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The Comparative Effect of Total Versus Partial Surgical Excision of Nictating Membrane on The Aqueous Tear Production and Ocular Surface Health in Donkeys (*Equus asinus*)

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Abstract

The precise role of the nictating membrane is uncertain and inconsistently discussed in the literature, it is still up for debate. Currently, there have been no extensive investigations on the effect of nictating membrane excision on tear production in donkeys. Therefore, the current study aimed to evaluate the effects of total or partial surgical resection of the nictitating membrane on aquous tear production, pH, and corneal integrity via fluorescein dye and histological examination in donkeys. Donkeys were allocated into two equal groups. Group I (5 donkeys): donkeys were subjected to complete resection of the third eyelid in the left eye and the right one was a control. Group II (5 donkeys), donkeys were subjected to partial resection of the third eyelid in the left eye, and the right one was a control. All animals were observed for three months postoperative. The following criteria (ophthalmic examination, Schirmer tear test I (STT I), tear pH, and fluorescein staining test) were used for the evaluation of the eyes weekly for the first two months, then every two weeks for the last month. There were no significant variations in the mean values of STT I and pH of the total resection of the third eyelid (group I) in comparison with the partial resection of the third eyelid (group II) ($P > 0.05$). In both eyes, there were no obvious clinical indicators of keratoconjunctivitis sicca (KCS). The histopathological examination of various regions such as eyelids, cornea, and conjunctiva in both groups compared to the control showed that all regions were perfectly normal. However, acinar atrophy, degeneration and periacinar fibrosis were visible in the glandular tissue of the third eyelid and cystic dilation and periductal fibrosis were visible in the nictitans gland's duct system. In conclusion, neither complete nor partial resection of the third eyelid causes the development of KCS or alters the ocular surface integrity.

KEYWORDS

Donkey eye, Third eyelid, Schirmer tear test, Tear pH, Fluorescein Staining Test.

INTRODUCTION

The third eyelid (membrana nictitans) is a movable, protective, and secretory structure that is located infero-medially in the anterior section of the orbit between both the eyelids and the globe. The structure consists of a T-shaped to an irregularly shaped piece of elastic cartilage covered by a conjunctival fold reflected from the palpebral and bulbar conjunctiva. The cartilage curled to fit the globe's anterior curvature. In horses, the third eyelid gland (TELG) serous in nature, was found at the baseline of the third eyelid, encircling the shaft of the T-shaped cartilage of the third eyelid (Prince *et al*., 1960; Samuelson, 1999).

In horses, movement of the third eyelid occurs passively, When the muscles of the retractor bulbi pull the eye into the orbit, which forced orbital fat and the third eyelid forward (Samuelson, 1999). The third eyelid has several critical functions, including the dispersion of the precorneal tear film, production of aqueous and immunoglobulin for the tear film, corneal protection, and used as a flap to cover corneal lesions, especially, corneal ulcers (Cooper, 2010).

The third eyelid gland and the lacrimal gland (LG) are the two intraorbital glands that emit the aqueous part of the tear film (TELG). These structures are essential for keeping ocular surface health because they contribute significantly to aqueous tear secretion. The tear film smooths the surface of the cornea, nourishes it, and plays a vital function in the ocular defense system. It acts as a conduit for the transport of air oxygen, other nutrients, and metabolite removal to the avascular cornea (dissolved carbon dioxide and lactic acid). Additionally, it has a good optical efficiency and provides proteins, growth factors, antimicrobials, and electrolytes. (Lamberts, 1994).

In horses, the orbital lacrimal gland is the primary production of tears, with the nictitating membrane gland making a minor contribution (Williams *et al*., 1979). Ocular disease may be caused by insufficient tear production or alterations to tear composition (Craig, 2002).

