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Histological and histochemical observations of the prostate gland at resting and stimulating status in adult local dog (*Canis familiaris*)

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Abstract

The present investigation was done to clarify the histological and histochemical characteristics of the well-developed prostate gland in the local adult dogs (*Canis familiaris*). The tissue specimens were taken from the prostate gland. Routine histological techniques and stained processed the samples. Histologically, the body of the prostate in resting was characterized by abundant amounts of fibrous tissue with minor glandular lobes surrounded by a very thick fibromuscular capsule. Each lobe was composed of numerous variable size lobules that contained several alveoli lined by simple cuboidal cells. While stimulating, the gland is characterized by much glandular tissue, thin fibro muscular capsule, and thin interlobar connective tissue. The disseminated part of the prostate gland was composed of diminutive scattered small size glandular lobules within the subepithelial cavernous tissue of the urethra about 1-2 cm post prostate body. Histochemical, the stimulating prostate showed marked intense magenta color, which referred to the presence of both acidic and neutral glycoprotein secretory products when stained with combine Alcian blue at 2.5 pH with PAS stain. In conclusion, this study showed the differences between the prostate glands in the resting and stimulating status in local breed dogs.

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Introduction

The dog (*Canis familiaris*) is the first and only large carnivore as domesticated carnivore type. It is part of the family Canidae, the genus Canis (1,2). The dog has been used in medical research as a model for humans. Also, the dog has proved to be a beneficial model in studies on human prostatic functions (3). The male reproductive system comprises several individual organs acting to produce spermatozoa and deliver them to the female's reproductive tract (4). The prostate is a gland present in all-male domestic mammals and is a mixed gland formed by serous and mucosal constituents. The prostate is a globular gland that surrounds the neck of the bladder and the urethra. During its union, it presents a body and two lobes or wolves (right and left), which discharge their contents to the urethra through prostatic ducts (5). The development and maintenance of prostatic secretory activity are guaranteed by hormone production. Testosterone penetrates prostate cells by diffusion and is metabolized into other steroids by enzymes (6). This study aimed to identify the histological observation and histochemical reaction in the prostate gland of adult male dogs to determine the type of secretion in the glands. The male dogs could breed during the different seasons, which harmed male reproductive fertility (7).

Materials and methods

Twelve healthy adult male indigenous dogs were collected from the AL-ghazel market in Baghdad-Iraq, the age of mature dogs is more than one year, and the average weight is about 25-30 kg. The study was conducted in the department of anatomy, histology at the College of Veterinary Medicine, University of Baghdad, during a period

extended from September to March 2021. Surgically small pieces of tissue were taken from the prostate gland (body and disseminate part) and fixed in 10% buffered formalin saline (7,8). The specimens were processed by routine histological method for paraffin technique and sectioned serially at 5-7 µm. The prepared sections were stained with the hematoxylin and eosin, Masson's trichrome, combined PAS and modified alcian blue stains, and aldehyde fuchsine stain (7-10). The histological observations were examined by using light microscope type (Olympus, BHC, Japan) and pictured by digital camera Sony type with a resolution of 2 megapixels (Optical Zoom, Japan) that USB connection with the computer slides which were pictured directly from the computer at various adjustment powers 100x, 40x and 10x (7,11-13).

Ethical approve

All the experiments had been conducted following the approval by the responsible bodies of the College of Veterinary Medicine at the University of Baghdad with the highest standard for the human and compassionate use of animals in biomedical research under approval No. 1300 on 11/10/2020.

Results

Compact portion (Corpus prostate)

According to the physiological statuses, there were two histological features can see within the glandular tissue of the corpus prostate; resting status and stimulating status.

