

HISTOLOGICAL AND HISTOCHEMICAL STUDY OF VESICULAR GLAND IN IMMATURE AND MATURE LOCAL BREED DONKEY (*EQUUS ASINUS*)

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Abstract

A total of ten samples of vesicular glands of immature and mature donkey were used. The histochemical parameters involved the general cytoarchitecture, thickness of fibro muscular tunica, diameters of lobules, diameters of alveoli, and epithelial height in addition the type and pH of glandular secretions of gland. Tissues were processed with paraffin technique and stained with Hematoxylin and Eosin stain and combine Alcian blue stain (pH 2.5)-PAS stain. The immature vesicular gland was surrounded by very thick tunica muscularis that measured $354.48 \pm 6.27 \mu\text{m}$. The immature glandular lobule was measured $271.18 \pm 4.73 \mu\text{m}$ in diameter, composes of simple mucosal folds that forming anastomosis feature and few alveoli which measured $78.39 \pm 3.59 \mu\text{m}$ in diameter. The immature alveoli lined by simple cubiodal epithelium which measured $3.46 \pm 0.1 \mu\text{m}$. The laminal propria of mucosal folds was composed of thin loose connective showed fibroblasts and fibrocytes. The mature vesicular gland was highly lobulated tubular gland which surrounded by slight thick muscular wall that measure was $213.25 \pm 4.51 \mu\text{m}$, the paranchyma of gland divided into numerous variable size lobules that significantly measured $436.20 \pm 3.59 \mu\text{m}$ in diameter. The mature lobule composed of numeroius variable sizes alveoli that significantly measured $115.81 \pm 2.60 \mu\text{m}$ in diameter. The mature alveoli lined by simple cubiodal-columnar epithelium that consisted of secretory cells, peg cells and basal cells, the secretory cells. Inter-lobular septa were composed of thick bundles of collagenous tissue. With Alcian blue (pH 2.5) stain, the histochemical sections of gland with combine Alcian blue-PAS stain revealed that the epithelial cells contain neutral secretory product. The statistical analysis revealed significant differences between the length, width and weight of theses glands in mature and those of immature donkey.

Key Word: Histology, Vesicular gland, Accessory sex gland, Alcian blue stain, Iraq

Introduction

Donkey or ass is a domestic animal in the equine species (Al-Gharban, 2017). It is from the African wild as *Equus africanus* and used as a numerous jobs as animal working for at least 5000 years (Britannica, 2021). The donkey as a herbivores species animals has many benefits, donkey milk has been used in as a food sources around for thousands of years and become common recently especially in many country of Europe (lisabetta *et al.*, 2012). In mammals, the accessory sex glands are revealed variety in numbers and histological structures (Abood *et al.*, 2018; Hassan, 2022;

Kumarasamy *et al.*, 2022). The dimensions of vesicular glands are difference according the physiological statue and the periods of immature and matter (Doohi, 2017; Mohammed and Doohi, 2017). The investigation of accessory sex gland of local breeds of mammals or farm animals were involve most species in Iraq as in ram and buck (Khalaf, 2009; 2010), bull (Hassan, 2021), gazelle (Doohi, 2017) and tom cat (Abood *et al.*, 2018). The current study aimed to providea basic knowledge and compare between immature and mature vesicular glands in donkey.

Materials and Methods

A total of ten samples of vesicular glands of immature and mature local breed donkeys were used in this work. The animals of study were obtained from department of anatomy and histology at college of veterinary medicine, University of Baghdad. The samples of vesicular glands immediately removed from the cadaver, washed up normal saline and immersed in 10% formalin for 72 hr. The tissue samples of gland processed with up grading concentrations of ethanol alcohol, then with paraffin technique. The paraffin blocks sectioned serially at 5-7 μm by rotary microtome. The prepared tissue sections stained with the Hematoxylin and Eosin stain and combine Alcian blue stain (pH 2.5)-PAS stain (Bancroft and Marilyn 2008.). The histometrical parameters of the study involved thickness of fibro muscular tunica, diameter of lobules, diameter of alveoli and the epithelial heights (Mohammed and Doohi, 2017; Hussan, 2021). Tissue sections have examined by light microscopy and microphotography has done using of Future Win Joe microscopic camera, the images have analyzed and scored by using Fiji image analyzer system (Suad *et al.*, 2018). Data were expressed as mean \pm standard error, and statistical analysis was carried out using two-way ANOVA at a significant difference of $p < 0.05$ (Gharban and Al-Shaeli, 2021; Gharban, 2022; Razooqi *et al.*, 2022).

