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Effects of Seasonal Alterations on Histological and Histochemical Structure of Poll Glands in Male Iraqian Camel (Camelus dromedaries)

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ABSTRACT
This study was conducted to comparatively investigate the hormonal, histological, and histochemical characteristics of the poll gland in adult male dromedary camels during the breeding season (December to March) versus the non-breeding season. Ten sexually mature male camels were used during the breeding season, and ten others during the non-breeding season.
Histological analysis focused on the general tissue architecture of the poll gland. Microscopic measurements included capsule thickness, alveolar lumen diameter, connective tissue thickness between alveoli, and epithelial thickness in both breeding and non-breeding seasons.
The glandular lobules were filled with large alveoli with narrow lumens, lined by tall simple cuboidal epithelium featuring apical blebs and surrounded by myoepithelial cells with dark-stained nuclei, followed by a thin interstitial connective tissue layer during the active season. In contrast, during the inactive season, the alveoli decreased in size, and the lining epithelium became simple squamous or low cuboidal, with lumen dilation and an increase in connective tissue thickness.Serial histological sections showed that the lobules drain into multiple excretory ducts that follow a convoluted path toward the surface and open at the upper parts of hair follicles.
Histochemically, the capsule and glandular structure exhibited strong reactions to hematoxylin and eosin staining. Smooth muscle cells showed positive reactivity with the PAS technique, and the cytoplasm of certain secretory cells reacted positively to Masson's

INTRODUCTION:

The genus Camelus is one of the basal genera of the Camelidae family, and is closely related to the extinct camel , the Arabian camel *(Camelus dromedarius)* . Camelus (Latin) means camel , Dromeus (Greek) means runner and the suffix -arius (Layin) means running camel , hence dromedarius (New Latin) means running camel **(Al-Mayahi *et al.*, 2023)** .

The Arabian camel is an important livestock resource in Arab world with enormous potential for milk , meat and transportation due to its unique physiology and considered one of the most resilient animals capable of surviving and performing under harsh arid and semi-arid conditions and However , poor reproductive performance is one of most important factors affecting camel productivity along with nutrition and disease , Therefore, maintaining high levels of reproductive performance in camels is essential not only for profitable production but also for providing ample opportunities for selection and genetic improvement **(Marai *et al* ., 2019) .**

Among the various domestic animal species, camels exhibit a unique reproductive pattern , Reproductive activity in male camels is inherently limited by relatively short breeding season (estrus) , during which the male exhibits numerous physiological , endocrine, and behavioral characteristics , the onset and duration of the breeding season have been found to vary from one geographic location to another, reflecting the influence of local environmental factors on sexual activity **(Agag *et al.*, 2020)** .

Material and Methods:

Samples were collected from the poll gland of sexually mature male Arabian camels (Camelus dromedarius), ranging in age from 8 to 12 years. The ages of the animals were verified using the dental ageing formula. The study was conducted during two seasons: the breeding season (December to March) and the non-breeding season (May to August).

The total number of animals included in the study was twenty. Samples were collected from different areas of veterinary slaughterhouses in Muthanna and Najaf Governorates, with two visits per week. The samples were transported to the Anatomy, Histology, and Embryology Laboratory at the College of Veterinary Medicine, Muthanna University, within two hours of slaughter, Immediately after slaughter, the poll gland was carefully excised using sterile dissection instruments (scalpel, scissors, forceps), then placed in clean, airtight containers. The samples were kept refrigerated until transported to the laboratory within 1–2 hours.

To prepare tissue for histological sectioning, tissue samples are taken from different parts of the poll gland. poll gland sections with a diameter of 0.5 to 0.8 cm were preserved for 48 h in 10% formalin. To prepare a 10% formalin solution, ten milliliters of 37 % formalin are mixed with ninety milliliters of distilled water (Vacca, 1985).

The histological sections were stained with Hematoxylin-Eosin stain (H&E) for general morphological features,

Masson trichrome stain for identifying connective tissue, PAS for identifying carbohydrates. The histological sections were observed with an Olympus microscope (Leica Galen III) and were photographed with a digital camera mounted to a microscope (Leica with Dinocapture 2).

