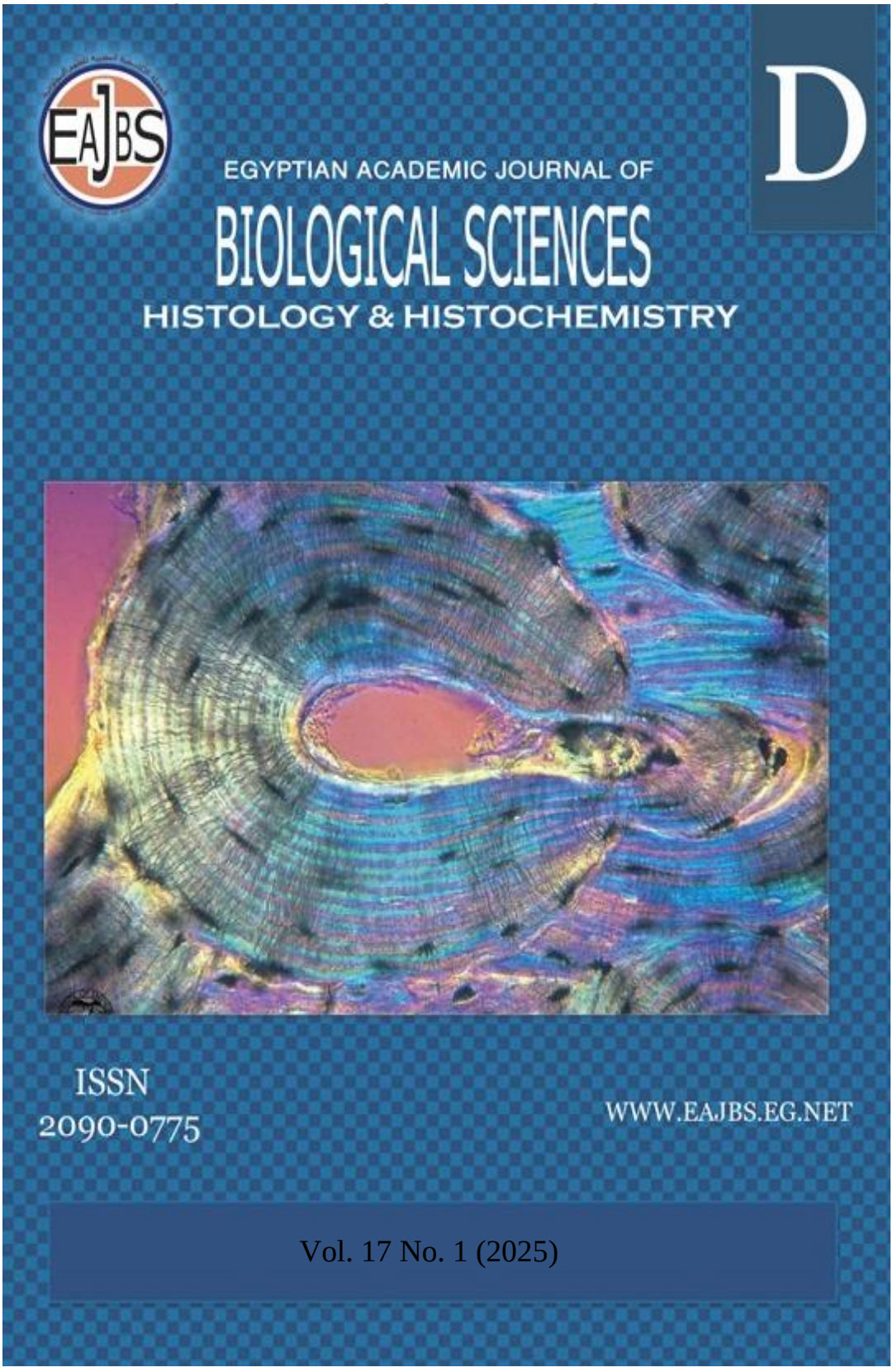
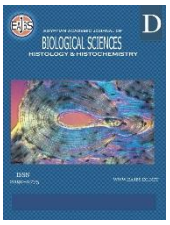


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.17(1) pp11-20(2025)*
DOI: 10.21608/EAJBSD.2025.410646

Egypt. Acad. J. Biolog. Sci., 17(1):11-20 (2025)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Microscopic Structure of The Tarsal Crypt System in Camels