Tear insufficiency causes the tear film to become hypertonic, the conjunctival and corneal epithelium to become dehydrated,

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and the corneal epithelium and subepithelial stroma to become hypoxic. Additionally, it results in inadequate ocular surface lubrication, secondary conjunctivitis, keratitis with stromal vascularization, and corneal ulceration (Ibrahim and Ahmed, 2021)

There are two types of tear production: reflex and basic. The Schirmer tear test II (STT II) after topical application anesthesia measures continuous or "basal" tears, which are produced continuously and allow normal functioning of the preocular tear layer. According to the Schirmer tear test I (STT I), reflex tears are produced in reaction to any stimulation of the cornea, conjunctiva, or nasal mucosa (Ollivier, 2004). Fluorescein dye is a hydrophilic substance that is absorbed by taken by the visible corneal stromain the presence of corneal epithelial abnormalities. Fluorescein stains intercellular gaps but not the typical healthy corneal epithelium or Descemet's membrane. Fluorescein dye serves to reveal corneal and conjunctival abnormalities, as well as inadequacies in the precorneal tear film (Strubbe and Gelatt, 1999).

The third eyelid may protrude unilaterally from its normal position secondary to uveitis or glaucoma. While, bilateral protrusion occurs secondary to systemic diseases such as lymphosarcoma or in debilitating animals (Martin *et al*., 2005). The third eyelid has been surgically removed in conditions of neoplasia, abscesses, or chronic inflammation (Misk, 2020). In dogs, surgical removal of the third eyelid resulted in no significant change in tear quantity as measured by STT I but decreased basal secretion volume as measured by STT II. The pH level is elevated, causing microinjury to the keratoconjunctival epithelium (Saito *et al*., 2001). We hypothesized that our results would be in close agreement with the previous studies, there was no significant variation in tear quantity as measured by STT I post-surgical resection of the third eyelid. Currently, no extensive research has been conducted on the effect of nictating membrane resection on donkey tear production. As a result, the current study aimed to evaluate the effectiveness of complete or partial surgical resection of the nictitating membrane on aqueous tear production, pH, and corneal integrity via fluorescein dye and histological examination in donkeys.

MATERIALS AND METHODS

Ethical Approval

The Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, has approved all procedures of this study according to the OIE standards for use of animals in research and education (aun/vet/3/0010).

Donkeys

The study included ten clinically healthy adult donkeys (5 nonpregnant and nonlactating females and 5 males). They weighed an average of 175 kg (range150-200kg) and were on average 4 years old (range 2–6 years). Based on a thorough investigation with the naked eye, STT I, tear pH, and fluorescein staining test, these donkeys were chosen because they have healthy eyes. Standard barns with unlimited access to food and water were utilized to house the donkeys.

Experimental study

Donkeys were separated into 2 equal groups. Group I (5 donkeys): donkeys were subjected to complete resection of the third eyelid in the left eye and the right one was left as a control. Group II (5 donkeys), donkeys were subjected to partial resection of the third eyelid in the left eye, and the right one was left as a control.

Donkeys were premedicated in a standing position with intravenous injection of xylazine HCl 2% (XylaJect® Adwia co, 10th of Ramadan City, Egypt) at a dose of 1.1 mg/kg body weight, then auriculopalpebral nerve was blocked via injection of 3 ml of lidocaine HCL 2% (Debocaine, AL-Debeiky pharmaceutical industries Co., Egypt) dorsolaterally to the highest point of the zygomatic arch, to block motor innervation of the upper eyelid and surrounding adnexal structures as well as local infiltration of lidocaine HCL 2% at the base of the third eyelid. Finally, 3 drops of 0.4% benoxinate HCL were applied to the ocular surface to provide surface analgesia (Benox, Eipico Pharmaceutical Industry Co., Egypt).

Surgical Excision of the nictating membrane

Prior to surgery, all donkeys were clinically checked to assess physiological indicators such as rectal temperature, heart rate, and breathing rate as well as an ophthalmic examination of both eyes using the naked eye, STTI, tear pH, and fluorescein staining test. The surgery was performed in a standing position. The area around the operated eye was prepared aseptically with alcohol and the eye itself was washed with sterile saline.

