethra. Some have considered the ampulla as a short-term storage site for sperm. The ampulla shares a duct with the vesicular gland on the same side and enters the urethra through a shared duct (Mohammed and Doohi, 2017). The seminal vesicle is a hollow tubular organ. The vesicular gland, found in all domestic animals and positioned on the dorsolateral portion of the neck of the urinary bladder, does not retain sperm. Dogs and cats lack it, while equine, bovine, ovine, caprine, and swine have highly developed ones. The ejaculatory duct is formed when the excretory duct connects to the deference terminal section of the duct. (Sarangi *et al.*, 2020; Al-Aaraji *et al.*, 2018).

The prostate gland may be found in almost all mammals. The first kind disseminates glandular tissue throughout the pelvic urethra (no body) but does not penetrate the voluntary urethral muscle that surrounds the pelvic region of the urethra. Sheep and buck have lobulated urethras (visible on the outside). The fibromuscular capsule and prostate stroma contract, releasing secretion into the urethra. At the same time, prostatic fluid increases the motility of spermatozoa expelled from the ductus deferens (Farooqui *et al.*, 2014). The bulbourethral glands release a lubricant-like material. In the majority of cases, sperm mix with secretions from these glands. When they were first activated during sexual stimulation, their secretions appeared as fine droplets in the urethral meatus of the erect penis (Emam *et al.*, 2016).

Aim: This study aims to study the histological and histochemical structure of the male accessory sex glands in caprine Iraqi breeds.

Materials and Methods

Sample Collection

The male accessory genital glands of ten adult males of Iraqi local breed buck weighing 4865 kg and 18-26 months old were obtained from the Al-Najaf slaughterhouse and used for histological examination.

To study the histomorphology and histochemistry of accessory glands, tissue samples were collected and fixed in 10% Neutral Buffer Formalin (NBF). After fixation, the samples were processed to prepare paraffin blocks, and paraffin sections of 3-5 μm were obtained on glass slides with a rotary microtome (Rotary Microtome CUT 4050 Rotary Microtome - Microtech Laborgeräte GmbH). The slides were stained with Hematoxylin and Eosin (H and E) to observe routine histomorphology, Periodic Acid Schiff (PAS) for demonstration of neutral mucopolysaccharides

and Masson's trichrome stain (Bancroft and Gamble, 2008). Stained slides were examined using a light microscope (OPTIMA® biological microscope G-206) with a digital camera USB connection with the computer slides pictured directly from the computer at various adjustment powers (100x, 40x, and 10x).

Results and Discussion

1-Ampulla

The histological examination of an ampulla in a buck reveal that the ampulla has to branch the tubular lumen due to the presence of several folds of lining epithelium pushed into the lumen of these tubules (Fig. 1). The lumen of the ampulla changed from narrow tubules with pseudostratified columnar epithelium to larger tubules with simple columnar epithelium and rounded nuclei (Fig. 1). This finding was in line with that of (Gofur, 2015). The seminal vesicle gland and ampulla share the ejaculatory apparatus.

The pseudostratified columnar epithelium was detected to line the duct of the ejaculatory apparatus. Ampulla secretion is merocrine, and the Ampulla glands drain into the deferens duct, providing fluid to the sperm and functioning as sperm storage (Fig. 1). In a buck sitting on a well-developed Lamina propria, the lining epithelium of the glandular tubule of ampulla has a compact collagen and elastic fiber that produces positive results with Masson's trichrome (Fig. 1).

The lining epithelium of the Ampulla tubule, on the other hand, was determined to be characterized by the presence of basal cells, and lipid droplets were also observed in the simple columnar epithelium of a buck, which is supported by Samuelson (2007); Ghonimi *et al.* (2014). Discovery of lipid droplet accumulations in the basal layer of the epithelium of a buck.

The Vesicular Gland

Folded epithelial mucosa with simple columnar epithelium and basal cells that are spherical to avoid nuclei may be seen in histological slices of a buck seminal vesicle (Fig. 2). The tubuloalveolar or complicated tubular gland is revealed in the section. A capsule of loose collagen and smooth muscle fibers surrounds the gland, which response favorably to Masson trichrome stain (Fig. 2). (Pathak *et al.*, 2012; Khan *et al.*, 2015) gave a similar recording. In humans, the alveoli of seminal vesicles are lined by a pseudostratified columnar epithelium (Nissar *et al.*, 2014; Farooqui *et al.*, 2014). According to the current results, a buck's seminal vesicle has capsule-like septa that split the gland into lobules (Fig. 2). The epithelium of the gland is supported by the lamina propria mucosa, a loose connective tissue composed of collagen and smooth muscle fibers (Alsafy *et al.*, 2021). In the buck, a simple columnar epithelium with oval nuclei lines the primary secretory duct of the seminal vesicle

(Sarangi *et al.*, 2021). In histological sections of the buck (Khan *et al.*, 2015), the mucosal epithelium of the alveoli has folded and projects into the alveolar lumen, resulting in numerous cavities of varying diameters (Fig. 2).