RESULTS AND DISCUSSIONS:

Capsule , septa and collagen fibers :

The current study revealed histological composition of poll gland an accessory gland of the male reproductive system in camels during both breeding and non-breeding seasons . The gland is surrounded by capsule of connective tissue with elongated shape composed of fibrous outer layer, consists mainly of dense collagen fibers that provide protection and structural support to the gland and an inner smooth muscle layer which contains smooth muscle cells. These results which is consistent with **(Ibrahim and Al-Kheraije ,2021)** who believed that it contributes to the expulsion of the gland's secretory contents during periods of increased hormonal activity which changed the form of gland capsule .

They observed a significant increase in the thickness of the smooth muscle layer, which they indicated contributes to enhancing the secretion of glandular contents. They also noted an increase in the degree of blood supply within and around the capsule which enhances metabolism and the transport of hormones to the glandular tissue . The capsule sends connective tissue barriers that divide the gland into lobes and lobules consisting mainly of collagen fibers **(Fig. 1,2).**

Alveoli and excretory ducts :

The present study demonstrated clear histological changes in structure of the camel head gland during breeding and non-breeding seasons . camel head gland between breeding and non-breeding seasons .These results agreed with **(Bhardwaj *et al* ., 2021)** who reported that glandular lobules during the breeding season are filled with large alveoli with narrow lumen lined with long narrow simple cuboidal epithelium contain distinct apical blisters surrounded by myoepithelial cells with dark nuclei followed by thin layer of interstitial connective tissue. In the non-breeding season , a decrease in alveolar size was observed and lining epithelium transformed into simple squamous or low cuboidal with marked increase in lumen diameter and connective tissue thickness **.** This result is disagreed with **(Ibrahim *et al* ., 2020)** which show Intralobular excretory ducts of poll gland alveolus were seen amongst secretory alveoli and were round or oval in transverse section and were lined by simple columnar epithelium and gradually changed to two layers of columnar cells especially in interlobular ducts and with **(Bargaa *et al.,* 2017)** which explain luminal bleb-like projections were also observed in these ducts , lobules were drained by several excretory ducts which pursued tortuous course towards surface and **(El Khasmi *et al* .,2015)** who reported that the gland undergoes seasonal histological changes influenced by levels of male hormones, especially testosterone **.**

The current study indicates that the gland consists of lobes containing vesicles lined with epithelial cells resting on a basement membrane and the camel's head gland is a modified sebaceous gland differing from conventional sebaceous glands in its large size and tendency to stain with acidic dyes and this was confirmed by the reaction of the cytoplasm with H&E staining and the appearance of distinct acidic granules **.** The results also showed that the basal cells close to the basement membrane were cuboidal and small, gradually transforming into transparent, polygonal cells filled with lipid droplets. These cells later rupture via a holocrine secretion mechanism, opening a main duct that opens into the hair follicle and then to outside of skin **.** These results were supported by **(Al Bulushi *et al.*,2019)** which showed the gland plays a distinct functional role during the breeding season . and with **(Sid-Ahmed *et al* ., 2018)** which described a seasonally active lobular and glandular structure . and with **(Padalino *et al.*, 2016)** which described in their metamorphosis and structural and functional changes of the gland during the breeding season . and **Tingari *et al* ., (2017)** which showed smooth muscle epithelial cells surrounding lobules , contributing to the propulsion of secretions . and **(Batjargal *et al* ., 2017)** which clearly identified using Masson's trichrome stain**(Fig.3,4)** Furthermore , PAS staining showed a positive reaction with the apical blebs and that gland secretes glycoproteins with a chemical communication function **. (Fig. 5,6).**

1. Secretory cells:

These cells as large with a central nucleus and granular cytoplasm and exhibiting positive reactivity to PAS which supporting the secretory role associated with pheromones during the breeding season **.**

2. Smooth muscle cells :

These cells as moderate with a apical nucleus and granular cytoplasm and exhibiting role in facilitating the secretion of secretions from the gland due to the influence of neurotransmitters such as acetylcholine and presence was confirmed by staining with special dyes and exhibiting positive reactivity to (masson stain) **.**

3. Immune cells :

Lymphocytes and plasma cells were observed within the connective tissue surrounding the alveoli and play immune components within the accessory gonads to help protect the secretory environment .