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ARTICLE INFO

Article History
Received:2/1/2025
Accepted:7/2 /2025
Available:11/2/2025

***Keywords*:**
Dromedaries, Tarsus, Tarsal crypt, Eyelids, Tear film.

ABSTRACT

The tarsal crypt system is formed by several invaginations in the conjunctiva that house secretory cells, mainly goblet cells, and played a role in the formation of a mucus layer of tear film. Little is known about this system in domestic animals and camels. The purpose of this study was to describe in detail the microscopic structures of the tarsal crypt system in dromedaries. A total of five pairs of eyelids were used for this purpose. The samples were harvested immediately after slaughter from the heads of apparently healthy, adult male camels. The tarsal crypt system in camels was not found only as direct invaginations within the tarsal margin, but also within the tarsal plate itself. A capillary invagination within the tarsal plate with numerous crypts was present. At the entrance of this capillary invagination, a large, wide crypt of about fourth to fifth times the size of the typical crypt was observed. Not all the crypts were typically tubular, some were in the form of shallow grooves. The secretory cells were mostly goblet cells, in addition, different secretory cells were also noticed. The mucosal-associated lymphoid tissue surrounded the perimeter of all crypts and was diffused in lamina propria. The secretory cells within the crypts as well as the associated lymphoid tissue suggested that the tarsal crypt system in Camel may not only have a role in the formation of tear film in dromedaries, but functions in immune defense as a part of the conjunctiva-associated lymphoid tissue strongly recommended.

INTRODUCTION

The tear film is a very thin layer of fluid on the ocular surface, comprised of an outer lipid layer, a middle aqueous layer, and an inner mucous layer (Davidson and Kuonen 2004; Mantelli and Argüeso 2008; Tiffany 2008). Topographically, it can be divided into two regions, the precorneal tear film and the pre-bulbar film (Willcox *et al.*, 2017). The precorneal tear film follows the contours of the cornea and is usually highly stable, while the pre-bulbar film follows the varying contours of the bulbar conjunctiva (Eliason and Maurice 1990; Bruce *et al.*, 1995; King Smith *et al.*, 2000; Willcox *et al.*, 2017). The mucous layer on the ocular surface provides stability, spread, and coherence of these two regions, particularly the precorneal tear film (King-Smith *et al.*, 2000; Mantelli and Argüeso 2008; Hodges and Dartt 2013). The precorneal thin mucus coating layer, sandwiched between the aqueous tear film and the corneal epithelium (Sharma, *et al.*, 1999; Zhang, *et al.*, 2004), and thus provides a hydrophilic base for even spreading of the aqueous tear film (Sharma and Ruckenstein 1985; Zhang *et al.*, 2004).

The break-up of this mucous layer may result in the opacity of precorneal tear film an important structure in vision (Sharma 1998; Sharma *et al.,* 1999; Willcox *et al.,* 2017; Dey *et al.,* 2019). As the mucus layer is pivotal for a healthy and refractive ocular surface, there is an integrated secretory system formed by goblet cells situated in a ring, around the cornea to provide an intact mucus precorneal layer (Kim *et al.,* 2000; Gipson and Argueso 2003; Davidson and Kuonen 2004). This system is formed by (1) Goblet cells in the greatest concentration along the eyelid margins (Shatos *et al.,* 2003; Agnifili *et al.,* 2018), (2) Goblet cells in the conjunctival fornix (Hodges and Dartt 2011; Gipson 2016), and (3) the tarsal crypts of Henle (Knop and Knop 2005; Knop *et al.,* 2012). Similarly, the mucous gland of Manz is found in the bulbar conjunctiva, near the corneoscleral junction (limbal junction) (Cholkar *et al.,* 2013; Fatt and Weissman 2013), present in pigs and ox (Ois and Rabaey 1951; Klećkowska-Nawrot and Dzięgiel 2008).

The tarsal crypt of Henle is found in the anti-marginal tarsal borders of the eyelid in man (Knop and Knop 2005; Knop *et al.,* 2012). It differs from glands known as Henle glands or crypts of Henle, in that it is located within the tarsal conjunctiva, while the Henle glands are located in the limbal conjunctiva (Steuhl 1989; Knop and Knop 2000, 2002, 2005).Little is known about the tarsal crypts of Henle in domestic animals, particularly the camels. It has been suggested that the tarsal crypts play an important role in the formation of the mucus layer of the tear film. Furthermore, studying the tarsal crypts of Henle would offer a better understanding of the physiology of tear film formation in this species, as well as pathophysiology- related ophthalmic problems.