For total resection, the third eyelid's leading edge was grasped, exteriorized by tissue forceps then crushed at its base with an artery forceps. The third eyelid was then excised by a scissor. The artery forceps was left in place for a few minutes followed by pressure using sterile gauze for hemostasis. The wound was not sutured (Fig. 1)

For partial resection, the third eyelid was grasped and exteriorized by tissue forceps and was crushed in the middle of the third eyelid with artery forceps. Then the free margin of the third eyelid was excised by a scissor. The artery forceps was left in place for a few minutes to prevent hemorrhage, the wound was left without suture (Fig. 2). Postoperative medications included topical oxytetracycline hydrochloride/polymyxin B sulfate ointment (Terramycin; Pfizer Pharmaceutical Industry Co., Egypt) was applied on the operated eyes twice daily for six days.

Clinical Examination

Periodically, the naked eye was used to inspect the eyes of the animals to check for any abnormalities such as hemorrhage, swelling, inflammatory exudate, conjunctivitis, or keratitis.

Schirmer Tear Test (STT-I)

Schirmer Tear Test I (without topical ocular anesthesia) was used to assess the tear. An individual, sterile, graduated (5 x 35 mm) Schirmer tear testing strip (OpStrip, Oph-technic, India) was inserted into the lower fornix at the center of the lower eyelid and was left for 1 minute. The moisture content was calculated using millimeters per minute (mm/min) (Crispin, 2000).

Tear pH

A pH paper test strip (ARC for chemicals, El Obour City, Egypt) was placed in the inferior fornix and left for 30 seconds. The tear pH was determined by comparing the color of the test strip with the color chart given with the indicator paper, then photos for the papers were taken to be analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) (Hussein *et al*., 2022).

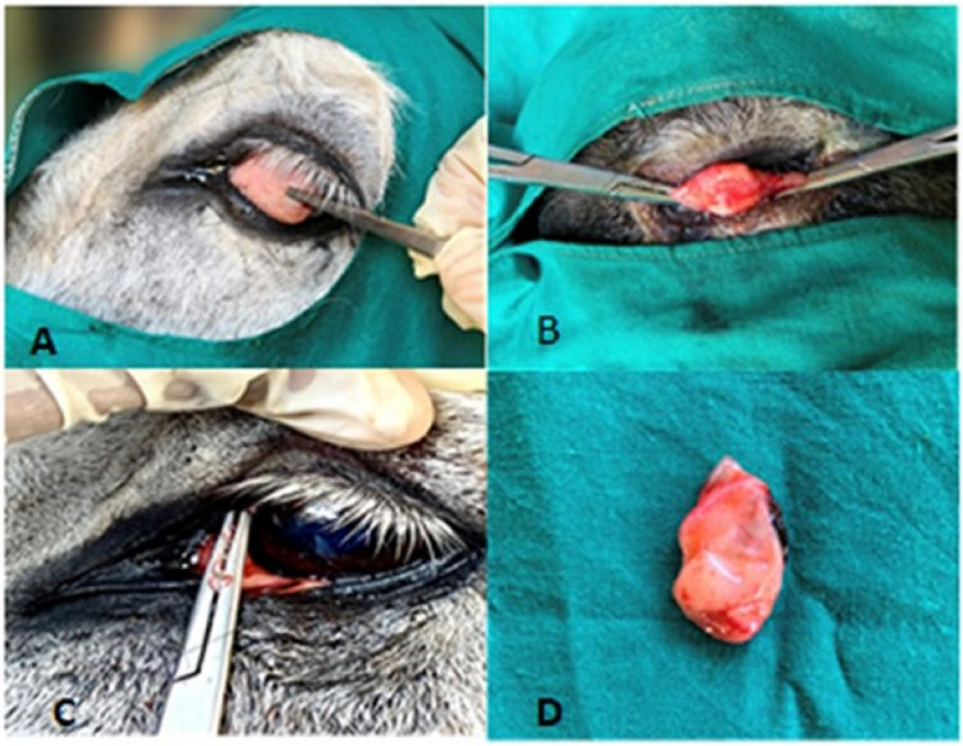

Fig. 1. Total resection of third eyelid (group I). A: Grasping of third eyelid by tissue forceps. B: Crushing of third eyelid at its base. C: Removal of third eyelid D: The third eyelid including its gland after total resection.