Resting status

The prostate was characterized by abundant amounts of fibrous tissue with minor glandular lobes. A thick fibromuscular capsule surrounded the body that sent very thick interlobar fibrous connective tissue septa to divide the glandular parenchyma into many lobes. Each lobe was composed of numerous variable-sized lobules containing several alveoli (Figure 1). The alveolus was lined by simple cuboidal cells, surrounded by myoepithelial cells and thin interalveolar connective tissue. The cuboidal epithelial cells had clear cytoplasm and contained round-flattened nuclei. Many urethral folds have appeared at the part of the prostate body closer to the urethra, lined by transitional epithelium propped by subepithelial cavernous tissue (Figure 2). The duct system of the prostate has initiated or arisen as a small-sized intralobular duct lined by simple columnar epithelium that drained secretion into a large interlobular duct. Interlobular duct drained common duct that opened into the urethral lumen. The interlobular and common ducts were padded by pseudostratified columnar epithelium. The prostate's epithelial cells' cytoplasm was contained under neutral mucopolysaccharide secretory products as seen in (Figure 3).

Stimulating status

The prostate body is characterized by abundant glandular tissue, thin fibro muscular capsule, and interlobar connective tissue. The body was surrounded by a fragile fibro muscular capsule that sent very thin interlobar fibrous connective tissue septa to divide the parenchyma of the gland into numerous lobules. Each lobule was composed of numerous large-sized lobules that contained numerous large-sized alveoli (Figure 4). Each alveolus was lined by a tall, simple cuboidal cell with eosinophilic cytoplasm that engorged with secretory products and showed secretory activities. A thin layer of fibrous connective tissue was separated among alveoli surrounded by myoepithelial cells and thin interalveolar connective tissue. A lot of urethral folds appeared at the part of the prostate body located closer to the urethra. It is padded by transitional epithelium supported by subepithelial hyperemic cavernous tissue (Figure 5).

Disseminate portion (Pars disseminate)

The disseminated part of the prostate gland was composed of diminutive scattered small size glandular lobules within the subepithelial cavernous tissue of the urethra about 1-2 cm posterior to the body of the prostate. The lobule consisted of a few tiny alveoli lined with simple columnar epithelium, revealing active secretory activities (Figure 6). The histochemical results of stimulating prostate showed that the cytoplasm of epithelial cells was revealed intense magenta color, which referred indicate the presence of both acidic and neutral glycoprotein secretory products (Figure 7).

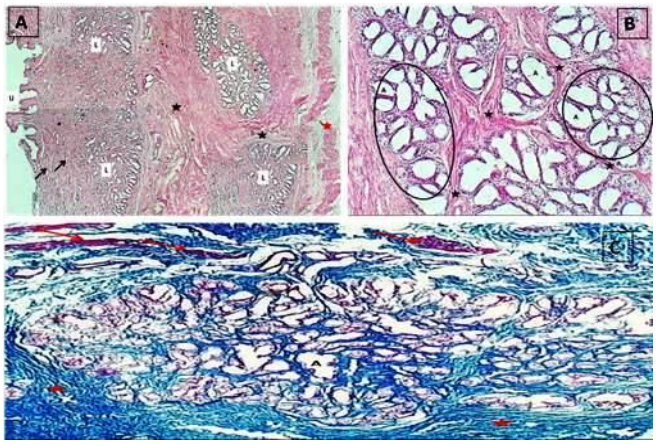

Figure 1: Histological section prostate (Body) at resting status (Adult dog) shows: (A) Sagittal section of the prostate (Body): lobes (L), thick interlobar connective tissue (Black asterisk), fibrous capsule (Red asterisk) urethral lumen (U) and cavernous tissue (Arrows) H&E stain, 40x. (B) Lobules (Black circular areas), interlobular tissue (Black asterisk), and alveoli (A). H&E stain, 100x. (C) Inter lobar collagenous tissue (Asterisks), fibromuscular capsule (Arrows), and alveoli (A). Masson's trichrome stain 40x.