The aim of this study was to define the microscopic structure of the tarsal crypts of Henle in dromedaries and discuss their possible functions.

MATERIALS AND METHODS

Animals:

This study was performed following the ethical guidelines approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, Benha University, Egypt BUFVTM01-10-24. A total of ten eyelids were obtained from the eye's adnexa of healthy adult one-humped male camels, immediately after slaughter from Toukh abattoir, El-Qalyobia, Egypt. Congenital or acquired abnormalities of the eye and the eyelids were considered exclusion criteria for studying the morphological and histological features of the lacrimal apparatus in camels.

Histological Examination:

Samples were taken from the margins of the eyelids near the nasal end and the puncta. These samples were collected in different orientations; sagittal, cross, and oblique to identify the structure of the different parts of the tissues, and small pieces of samples were fixed in 10% neutral buffered formalin. The fixed specimens were dehydrated in ascending grades of ethyl alcohol, cleared in several changes of xylene, impregnated, and embedded in paraffin wax. The tissues were blocked, and 5-7 μm thick sections were cut using a rotary microtome. The general histological observations were carried out on paraffin sections stained with hematoxylin and eosin (H&E) (Bancroft and Gamble, 2008).

RESULTS

The tarsus of the eyelid was an elongated plate of dense connective tissue, located directly above the eyelid margin (Fig. 1) within the anti-marginal tarsal border, there was a notable system of crypts (Fig. 1). These crypts were microscopic pockets that were lined with secretory cells, mostly goblet cells (Fig. 2). The crypts were not located directly on the anti-marginal border, instead, they were found within an invagination of

about 2.5 mm in depth. This capillary invagination was situated in the midtarsal region, near its opening into the surface (conjunctival Cul-de-sac) there were some papillary projections of about 0.5 mm in length lined with goblet cell clusters.

The cryptal invaginations were polymorphic in shape, Extension into lamina propria, and the lining epithelium. some crypts were tubular, with a mouth-like opening and a deeply enfolded part into lamina propria. These microscopic features were typically the crypt of Henle. However, the lining epithelium presented not only goblet cells but also a number of unidentified secretory cells. These secretory cells were rounded and plumper than the goblet cells, with vacuolated cytoplasm (Fig. 2). Other crypts had less deeper pockets that opened widely into the margin. These pockets were in the form of wide grooves or small pouches that were lined mainly by goblet cell clusters (Fig. 3A, B).

The mucosal -associated lymphoid tissue was of a net-shaped arrangement that surrounded the enfolding part of the crypt into the lamina propria (Fig. 3A, B). Inter-cryptal aggregations of the lymphoid tissue cells were also observed (Fig.3A, B).

The tubular crypts reach 25-50 µm in depth with openings of about 10-20 µm in diameter, while the groove-like crypts had an average depth of about 50 µm. There were wide and large tubular crypts near the ocular surface, four to five times larger than the typical crypts, 200-250 µm.

The tarsal marginal area itself was rich in intraepithelial secretory aggregations (Fig. 4A, B), which includes goblet cell clusters and/or intraepithelial mucus secretory cells (Fig. 4C, D). Numerous intraepithelial mucus clefts or furrows were also noticed in between these secretory aggregations (Fig. 4C, D). Not all goblet cells within the crypts and those of cluster formation were typically identified. Numerous goblet cells were pear-shaped to slender cells, with eosinophilic granules dispersed in the cytoplasm (Fig. 5).