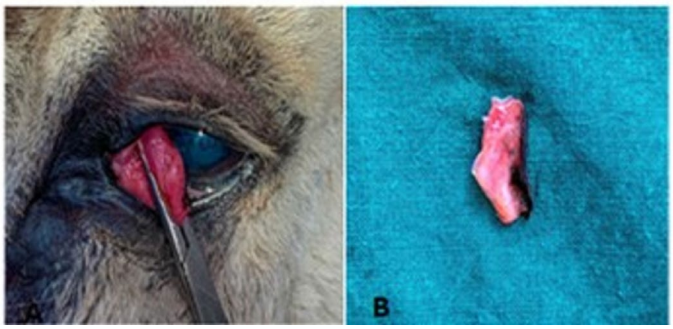

Fig. 2. Partial resection of third eyelid (group II). A: Crushing the third eyelid at its middle. B: Free margin of the third eyelid after partial resection.

Fluorescein Stain Test

One drop of 2% fluorescein solution was put on the ocular surface of the eye for 1 min to detect corneal ulcers. Then the eye was cleansed with sterile normal saline to eliminate any remaining stain.

Clinical examination, Schirmer tear test I, tear pH, and fluorescein stain test were evaluated weekly for the first two months, then every two weeks for the last month.

Histopathological examinations

Donkeys were euthanized at the end of the examination period after being sedated with an intravenous injection of xylazine hydrochloride 2% at a dose of 1.1 mg/kg, followed by a rapid intravenous injection of thiopental sodium (Thiopental sodium 1 g vial, EPICO, Egypt) at a dose of 35 mg/kg (Knottenbelt and Malalana, 2014). For the histopathological examination, collect the eyes of four randomly selected animals from each group.

For histological sections preparation, small samples from the third eyelid (nictitating membrane), nictitating gland, upper and lower eyelids, conjunctiva, and cornea of the control groups have been fixed in 10% buffered formalin solution (pH 7.0) for 2-3 days. The operated eye in group I endured complete resection of the third eyelid; the samples have been collected from the upper and lower eyelids, cornea, and conjunctiva. The operated eye in group II endured a partial resection of the nictitans gland and third eyelid cartilage; the specimens were collected from the third eyelid, the upper and lower eyelids, the cornea, and the conjunctiva. The obtained samples were dehydrated using progressively higher levels of alcohol, including 70%, 80%, 90%, and 100%. The samples were washed using the conventional procedure previously described, which involved submerging them in xylene before immersing them in paraffin wax (Sigma Aldrich, USA) utilizing the established approach already mentioned by (Hussein and Abdel-Maksoud, 2020). Step serial transverse and meridian sections were slice at 4-5 µm thickness via a microtome (Richert Leica RM 2125, Germany) and mounted on glass slides. Ocular slices were embedded in paraffin using xylene, rehydrated with progressively lower alcohol concentrations, and then washed in distilled water. The slices were then dyed with Alcian blue/periodic acid Schiff (AB/PAS), Crossman's trichrome, and hematoxylin/eosin (H&E). The general tissue architecture were described using H&E staining. The presence of muscle fibers and collagen was determined using Crossman's trichrome stain. The periodic acid-Schiff staining (PAS) was used to establish the presence of neutral mucopolysaccharides, while Alcian blue was used to demonstrate the presence of acidic mucopolysaccharides (AB, pH 2.5) (Abdel-Maksoud *et al*., 2019; Hussein and Abdel-Maksoud, 2020). All obtained slides were examined and photographed by CX-43 Olympus for histological and histochemical description.

Statistical analyses

The pH and schirmer tear test results were described using the mean and standard deviation (mean±SD). A statistical software for the social sciences for Windows was used to conduct the analyses (SPSS software, Version 21, IBM Corp., Armonk, NY). Statistical analysis of the data by one-way ANOVA followed by Tukey's HSD post hoc test. An independent samples t-test was utilized to compare STT I and pH values between the left (operat-

ed) and right(control) eyes of both groups and to compare mean values of STT I and pH between the left (operated) eyes of both groups (after complete resection and after partial resection). Values of P < 0.05 were deemed significant.