Results

Immature vesicular gland

The vesicular gland was large gland surrounded by very thick fibro-muscular wall (*Tunica muscularis*) that measured $354.48 \pm 6.27 \mu\text{m}$. The muscularis was sent numerous septa into the parenchyma of the gland that divided gland into numerous variable size lobules (Figure 1). The lobule was measured ($271.18 \pm 4.73 \mu\text{m}$), had very wide lumen and composes of simple mucosal folds that forming some figures of anastomosis and few alveoli which measured ($78.39 \pm 3.59 \mu\text{m}$) in diameter. The alveoli and folds were line by simple cubiodal epithelium which height measured ($3.46 \pm 0.1 \mu\text{m}$), the epithelium was consisted of two types of cells: simple cuboidal cells and basal cells. The laminal propria of mucosal folds was composed of loose connective showed fibroblasts and fibrocytes (Figures 2, 3). Inter-lobular septa were composed of thick bundles of smooth muscle fibers that surrounded lobule and fibrous connective tissue (Figures 3, 4). The tunica muscularis was composed of two layers; inner circular layer of smooth muscle and outer longitudinal layer of smooth muscle (Figure 5). The tunica serosa was composed of areolar connective tissue which covered by mesothelium and showed newly produced immature collagen fibers. With Alcian blue (pH 2.5) stain, the histochemical sections of gland with PAS stain and combine Alcian blue -PAS stain the result revealed that the epithelial cells contain neutral secretory product (Figure 6).

Mature vesicular gland

The vesicular gland was lobulated tubular gland that has central duct surrounded radially by branching tubular to tubule-alveolar type. The gland was very large gland surrounded by slight thick muscular wall that measured ($213.25 \pm 4.51 \mu\text{m}$) which sent septa into the parenchyma of gland and the parenchyma of gland was divided into numerous variable size lobules (Figure 7). The lobule was measured ($436.20 \pm 3.59 \mu\text{m}$) in diameter build up by numerous variable sizes alveoli which displayed very wide lumen and measured ($115.81 \pm 2.60 \mu\text{m}$) in diameter. The alveoli lined by simple cuboidal-columnar epithelium that consisted of three types of cells: secretory cells, peg cells and basal cells, the secretory cells contained large rounded nucleus and eosinophilic cytoplasm (Figure 8). Inter-alveolar tissue was composed of collagenous connective showed fibroblasts and fibrocytes (Figure 9). Inter-lobular septa were composed of thick bundles of collagenous tissue (Figure 9). The tunica muscularis was composed of two layers; inner circular layer of smooth muscle and outer longitudinal layer of smooth muscle (Figure 9). The tunica serosa was composed of areolar connective tissue which covered by mesothelium showed newly produced immature collagen fibers (Figure 9). The histochemical results of the vesicular gland showed that, with PAS stain, combine Alcian blue (2.5 pH)-PAS stain the histochemical sections of vesicular gland showed that the epithelial cells of alveoli secreted weak neutral mucopolysaccharides (Figure 10).

Discussion

Despite of the vesicular gland was slight similar in immature and mature periods, but the current results revealed marked important differences in cytoarchitecture between both immature and mature that obvious in the measurements which showed marked differences, these differences were associated with the physiological status of the gland in mature glands which revealed epithelial secretory activities and active stromal tissue that associated with active fibroblast (Hassan, 2021). The current results revealed very thick fibromuscular wall in immature vesicular gland in compared with that of mature gland, the current study suggest that the physiological hyper-atrophy or enlargement which associated with significant increases in the histometrical measurements of the epithelial height, lobular diameters and alveolar diameter (Table 1), in addition for epithelial secretory activities and active stromal tissue that associated with active fibroblast growth factors, all these lead to stretch the fibromuscular wall consequently causes more thinning wall. As in all most domestic animals the vesicular gland of local breed donkey was lobulated tubular gland (Badia *et al.*, 2006 b; Eurell and Frappier, 2013; Varner and Schumacher, 2011; Farooqui *et al.*, 2014; Nissar *et al.*, 2014; Gofur, 2015; Nagasailaja *et al.*, 2016; Mohammed and Doohi, 2017; Hassan, 2021).