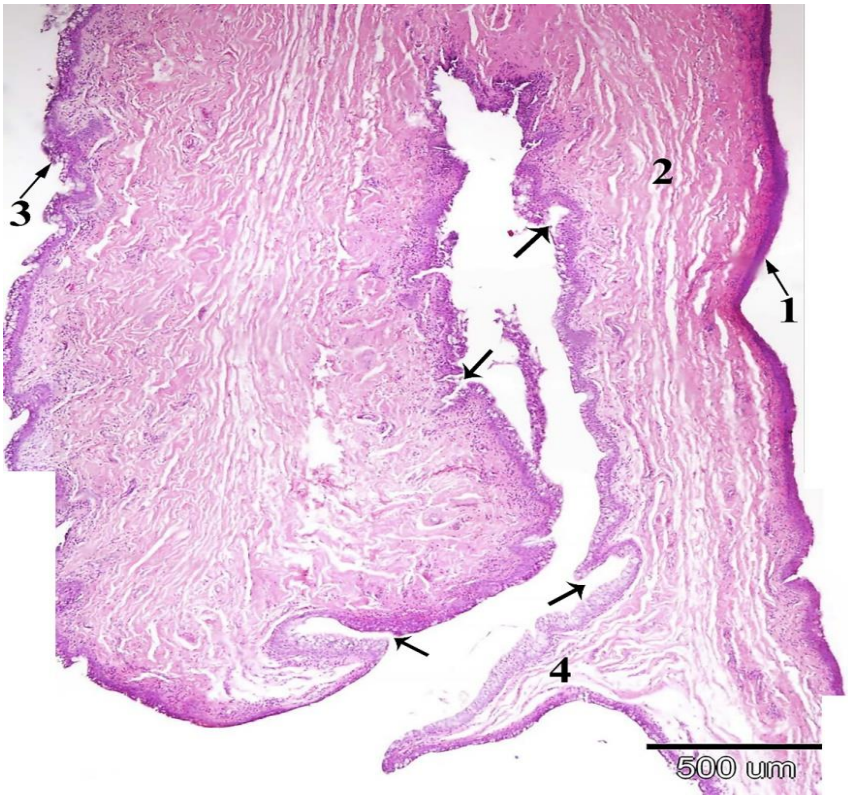

Fig. 1: A light micrograph showing a sagittal section of the camel-eyelid, H&E stain: eyelid margin (1), tarsus (dense connective tissue in the lamina propria (2), anti-marginal tarsal invagination with many crypts (black arrows) (3), papillary projection lined with goblet cells (4).