RESULTS

Clinical findings

A gross examination of the group I (total resection of the third eyelid) showed a normal appearance of the conjunctiva. However, mild hyperemia of the bulbar conjunctiva without inflammatory exudate was observed in one donkey during the first week then disappeared again in the second week following surgery. A gross examination of the group II (partial resection of the third eyelid) showed a normal appearance of the conjunctiva. However, mild hyperemia of the bulbar conjunctiva without inflammatory exudate was noted in 3 donkeys during the first week after surgery and subsided in the second week. Additionally, the controlled eyes (right eyes) of both groups had no significant changes during the period of study. Cornea had a normal appearance in donkeys (operated and controlled eyes) of both groups during all weeks post surgery (Fig. 3).

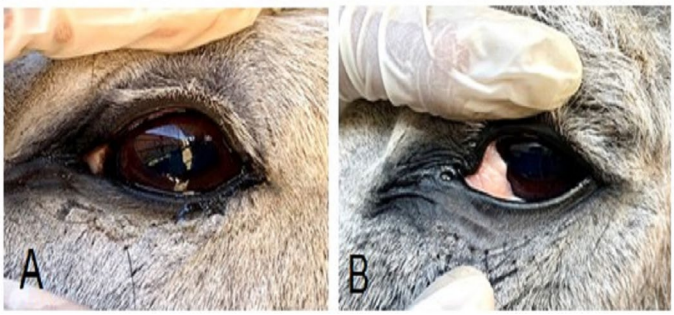

Fig. 3. The resection of the third eyelid. A: The operated eye after 3 month following total resection of third eyelid (group I). B: The operated eye after 3 month following partial resection of third eyelid (group II).

Schirmer Tear Test I (STT I)

In group I, there were non significant variations in the values of STT I of the left (operated) eyes compared to the right (control) eyes (P > 0.05). In the first week post surgery, the schirmer tear test I values were non significantly increased (19.0±1.0) followed by a non significant decrease until the sixth week, then non significant increase until the end of the study (18.2±0.83) compared with the baseline values (17.4±1.14).

In group II, there were non-significant variations in the values of STTI of the left (operated) eyes compared to the right (control) eyes (P > 0.05). In the first week post surgery, the schirmer tear test I values showed a non significant increase (18.8±1.3) followed by a non significant decrease until the end of the study (17.4±0.89) compared with the baseline values (17.6±1.14).

There were non significant variations in the mean values of STT I of the total resection of the third eyelid (group I) in comparison with the partial resection of the third eyelid (group II) (P > 0.05) (Table 1).

Tear pH

In group I, there were non significant variations in the values of tear pH of the left (operated) eyes compared to the right (control) eyes (P > 0.05). There were non significant variations in the values of tear pH of the operated eyes during the study compared with the baseline values (7.6±0.22) (P > 0.05). In group II, there were non significant variations in the values of tear pH of the left (operated) eyes compared to the right (control) eyes (P > 0.05). There were non significant variations in the values of tear pH of the operated eyes during the study compared with the baseline values (7.6±0.1) (P > 0.05). There were non significant variations in the mean values of tear pH of the total resection of third eyelid (group I) in comparison with the partial resection of third eyelid (group II) (P > 0.05) (Table 2).

Table 1. Comparison between the mean±SD of Schirmer tear test I after total resection of the third eyelid (group I) and partial resection of the third eyelid (group II).

Time	Total resection of the third eyelid (group I)	Partial resection of the third eyelid (group II)
Zero time (Before surgery)	17.40±1.14	17.60±1.14
1st week	19.00±1.00	18.80±1.30
2nd week	17.60±0.89	17.80±1.30
3rd week	17.60±1.14	17.60±0.54
4th week	17.40±0.89	17.20±0.83
5th week	17.80±1.64	17.40±1.14
6th week	17.60±1.14	17.40±0.89
7th week	18.20±0.83	17.40±1.34
8th week	18.20±0.83	17.40±0.54
10th week	18.00±1.00	17.20±1.09
12th week	18.20±0.83	17.40±0.89

Table 2. Comparison between the mean±SD of tear pH after total resection of the third eyelid (group I) and partial resection of the third eyelid (group II).