The current study revealed marked differences between the epithelium of the immature and mature glands, this differences beyond the differences between the resting and stimulating status of epithelial cells. On the other hand the epithelium type of the mature vesicular gland in current result was similar that recorded by Ghonimi *et al.* (2014) and Hassan (2021) in bull and Badia *et al.* (2006) and Nagasailaja *et al.* (2016). Meanwhile the current result is dissimilar that recorded

(Eurell and Frappier, 2013; Verma *et al.*, 2013; Adhikary *et al.*, 2015; Farooqui *et al.*, 2014; Nissar *et al.*, 2014; Gofur, 2015; Emam *et al.*, 2016) who refereed for pseudo stratified columnar cells while in stallion the epithelium is simple cuboidal to pseudo stratified type (Eurell and Frappier, 2013).

The muscular wall of donkeys vesicular gland made up by double layer of fibromuscular tissue of circular smooth muscles fibers that similar that recorded by (Verma *et al.*, 2013; Eurell and Frappier, 2013; Farooqui *et al.*, 2014; Nissar *et al.*, 2014; Gofur, 2015; Emam *et al.*, 2016; Mohammed and Doohi, 2017) meanwhile the tunica muscularis is a single layers of the smooth muscle bundles arranged circularly in bull (Ghonimi *et al.*, 2014; Adhikary *et al.*, 2015, Hassan, 2021). In addition, collagenous fibers were observed within great amount of smooth muscle fibers that demonstrated in the interstitial connective tissue of the vesicular gland as well as that in gland of camels (Ali *et al.*, 1978)and stallions (Wrobel and Dellmann, 1993).

Table (1): Thickness of muscular wall, diameters of lobules, alveoli andepithelial height of vesicular gland in immature and mature male donkey

Parameters	Immature M ± SE	Mature M ± SE
Thickness of muscular wall /µm	354.48±6.27 A	213.25±4.51 B
Diameters of lobules / µm	271.18±4.73 A	436.20±3.59 B
Diameters of alveolus/ µm	78.39±3.59 A	115.81±2.60 B
Epithelial height/ µm	3.46±0.11 A	10.60±0.05 B

(*) Different capital litters (AB) represents significant differences between glands at 0.05

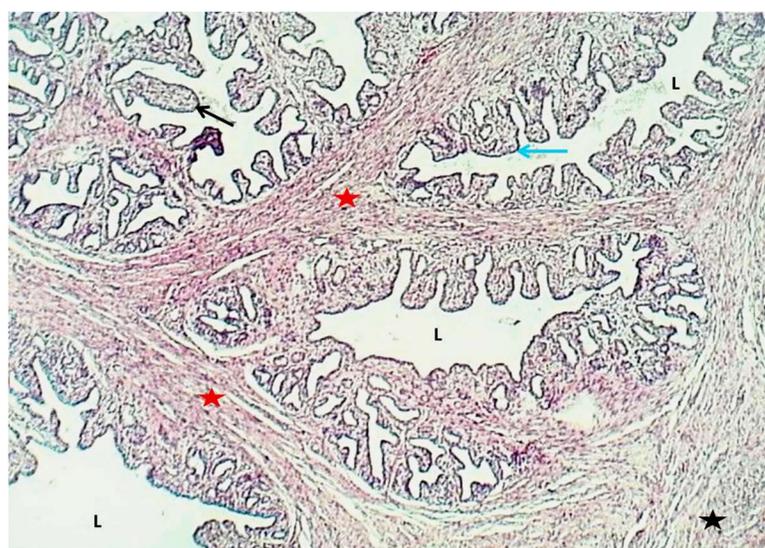


Figure 1: Histological section of vesicular gland (immature donkey) shows: fibro muscular layer (Black asterisk), inter lobular fibro muscular septa (Red asterisk), lobules (L), simple folds (Black arrows), anastomosing folds (Blue arrow). H&E stain 40x.