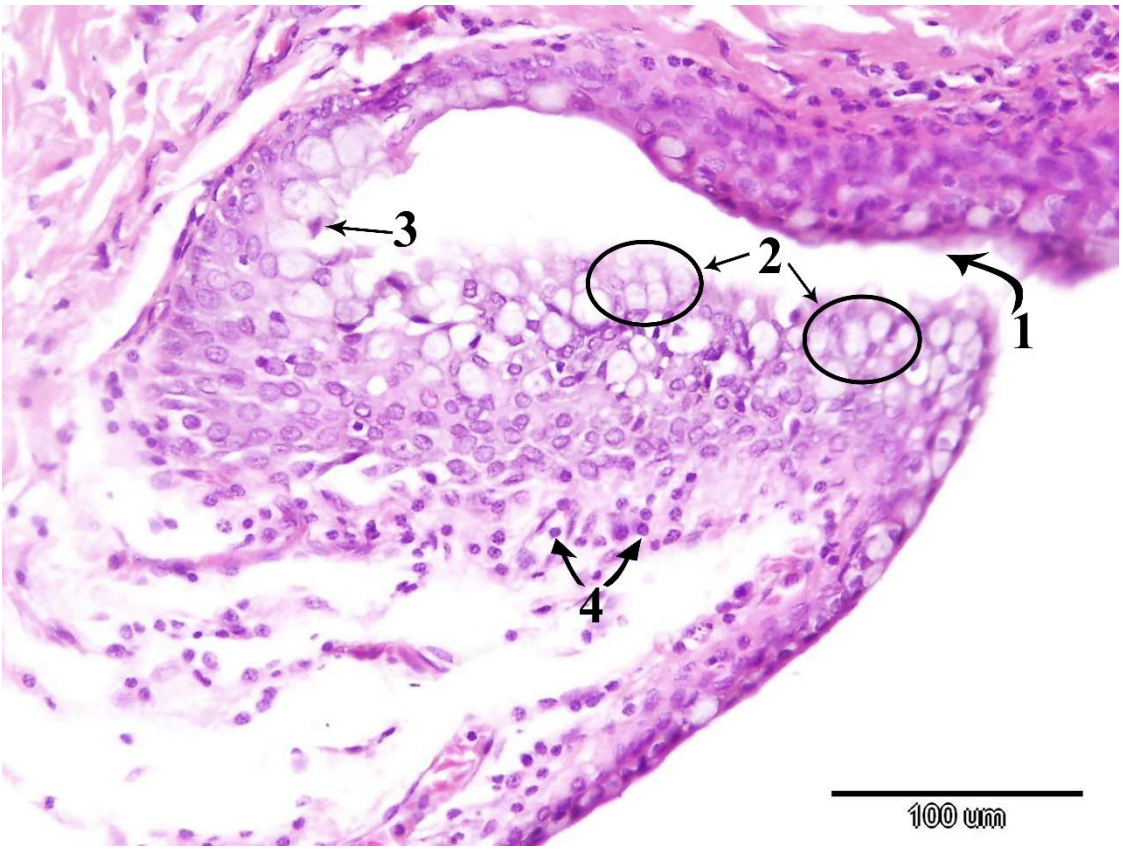

Fig. 2: A micrograph showing typical crypt of Henle in camels, H&E stain: mouth-like opening of the crypt (1), the goblet cells aggregations (2), the rounded secretory cells (3), the mucosal-associated lymphoid tissue, forming a pre-cuffing network within the lamina propria (4).

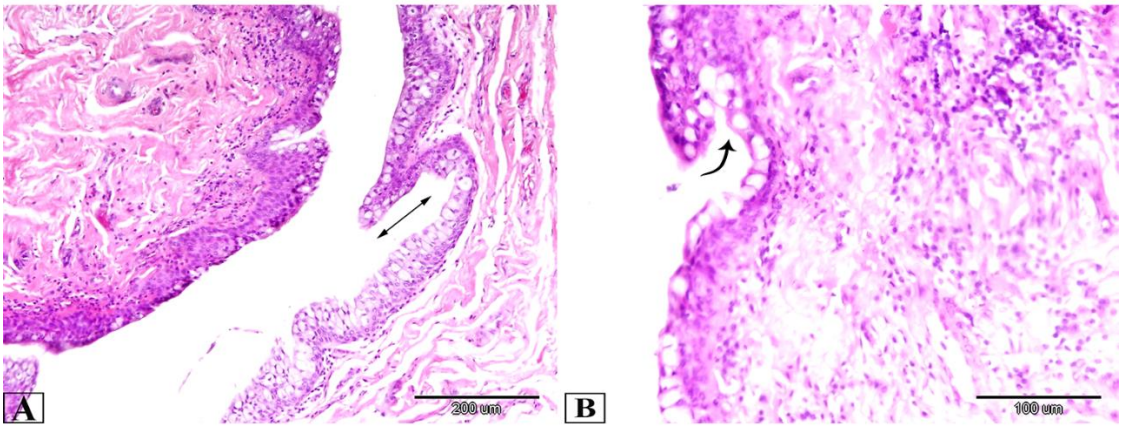

Fig. 3: A micrograph of atypical crypt of Henle (A, B) showing: Note, the wide groove-like crypt, which lined by classical goblet cells (A), this form usually found near the ocular surface, and it thought to help in the stability of tear film via increasing the surface area of mucous secreting cells. Also, the small pouch-like crypts are not typical of classical tubular type (B).