Table 1 : Comparison of Histomorphometric Parameters of the Camel Poll Gland between Breeding and Non-Breeding Seasons

	Histomorphometric Parameter	Breeding Season	Non-Breeding Season	T-test Value	Significance (P-value)
1	Capsule thickness (mm)	1.900 ± 0.2000	4.467 ± 0.9291	0.646	P < 0.05
2	Alveular diameter (mm)	4.971 ± 0.9456	6.314 ± 1.3692	1.416	P < 0.05
3	Lumen diameter (mm)	2.185 ± 0.7883	3.371 ± 0.6575	1.450	P < 0.05
4	Epithelial cell height (mm)	0.771 ± 0.1202	0.751 ± 0.2907	—	P < 0.05 (assumed
5	Interlobular tissue thickness (mm)	1.900 ± 0.2000	4.467 ± 0.9291	0.890	P < 0.05

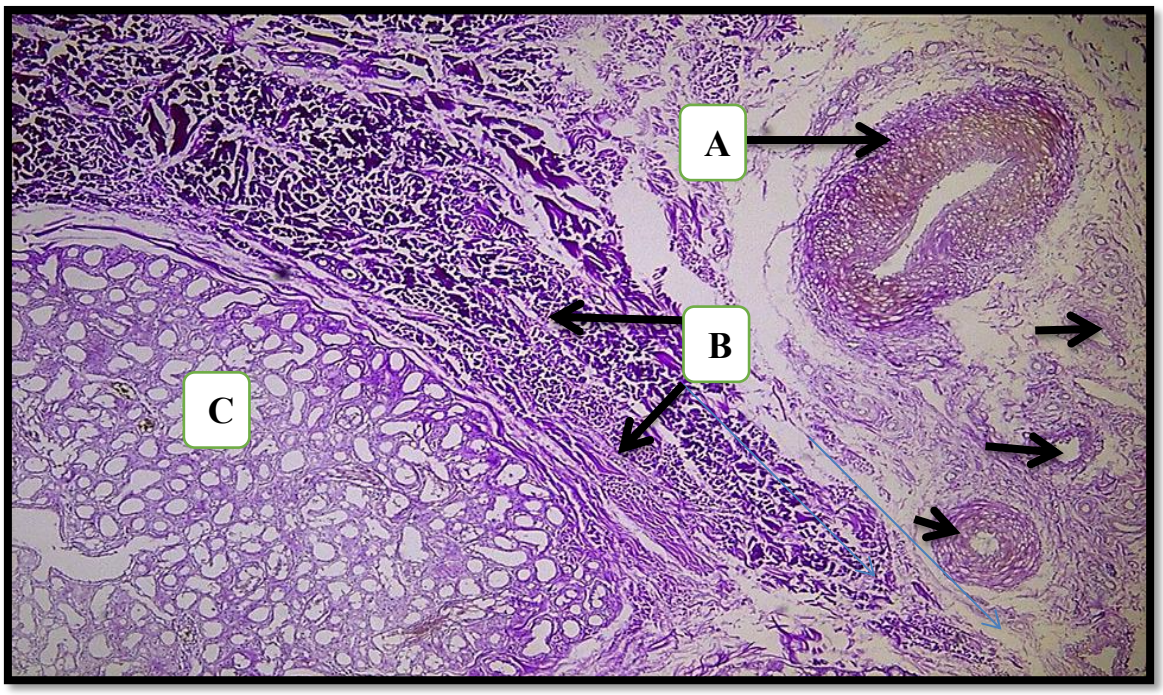

Fig.1. Microscopic section of the camel poll gland. Shown**: (A)**blood vessels**. (B)** show Thick muscular layer **.(C)** lobule with croded alveular glandular and thin inter-alveolar tissue thickness **.X100 H&E, Breeding Seasonal .**