Time	Total resection of the third eyelid (group I)	Partial resection of the third eyelid (group II)
Zero time (Before surgery)	7.60±0.22	7.60±0.10
1st week	7.66±0.19	7.58± 0.08
2nd week	7.62±0.21	7.58 ±0.04
3rd week	7.64±0.20	7.60±0.07
4th week	7.62±0.10	7.60±0.10
5th week	7.68±0.13	7.60±0.07
6th week	7.64±0.11	7.60±0.07
7th week	7.60±0.07	7.64±0.08
8th week	7.62±0.10	7.62±0.08
10th week	7.58±0.04	7.60±0.10
12th week	7.58±0.08	7.62±0.08a

Fluorescein Staining Test

No evidence of keratoconjunctivitis sicca was detected by negative fluorescein staining test in donkeys of both groups.

Histological findings

The third eyelid consisted of a conjunctival fold which was supported by a cartilaginous plate and fibrous tissue composed of elastic and collagen fibers and abundant blood vessels. The donkey's third eyelid cartilage was found to be of elastic type (Fig. 4A). Cartilage is composed of specialized cartilage cells called chondrocytes and a limited amount of intercellular matrix. This matrix is composed of glycosaminoglycans and proteoglycans.

In elastic cartilage, the elastic fibers form a threadlike network in the matrix (Fig. 4B). The base of the cartilage in the donkeys was surrounded by a massive adipose tissue (Fig. 4C). The bulbar and palpebral conjunctival epithelium was simple squamous epithelium and stratified columnar epithelium respectively (Fig. 4D). A few aggregated lymphoid nodules were observed in the lamina propria that located adjacent to the bulbar conjunctiva (Fig. 4E). Numerous goblet cells were dispersed among the palpebral conjunctival epithelium. However, few goblet cells were demonstrated at the bulbar surface near the tip of the third eyelid. The goblet cell reacted positively to PAS/ AB (purple color) (Fig. 4F).

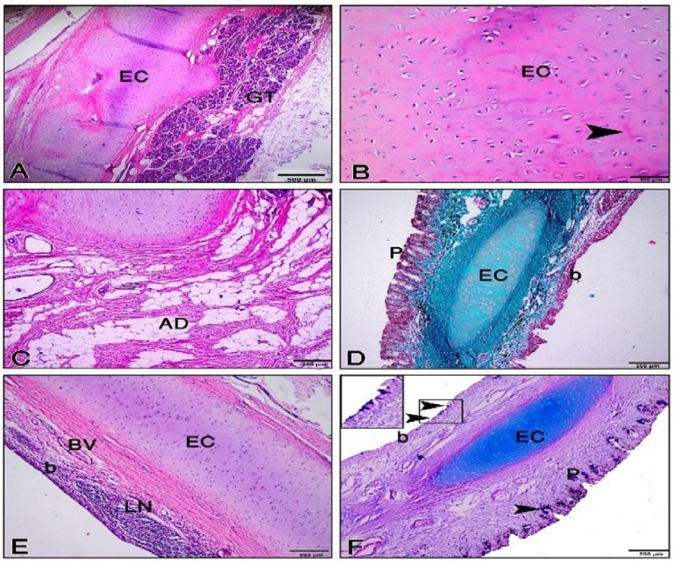

Fig. 4. Histology of the nictitans membrane and gland. A, C: Paraffin section stained with Hx and E showing the third eye lid composed of elastic cartilage (EC) and glandular tissue (GT). B: The third eyelid cartilage was elastic (, arrowhead, EC). C: The base of the cartilage was surrounded by a massive adipose tissue (AD). D: the bulbar (b) and palpebral conjunctival (P) epithelium was simple squamous epithelium and stratified columnar epithelium respectively. E: A few aggregated lymphoid nodules (LN) were observed adjacent to the bulbar conjunctiva. F: Numerous goblet cells showed positive reactivity to PAS/ AB (purple color) were dispersed among the palpebral conjunctival epithelium (arrowhead). However, few goblet cells were demonstrated at the bulbar surface near the tip of third eye lid (inset, arrowheads). Abbreviation; BV: blood vessel.