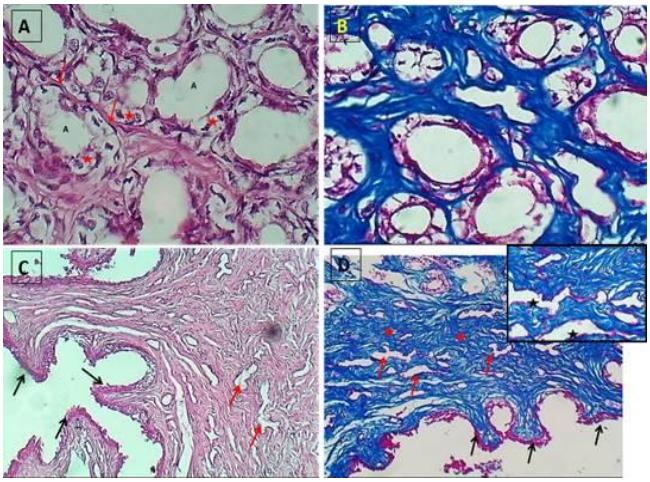

Figure 2: Histological Section of resting prostate (Adult) shows: (A) lobule of resting prostate (Adult) shows: small size alveoli (A) lined by simple cuboidal cells (Asterisks) and surrounded by myoepithelial cells (Arrows). H&E stain, 400x. (B) Alveoli of seminal vesicle shows Alveoli (A) and inter-alveolar collagen septum (Arrows). H&E stain, 400x. (C) Urethral folds (Black arrows) and cavernous tissue (Red arrows). H&E stain, 40x. (D) Urethral folds (Black arrows), cavernous tissue (Red arrows), collagen bundles (Red asterisks), and blood channels (Black asterisks). Masson's trichrome stain 100 and magnified section 400x.

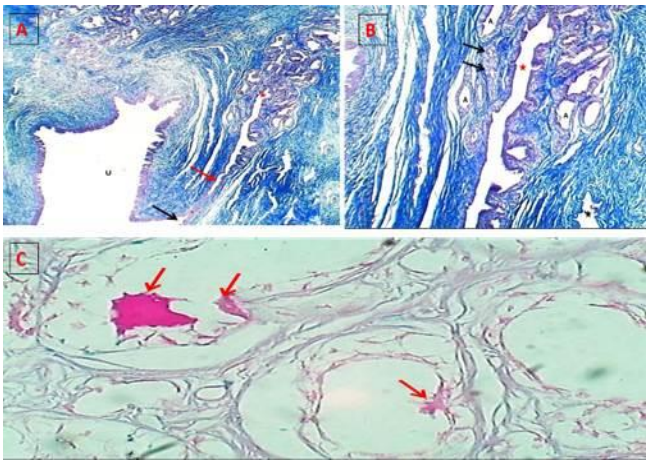

Figure 3: Section of resting prostate-body of shows: (A) Alveoli (a), interlobular duct (Asterisk), common duct (Red arrow), the opening of duct (Black arrow), and urethral lumen (U). Masson trichrome stain 40x. (B) Prostate lobule shows Alveoli (A), intralobular duct (Arrows), interlobular duct (Red asterisk), and blood channel of cavernous tissue (Black asterisk). Masson trichrome stain 100x. (C) Alveoli of resting prostate (Adult) shows neutral mucopolysaccharide secretion (Arrows). Combine Alcian blue (pH 2.5) PAS stain 400x.