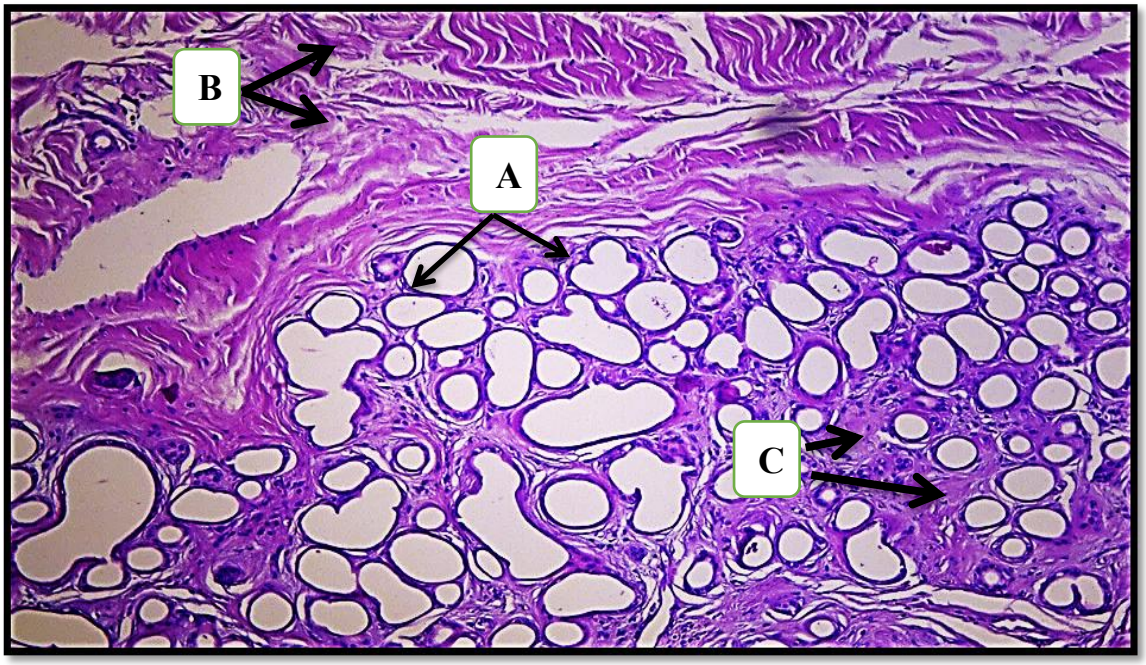

Fig.2. Microscopic section of the camel poll gland. Shown**: (A)** alveular liend with simpl squemuose epitheliuem. **(B)** Thick conneactive tissues with immune cells **. (C)** inter- lobular ducts tissue thicknes. **X100 H&E, Non- Breeding Seasonal .**

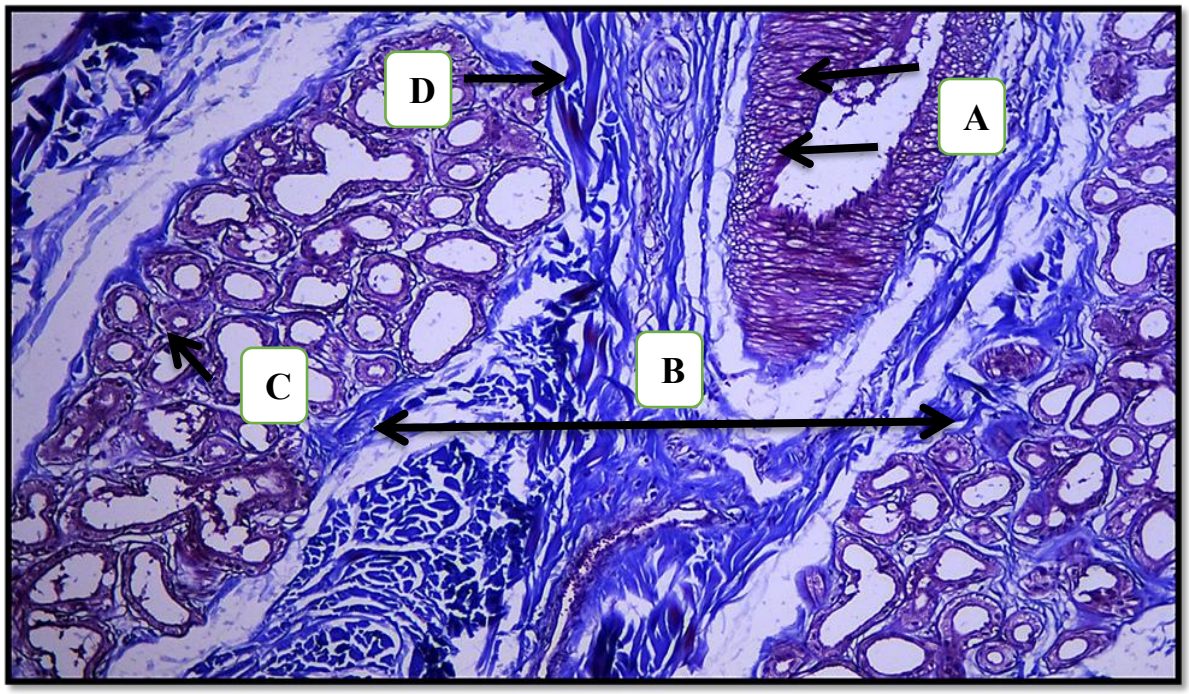

Fig.3. Microscopic section of the camel poll gland. Shown**: (A)** Capsule with Blood vessels (thick tunica) . **(B)** Setpta of Connactive tissues**.(C)** lobule . **(D)** Smoothe .M **X100 Masson's Trichrome, Breeding Seasonal .**