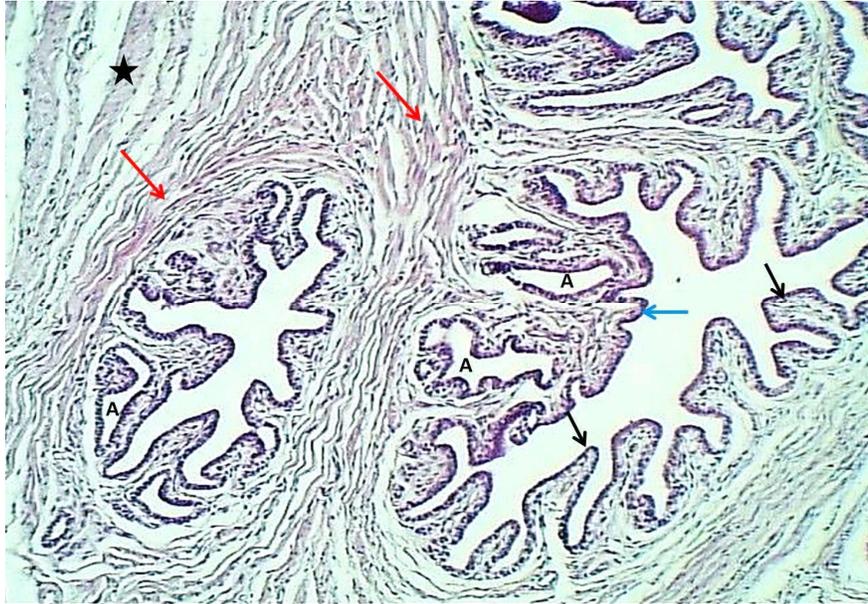


Figure 2: Histological section of alveoli of vesicular gland (Immature donkey) shows: inter alveolar smooth muscle (Red arrows) fibrous tissue (Asterisk), alveoli (A), simple fold (Black arrows) & anastomosing folds (Blue arrows). H&E stain 100x.

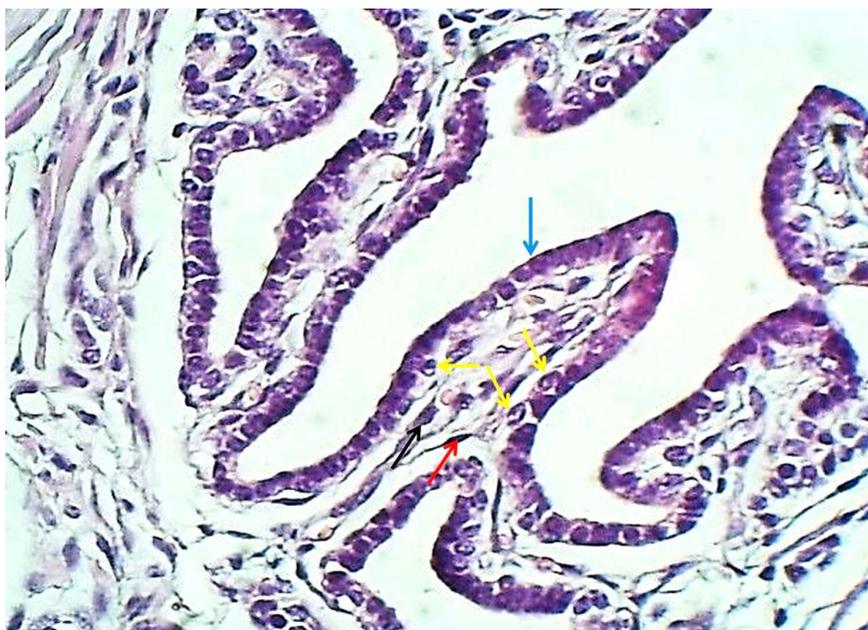


Figure 3: Histological section of lobules of vesicular gland (Immature donkey): simple cuboidal epithelial cells (Blue arrow), basal cells (Yellow arrows), lamina propria with fibroblast (Black arrow) & fibrocytes (Red arrow). H&E stain 400x.