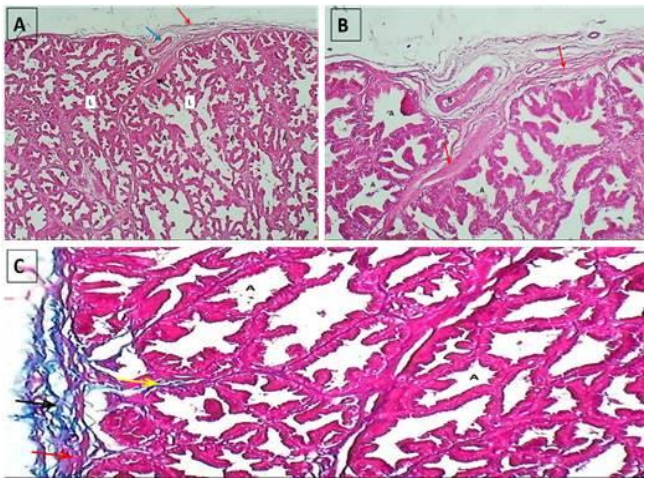

Figure 4: Histological section of stimulating prostate –body (Adult dog) shows: (A) thin fibro muscular capsule (Red arrow), blood vessel (Blue arrow), interlobar connective tissue (Black arrow), lobes (L), and alveoli (A). H&E stain, 40x. (B) The wall of stimulating prostate (Adult dog): smooth muscle (Red arrows), alveolus (A), and blood vessels (B). H&E stain, 100x. (C) Smooth muscle (Red arrows), collagen fibers (Black arrow), thin interlobar tissue (Yellow arrow), and alveolus (A). Masson's trichrome stain 100x.

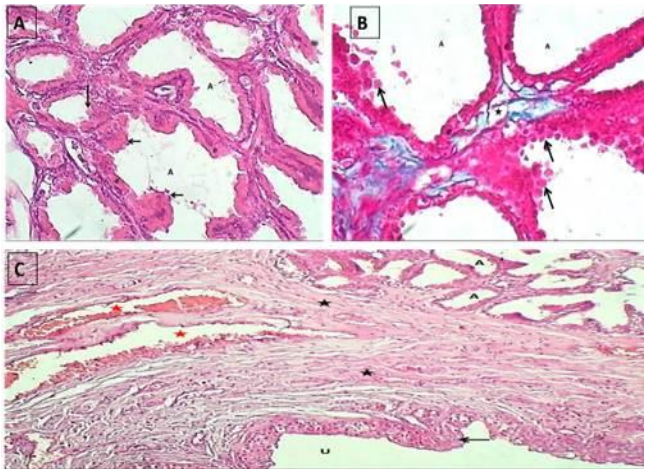

Figure 5: Histological section of stimulating prostate alveoli (Adult dog): (A) large size alveoli (A), epithelial cells (e), and epithelial secretory activities (Arrows). H&E stain, 400x. (B) Large size alveoli (A), epithelial cells (e), and epithelial secretory activities (Arrows). Masson's trichrome stain 400x. (C) Alveoli (A), urethra (U), the urethral epithelium (Arrow), hyperemic cavernous tissue (Red asterisks), and subepithelial fibrous tissue (Black asterisks). H&E stain, 100x.

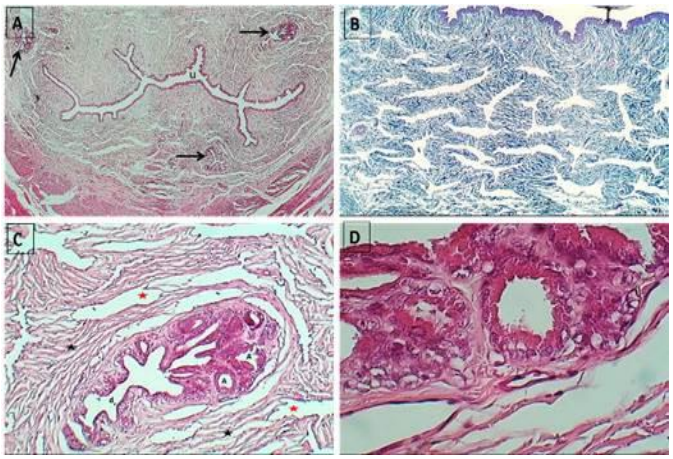

Figure 6: Histological section of the urethra (Adult dog) shows: (A) disseminate part of prostate (Arrows) within subepithelial cavernous tissue of urethra (U). H&E stain, 40x. (B) Subepithelial cavernous tissue of the urethra. Masson's trichrome stain 100x. (C) Lobule of prostate disseminates part (Adult dog) shows alveoli (A), subepithelial cavernous tissue (Black asterisks), and blood channels (Red asterisks). H&E stain, 100x. (D) Alveoli of prostate disseminates part (Adult dog) shows small alveoli with active secretory status. H&E stain, 100x.