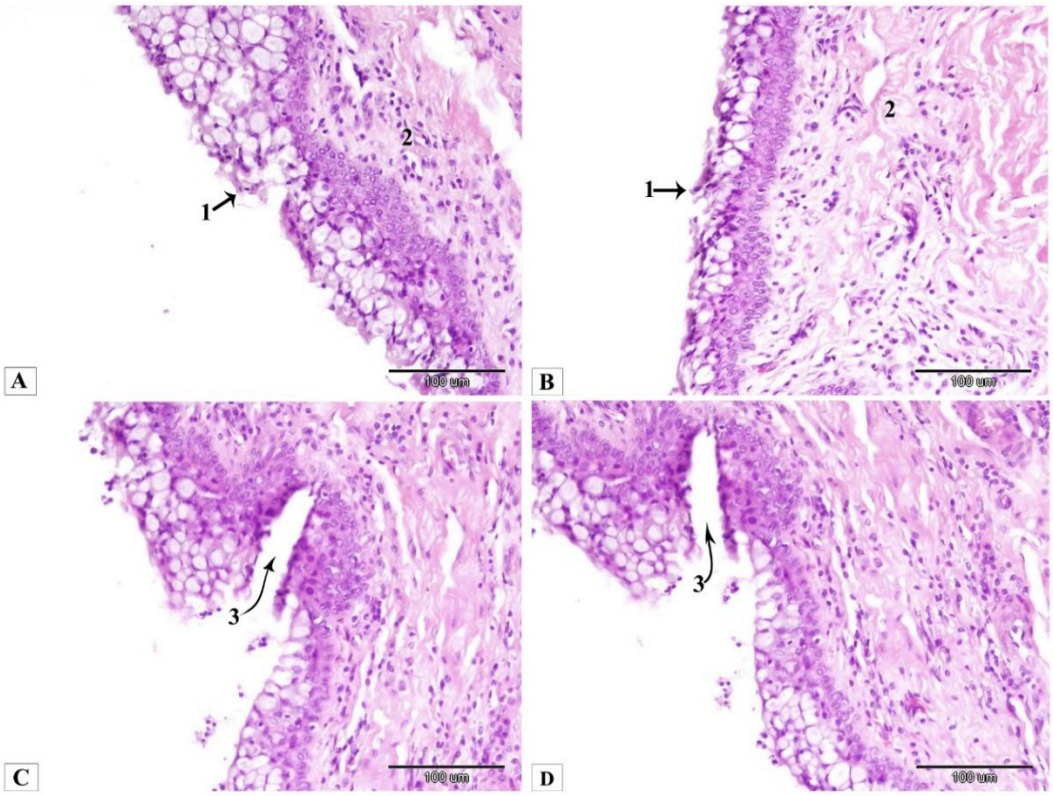

Fig. 4: A micrograph (A-D) showing the marginal intraepithelial glands in the tarsus of camels, Note, that the intraepithelial mucus glands (1), didn't extend deeply into the lamina propria (2), as the crypts do, and only have furrow-like clefts found between each other (3).