The Prostate Gland

The diffuse component of the prostate gland in the buck is spread over the submucosal layer of the pelvic urethra. The prostate glands are tubuloalveolar with well-developed alveoli on histological examination (Fig. 3). According to (Dyce *et al.*, 2009), these alveoli are bordered with simple columnar epithelium (Alsafy *et al.*, 2021) in ruminants of a small size. According to the current results, sections stained with PAS stain revealed the lining epithelium of the prostate gland duct converted into the pseudostratified columnar epithelium. The presence of round, oval nuclei characterize this epithelium. Myoepithelial cells grew between the gland's alveoli (Fig. 3). These findings have never been reported in the buck journal before. The kind of prostate gland secretion in buck is seromuroid due to strong reactivity with PAS stain in sections of the prostate gland (Fig. 3). This result is in line with those of (Samuelson, 2007; Pathak *et al.*, 2012). The parenchyma of the dispersed buck's prostate gland is surrounded by the fibromuscular band and smooth muscle fibers. Histological investigation reveals that most alveoli contain tiny lumina flanked by the columnar epithelium. This result mirrored the findings of (Mohammed and Doohi, 2017). The lining epithelium of the prostate gland's duct was recognized as pseudostratified columnar epithelium, particularly where the duct connected to the pelvic urethra (Fig. 3). This contradicts observations in bull (Sarangi *et al.*, 2020) that the epithelium of the gland is pseudostratified columnar epithelium in humans. In a buck, the prostate gland secretions are merocrine (Fig. 3) (Farooqui *et al.*, 2019).

The Bulbourethral Gland

The secretory units of the bulbourethral gland in the buck came in various shapes and sizes, with most having an alveolar shape and others having a saccular or tubular shape, leading to the gland being classified as a tubuloalveolar gland (Fig. 4).

Collagen, elastic, smooth, and striated muscle fibers make up the bulbourethral gland capsule. The gland is divided into tubules by septa that pass between the secretory units and connect to the lamina propria of the submucosa (Fig. 4). The urethral or bulbocavernosus muscles are the source of striated muscle fibers in histological sections because they surround the gland. The gland's duct is lined with a simple columnar epithelium, which contains spherical or oval nuclei (Fig. 4). This was in contrast to (Alsafy *et al.*, 2021). Camels have a seromucous bulbourethral secretion that responds favorably to PAS stain (Fig. 4). (Samuelson, 2007)

remarked on recent research. The bulbourethral gland in humans secretes mucus (Junqueira and Carneiro, 2005). The simple columnar epithelium that lines the excretory duct of a buck bulbourethral gland was identified. The secretory units are surrounded by various epithelium, ranging from simple columnar epithelium to simple cuboidal epithelium, as well as round or oval nuclei found at the base (Fig. 4).

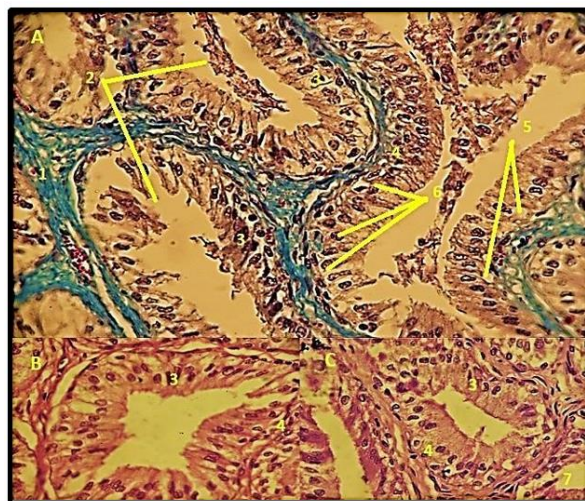


Fig. 1: Micrograph of the ampulla showing in the buck: 1- Trabecula between alveoli, 2- Lumen of secretory alveoli, 3- Secretory epithelia (The pseudostratified columnar epithelium), 4- Lamina propria, 5- Lipid droplets, 6- Basal cells, 7- Capsule of the gland. (A) Masson trichrome stain 100X. (B) H&E stain 100X. (C) PAS stain 100X 100 X