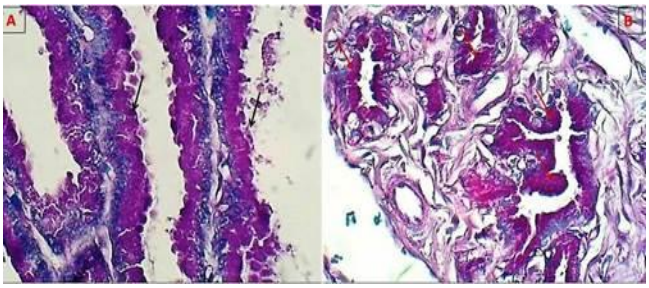

Figure 7: (A) Histological section of alveoli of body prostate (Adult dog) shows magenta color, which referred for acidic and neutral glycoproteins secretions in the cytoplasm of alveolar cells (Arrows). Combine Alcian blue (pH 2.5) PAS stain 400x. (B) Section of alveoli of prostate disseminates part (Adult dog) shows small size alveoli contained acidic and neutral glycoproteins secretions (Arrows). Combine Alcian blue (pH 2.5) PAS stain 400x.

Discussion

A recent study revealed that the prostate has two well-distinguished portions: compact portion and disseminate portion. This result was compatible with the previous result in some mammals like a bull (14), while not similar to Black Bengal bucks and indigenous gazelle, composed only by the disseminate portion (15,16), which may be due to species differences.

Compact portion (Corpus prostate)

Resting status

It is characterized by much fibrous tissue with small glandular lobes surrounded by a thick fibromuscular capsule that divides the glandular parenchyma into many lobes. This result was parallel with canine species and tomcat (17-21). The alveolus is covered by a simple cuboidal cell surrounded by myoepithelial cells and thin interalveolar connective tissue. The cuboidal epithelial cells had clear cytoplasm and contained round-flattened nuclei. This result disagrees with Hasan and Hamza (14) in bull, who mentioned that the alveoli are small-sized and narrow luminal structures lined with simple columnar epithelium, and their cytoplasm contained lightly stained mucous secretions while compatible with them the indigenous gazelle (16). The duct system began with a small-sized intralobular duct, which lined simple columnar epithelium that drained secretion into a large interlobular duct, then to a common duct that opened into the urethral lumen. The interlobular and common ducts were padded by pseudostratified columnar epithelium. This result was incompatible with the duct system in the donkey (22) and the indigenous gazelle (16), which lined with simple cuboidal epithelium.

On the other hand, the subtotal intracapsular prostatectomy procedure indicated that the male does not lose their fertility, and fewer complications occur compared with complete prostatectomy (23). This difference may be due to the type of secretion and activity of the prostate gland in the dog being different from the ruminant. Most seminal fluid is coming from the prostate gland in the dog, while the ruminant has more than one gland that leads to the physiology of secretion had another mode of action.

Stimulating status

At this status, it has much glandular tissue, thin fibro muscular capsule, and thin interlobar connective tissue, surrounded by a fragile fibro muscular capsule that divides the parenchyma of the gland into numerous lobules. This result disagreed with previous results in indigenous bulls of Bangladesh by Adhikary *et al.* (24). He mentioned that the capsule is composed of collagen and reticular connective tissue fibers. The large smooth muscles arranged inner circularly and outer longitudinally black Bengal bucks the capsule has been sent thick bundles of trabecular connective tissue which divided the glandular parenchyma into individual lobules. It may be due to species differences that the body of the prostate gland was well-developed in dogs. Each lobe was composed of numerous large-sized lobules that contained numerous large-sized alveoli covered by tall, simple cuboidal cells with eosinophilic cytoplasm that engorged with secretory products and showed secretory activities. This observation was incompatible with Hasan and Hamza (14) in bull and Gofur (15) in black Bengal bucks who found that alveoli are small-sized and narrow luminal structures and lined with simple columnar epithelium and

their cytoplasm contained lightly stained mucous secretions, while parallel indigenous gazelle (16) who recorded that the alveoli are vast structures and lined with simple cuboidal epithelium. This may be due to the seasonal activity in the dog's prostate gland during the stimulating status, which affects the type of secretion from the prostate gland.