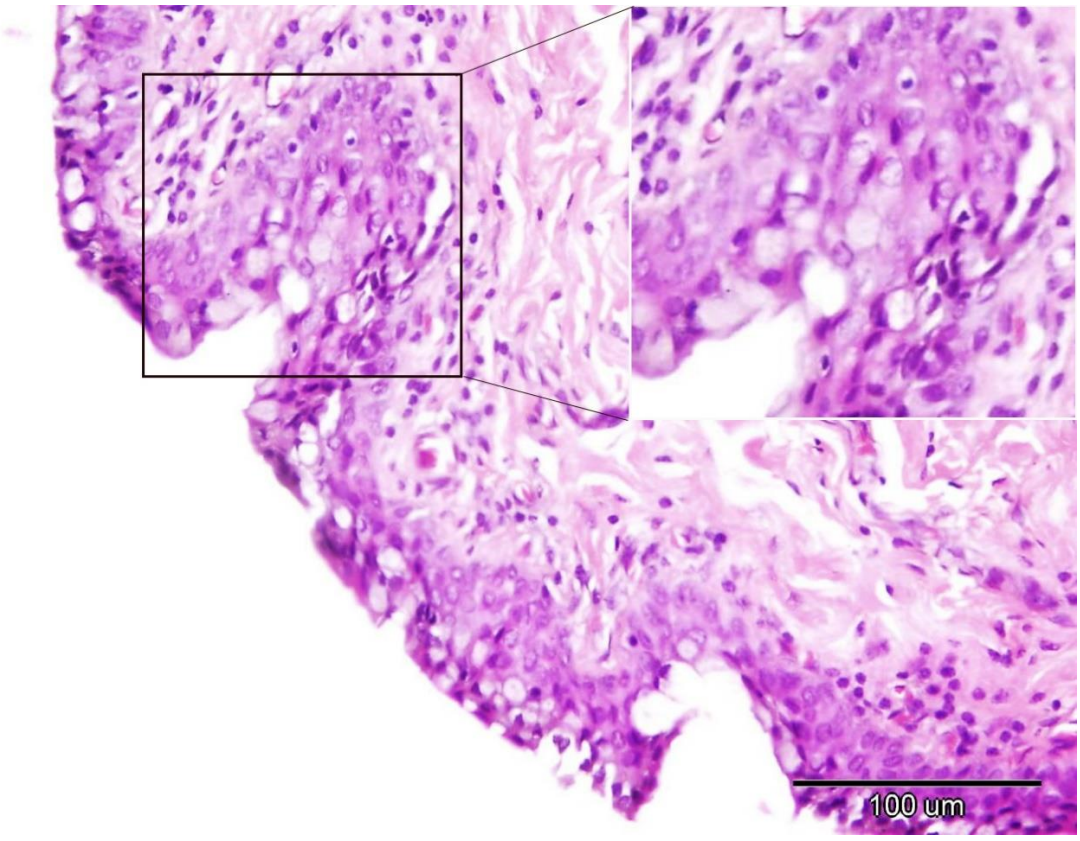

Fig. 5: A micrograph showing the crypts of Henle, stain H&E, note the presence of numerous goblet cells with eosinophilic staining. These cells aren't a classical goblet cells and may be belong to a special type known as serous-goblet cells.

DISCUSSION

The present study revealed that the anti-tarsal border of the eyelids in camels has an interesting crypt system formed mainly by typical crypts of Henle. These crypts were found within a capillary invagination within the tarsal plate. An immune-associated lymphoid tissue was found around the perimeter of each crypt and diffused in the lamina propria.

Grossly, the conjunctiva is a smooth glistening membrane, however under microscopy, a complex system of crypts appears (Knop and Knop, 2000). These crypts are usually found directly on the anti-marginal border of the tarsal plate(Knop and Knop, 2000), however, in camels, it was also found indirectly within the tarsus. These narrow and deep invaginations may act as capillary tubes, which may attract the lacrimal secretions inside its cavity, and thus into the cryptal lamina, this may help in the fixation and integrity of the aqueous layer, especially near the puncta (Knop and Knop 2002; Yokoi *et al.*,2014).

The secretory cells within the crypts were of both classical and non-classical types. The classical cells of typically goblet form were the most cells, especially at the wide grooves-like crypts and the large crypts, near the ocular surface. The non-classical goblet cells were pear-shaped goblet cells, which may be of the type known as "serous-goblet cells" (Mokhtar 2015; Anwar *et al.,* 2021), especially when we take in consideration the eosinophilic cytoplasm which indicate the presence of serous-type of secretion (Brandtzaeg 1974; Klećkowska-Nawrot *et al.,* 2013) However, further investigations were still required to identify the different types of secretory cells within the crypts.

The intraepithelial mucus cells found within the tarsal margin in this study were similar to those found in the Nasal septal island in dromedaries (Abo-Ahmed, *et al.,* 2021). These intraepithelial glands and the goblet cells may have a role in the formation of tear film, namely the mucus layer. It's well established that goblet cells secretes Gel-forming mucins (Knop *et al.,* 2012; Portal *et al.,* 2019), this Gel-forming mucins may help in the formation of the mucous layer of the tear film, and consequently, help in lubrication, protection, and stability of the tear film (Mantelli and Argüeso 2008; Hodges and Dartt 2013; Ruponen and Urtti 2015).

In man, the average depth of the crypt of Henle was about 50-100 μm (Knop and Knop 2000). This was nearly the average depth found in camel specimens; however, a notably deeper and larger crypts were found near the surface.

The presence of mucosal (conjunctival) associated lymphoid tissue within the lamina propria and around the crypts strongly suggested a possible immunological role of these crypts (Knop and Knop 2005; Kuloglu 2022).

The tarsal crypt system as a whole may have a role in maintaining the ocular surface healthy and refractive via its secretory products and the associated lymphoid tissue (Martín and Corrales, 2013)

Conclusion

This study provides describing the tarsal crypt system in camels, a species for which this area has been largely unexplored thus the study fills a gap in the knowledge about this crypt system and its possible role in tear film formation. The crypt structure was variable in shape and size as well as its relation to the goblet cells. Importantly, the mucosal-associated lymphoid tissue surrounded the whole perimeter of each crypt, strongly suggesting an immunological role. The suggestion that the tarsal crypt system in camels may have an immunological role in addition to its function in tear film formation is an intriguing hypothesis that could open avenues for further research.

Declarations:

Ethics Approval: This study's techniques were executed in agreement with the standards for animal use and

care approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt (BUFVTM01-10-24.

Conflict of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request**.**

Funding Information: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgment: Not applicable.

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