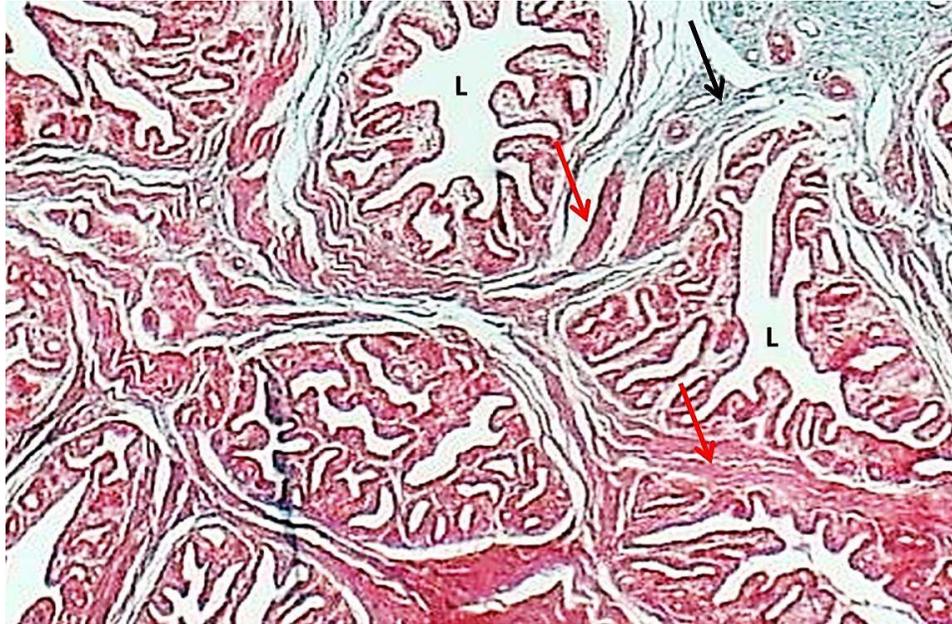


Figure 4: Histological section of vesicular gland (Immature donkey) shows: lobules (L), arrows), fibrous connective tissue (Black arrow), muscular tissue (Red arrows). Massonstrichrom stain 100x.

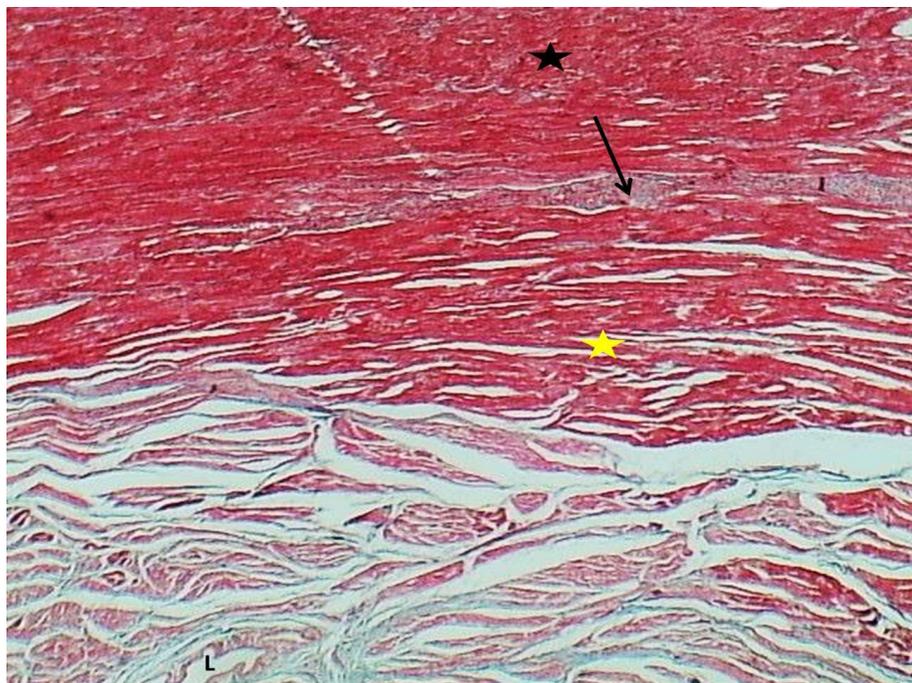


Figure 5: cross section of vesicular gland (Immature donkey) shows: lobule (L), inner circular smooth muscle (Yellow asterisk), outer longitudinal smooth muscle (Black asterisk) of tunica muscularis, inter muscular fibrous tissue (Black arrows). Massons trichrom stain. 40x.