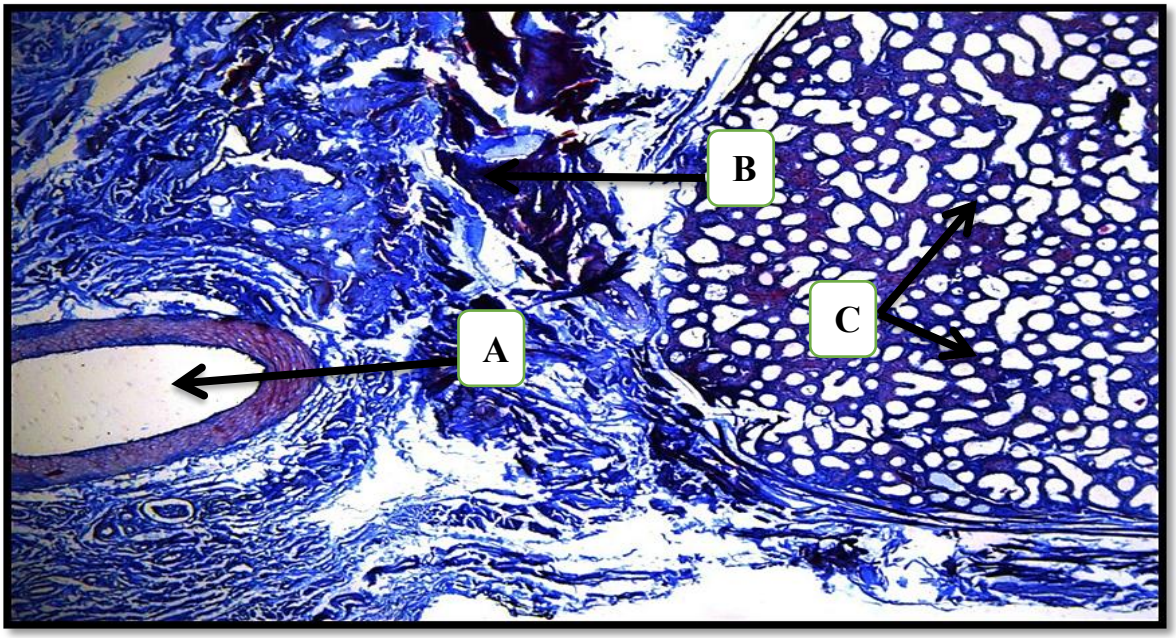

Fig.4. Microscopic section of the camel poll gland. Shown**: (A)** Blood vessels (thin tunica) . **(B)** thick Connactive tissues and collagen fiber with Smoothe .M**. (C)** secretory acini with widespread. **X40 Masson's Trichrome, Non -Breeding Seasonal**