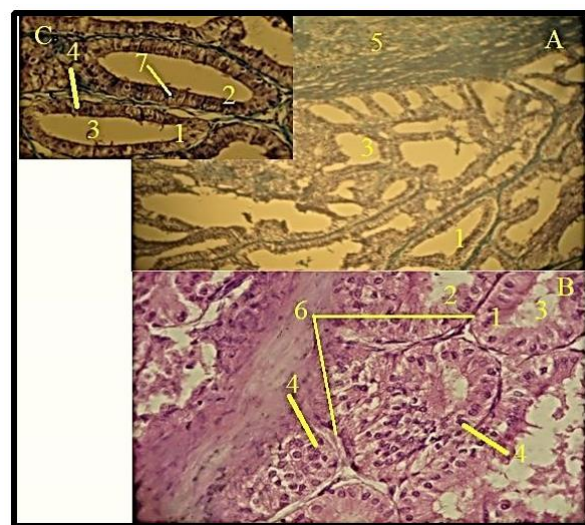


Fig. 2: Micrograph of vesicular gland in buck showing: 1- Secretory alveoli of the gland, 3- Lumen of secretory alveoli, 4- Basal cells, 5- Capsule of the gland, 6- Intra-alveolar septa, 7- Secretory epithelia (Simple columnar epithelia). (A) Masson's trichrome stain 10X. (B) PAS stain 40X. (C) Masson's trichrome stain 100X

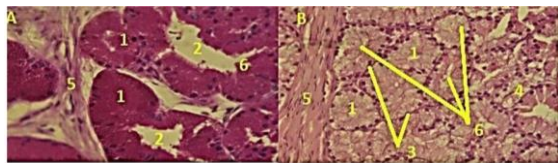


Fig. 3: Micrograph of the prostate gland in the buck showing: 1- Secretory alveoli (Merocrine secretion), 2- Lumen of alveoli, 3- Basal cells, 4- Intralobular duct, 5- Intralobular septa, 6- Secretory epithelia (Simple columnar epithelia). (A) PAS stain 40X. (B) H and E stain 40X

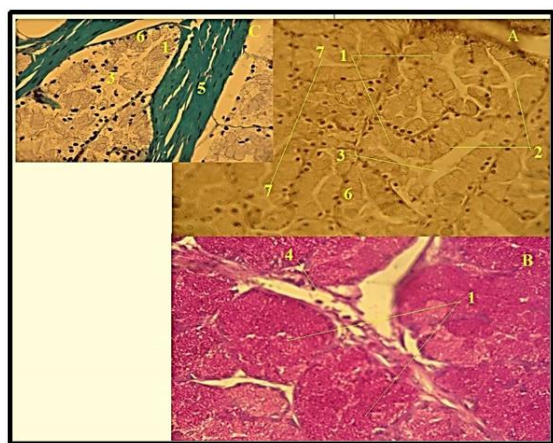


Fig. 4: Micrograph of bulbourethral gland in buck showing: 1- Secretory alveoli, 2- Secretory epithelia (Simple columnar epithelia), 3- Lumen of secretory alveoli, 4- Intera-alveolar duct, 5- Collagen fibers of intra-alveolar septa, 6- Basal cells, 7- Mucus secretion. (A) H&E stain 10X. (B) PAS stain 40X. (C) Masson's trichrome stain 40X

Like (Pathak *et al.*, 2012; Neves *et al.*, 2013), Bucks made a similar observation. Myoepithelial cells with star-shaped to fusiform nuclei (Fig. 4). Alsafy *et al.* (2021) found similar discoveries. Myoepithelial cells surround the alveoli and exert a contractile impact on the bulbourethral gland's alveoli, which helps explain why bulbourethral secretion is discharged into the urethra. Consequently, its secretion neutralizes pH, flashes, and lubricates the urethra as it passes through the pelvic urethra, allowing sperm to pass through more easily during ejaculation (Mohammed and Doohi, 2017; Al-Hussaniy *et al.*, 2022).

Conclusion

The seminal vesicle, bulbourethral glands, and vas deferens ampulla are well-developed and paired. Bucks have scattered prostate glands. The pseudostratified columnar epithelium lines the branching tubular ampulla. Simple columnar epithelium and basal cells cover the mucosa of the seminal vesicle.

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Author's Contributions

Waleed J. A. Al-Kelaby: Preparation and conducted the research.

Wafaa Hadi Mousa AL-Hashem and Zahraa S. M. Al Kaabi: Analyzed results.

Hany Akeel Al-Hussaniy: Proofreading, helped in publication, and respond to the journal requirements.

Ethical Regulation

We have received 2 ethical approvals, one from the university of Kufa, College of Veterinary Science (117)-2020 and the second from the Iraqi medical research center (238- May- 2020).

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