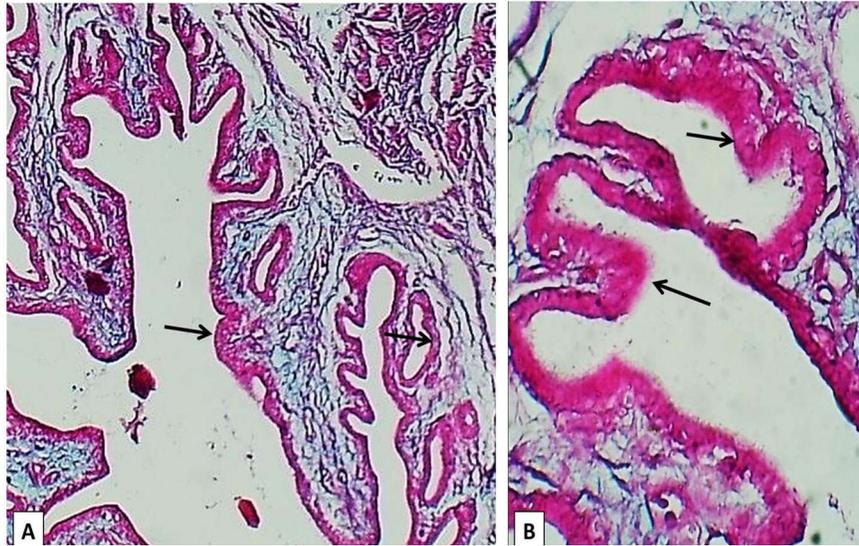


Figure 6: Histochemical section of vesicular gland (Immature donkey) shows: the epithelial cells contained neutral secretory products (Arrows).combine alcian blue (pH2.5) –PAS stain. Micrograph (A) 100x&(B) 400x.



Figure 7: section of vesicular gland (Mature donkey) shows; fibro muscular tunica (Asterisk), inter lobular connective tissue (Arrows), lobules (L) & alveoli (A) .H&E stain 40x.

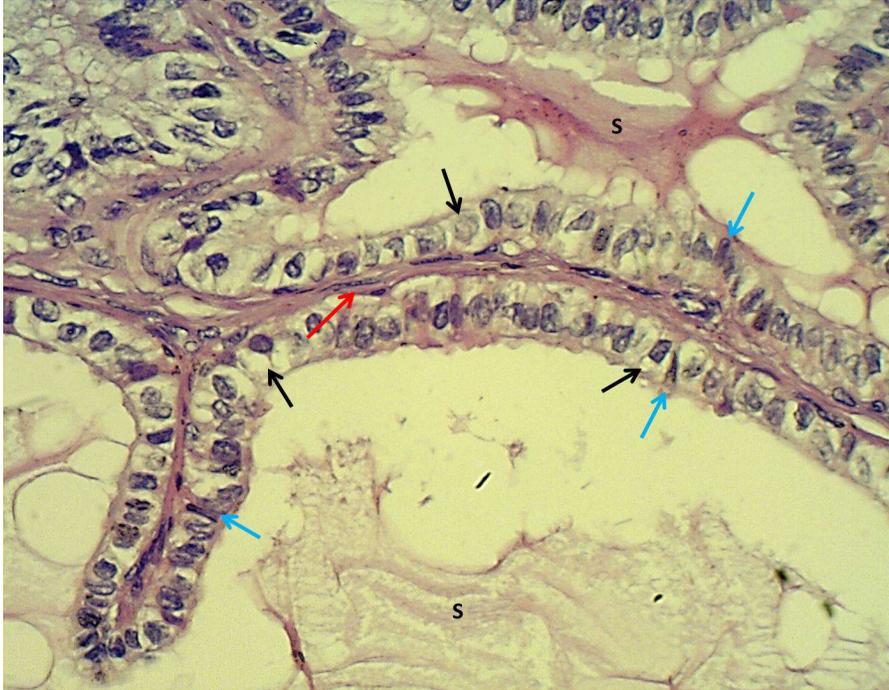


Figure 8: section of vesicular gland (Mature donkey) shows; thin inter alveolar connective tissue (Red arrow), secretory alveolar cells (Black arrows, Blue arrows) & secretion (S) .H&E stain 400x.