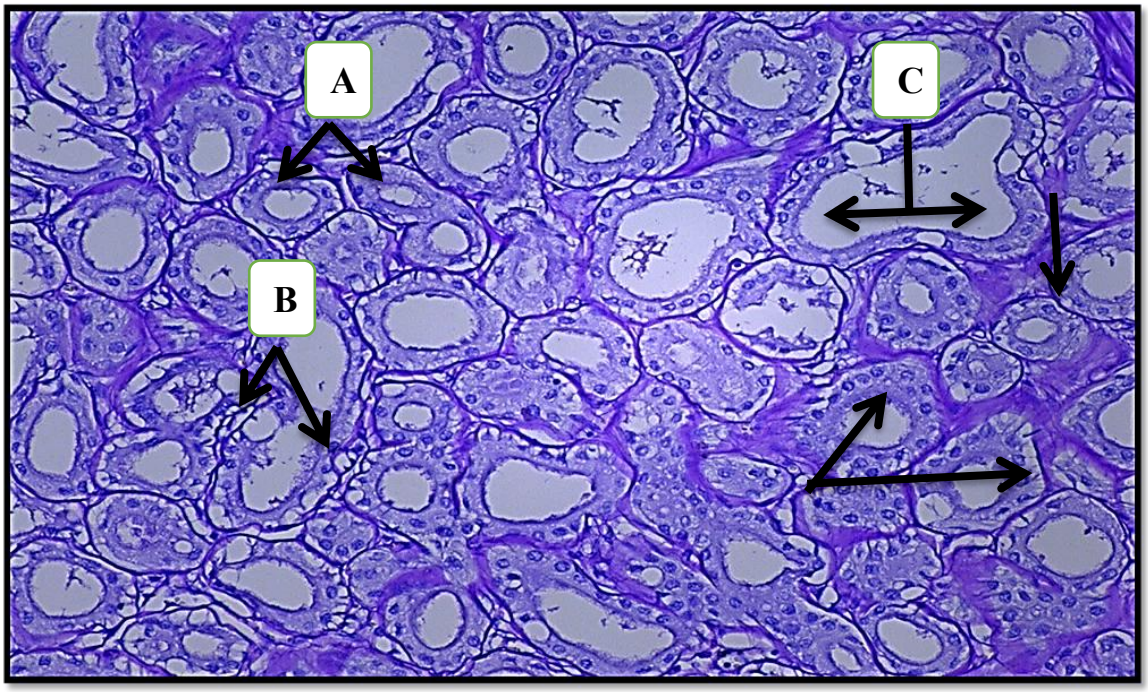

Fig.5. Microscopic section of the camel poll gland. Shown**: (A)** The lobules shown diameter crowded with reduce conneactive tissues. **(B)** vacule with myioed cells **. (C)** intre lobular ducts with nurrow lumen. **X200 PAS, Breeding Seasonal .**

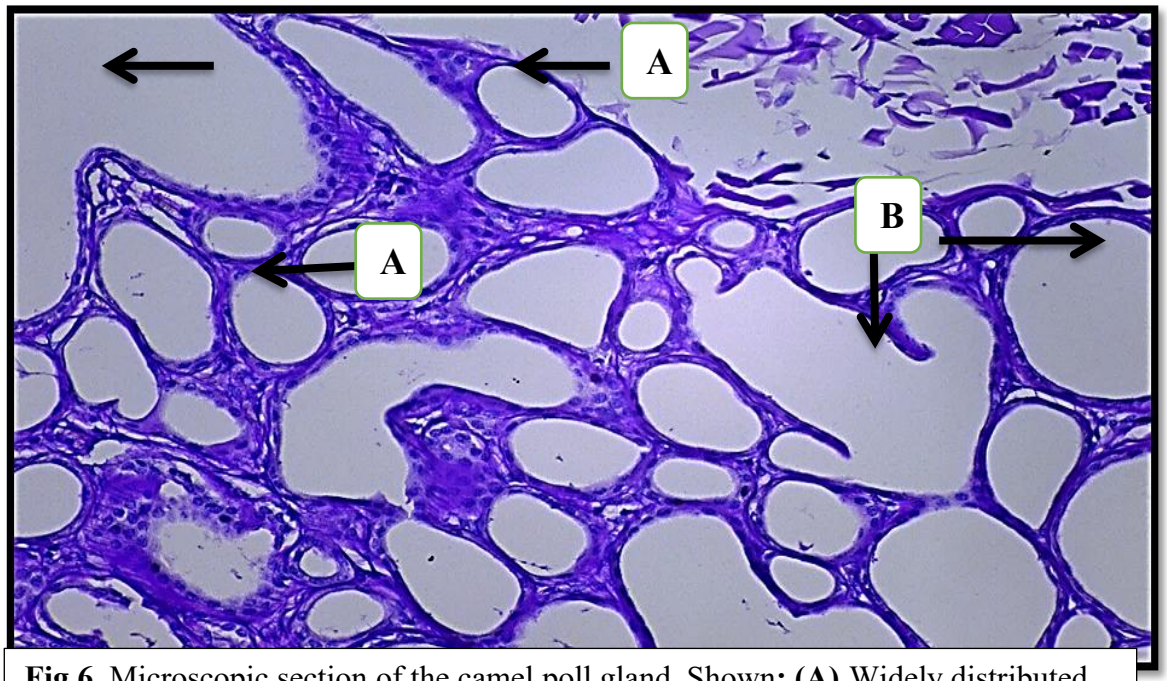

Fig.6. Microscopic section of the camel poll gland. Shown**: (A)** Widely distributed acini with a large lumen. **(B)** intre lobular ducts with simple squamous epithelium**. X200 PAS, Non _ Breeding Seasonal .**

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