Disseminate portion (Pars disseminate)

It is composed of petite scattered small size glandular lobules within the subepithelial cavernous tissue of the urethra about 1-2 cm Posterior to the body of the prostate. Each lobule was consisting of a few tiny size alveoli lined with simple columnar epithelium. This result was not parallel with the observation in a bull by Hasan and Hamza (14), who mentioned that it has variable size glandular lobules within the loose connective tissue of the urethra. In ram (*Ovis aris*) and buck (*Cyprus hircus*), the glandular tissue has well-developed alveoli, which lining by simple columnar epithelium and are characterized by the presence of round, oval nuclei (24). This difference may be due to the body of the prostate gland in a dog being well developed compared to the disseminating part, which leads to secretion being more in the body than the disseminating part. Histochemical, at the stimulating status, the cytoplasm of epithelial cells has marked intense magenta color, which refers form both acidic and neutral glycoprotein secretory products. This observation was incompatible with Abou-Elhamd *et al.*, (22) in donkeys which showed acidophilic secretory materials, and Hasan and Hamza (14) in bull, the secretory units of prostate gland lobule have neutral mucopolysaccharide secretion. On the other hand, its parallel with the results in indigenous gazelle has strongly reacted with PAS and reacted negatively with Alcian blue stain (16). This difference in the type of secretion may be that prostate fluid provides an optimal environment for survival and sperm motility and provides a physiological buffer against the acidic environment of the female tract. The prostate gland was responsible for producing most of the seminal plasma, which contains large amounts of proteins and enzymes, cholesterol, and lactate due to lacking the other accessory sex glands. The prostate fluid was neutralized the urethral acidity, which maybe help the activation of sperm, give consistency to the seminal fluid, and give the characteristic smell of semen.

Conclusion

The present observation showed that the body of the prostate in the resting status of a mature dog have much amount of fibrous tissue with minor glandular lobes, while in stimulating status, a much amount of glandular tissue, thin fibro muscular capsule, and thin interlobar connective tissue was present.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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المشاهدات النسجية والكيميائية النسجية لغدة البروستاتا عند حالة التحفيز وعدم التحفيز في الكلاب المحلية البالغة

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الخلاصة

تم إجراء هذا البحث لتوضيح الصفات النسيجية والكيمياء النسيجية لغدة البروستاتا المتطورة في الكلاب المحلية البالغة. تم أخذ عدد من العينات النسيجية من غدة البروستات، بعد ذلك تم تمرير العينات خلال التقنيات النسيجية الروتينية ومن ثم صبغها. من الناحية النسيجية، تميز جسم البروستاتا أثناء الفترة الغير محفزة بكميات كبيرة من الأنسجة الليفية مع القليل من الفصوص الغدية التي تحيط بها محفظة سميكة جدًا من الألياف العضلية. يتكون كل فص من العديد من الفصيصات المتغيرة الحجم التي تحتوي على العديد من الحويصلات التي تحاط بواسطة الخلايا الطلائية المكعبة البسيطة وأثناء وجودها في حالة التحفيز، كانت تتميز بوجود كمية كبيرة من الأنسجة الغدية والمحفظة العضلية الليفية الرقيقة والنسيج الضام الرقيق بين الفصوص. يتكون الجزء المنتشر من غدة البروستاتا من فصيصات غدية قليلة متناثرة صغيرة الحجم داخل النسيج الكهفي تحت الظهارية للإحليل حوالي ١-٢ سم بعد جسم البروستاتا. الكيمياء النسيجية، أظهرت البروستاتا المحفزة لونًا أرجوانيًا شديدًا ملحوظًا ويشير هذا إلى إفراز بروتين سكري حامضي ومتعادل وذلك عند صبغها بالألشيان الأزرق عند ٢,٥ درجة حموضة مع صبغة حامض شف الدوري. أستنتج من هذه الدراسة أن هناك فروقا بين غدد البروستاتا في حالة الراحة والتحفيز في الكلاب المحلية.

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