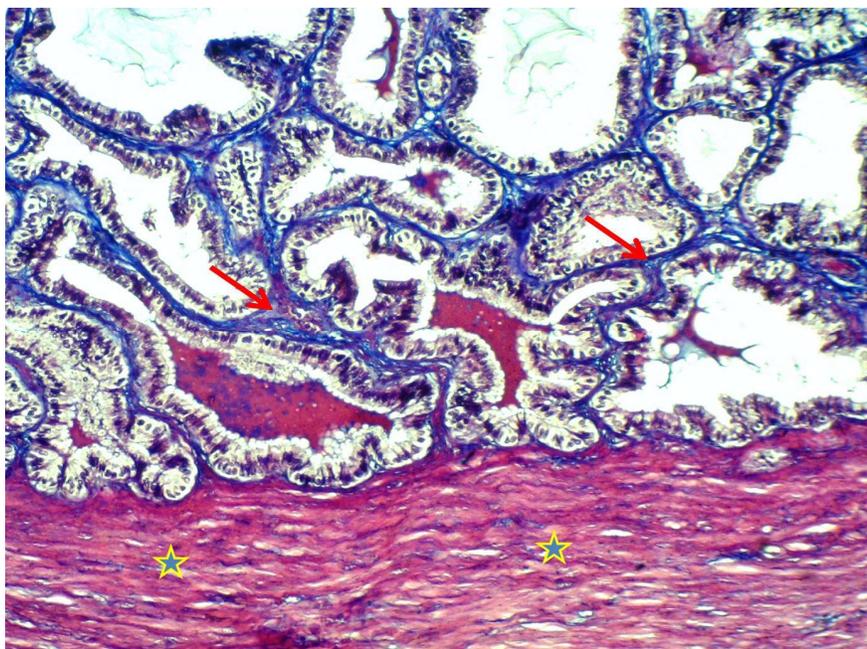


Figure 9: section of vesicular gland (Mature donkey) shows; inter alveolar collagenous connective tissue (Red arrows), muscular wall (Asterisks) .H&E stain 100x.

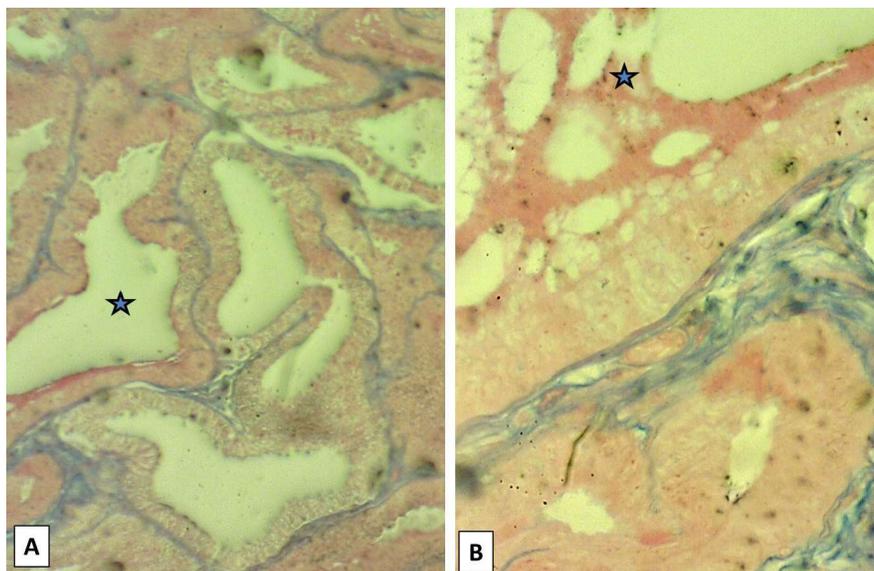


Figure 10: Histochemical section of vesicular gland (Mature donkey) shows alveoli filled with neutral mucopolysaccharids (Asterisks), the epithelial cells contained weak neutral mucopolysaccharids (Arrows). Combine alcian blue pH 2.5) - PAS stain. Micrograph (A) 100x & (B) 400x.

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