The third eyelid (nictitans) gland in donkeys is a multilobular, tubinoacinar that is located adjacent to and surrounding the third eyelid cartilage. Numerous fat cells were observed among the glandular tissue (Fig. 5A). Both mucous and serous glands were visible due to the gland's mixed seromucous discharge (Fig. 5B). Tall columnar cells with light acidophilic cytoplasm and basal oval or flat nuclei lined the mucous gland, which had a broad lumen. However, the serous gland is lined with pyramidal-shaped cells with strongly acidophilic cytoplasm and rounded basally situated nuclei. It possessed a tiny lumen, and the cell borders were barely discernible (Fig. 5C). The secretory end-pieces showed positive staining for the mixture of Alcian blue (AB) and Periodic Acid Schiff (PAS), therefore, the glands showed purple color (Fig. 5B, D). Surprisingly, glandular tissue was observed within the cartilaginous plates of the third eyelid (Fig. 5D).

The secretory end-pieces enter into intralobular ducts easily that are lined with cuboidal epithelium (Fig. 6A). Low cuboidal or flat squamous cells bordered the interlobular ducts where the intralobular ducts open (Fig. 6B, C). The bulbar conjunctiva is where the interlobular duct finally opens (Fig. 6D).

In the control eye, the outer surface of the eyelids is bordered by the skin; however, the underlying dermis is made up of fibrous connective tissue and skeletal muscle fibers (Fig. 7 A). Adnexal specializations such as cilia and glands are also present. The Moll glands, which are apocrine sweat glands, were investigated near the base of the eyelashes (Fig. 7 B). The tarsal or meibomian glands are modified sebaceous glands with multilobed acini and are occupied by lipid droplets and resemble a huge cluster of grapes (Fig. 7 C). The palpebral conjunctiva consisted of stratified epithelium that changed to stratified columnar with goblet cells. Goblet cells were big, spherical cells that contained glycoprotein (mucin). The glycoprotein showed a positive reaction for both AB/ PAS (purple color) (Fig. 7 D, E). The bulbar conjunctiva consisted of a stratified squamous cell (Fig. 7D). The cornea of donkeys consisted of four layers from outside: non-keratinized stratified squamous epithelium was observed, the thick layer of collagen fibers that run parallel to each other, descemet's membrane which is an acellular layer made of type IV collagen and endothelium which consisted of flat squamous cells (Fig. 7 F).

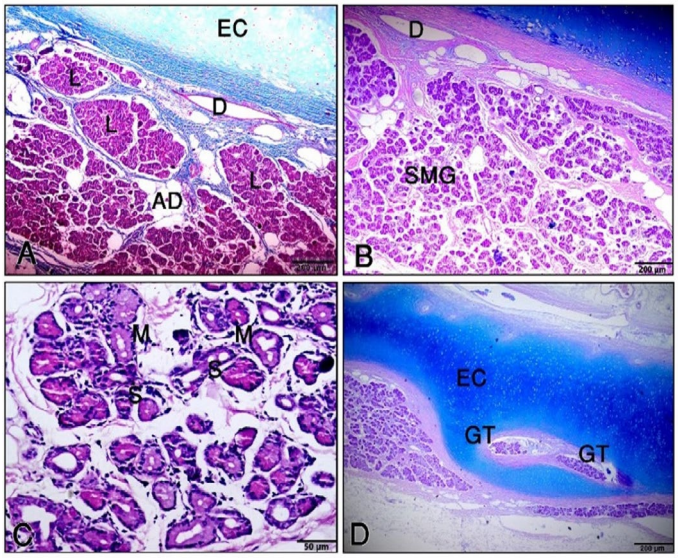

Fig. 5. Histology of the nictitans gland. A: Paraffin section stained with Crossman's trichrome showing the third eyelid (nictitans) gland in donkeys is a multilobular (L), tubinoacinar that is located adjacent to and surrounding the third eye lid cartilage (EC). A: numerous fat cells (AD) were observed among the glandular tissue. B: PAS/ AB staining showing the mixed secretion (purple color) of the seromucous gland (SMG). C: Hx and E stained section showing the mucous gland (M) and the serous gland (S). D: surprisingly, a glandular tissue (GT) was observed within the cartilaginous plates (EC) of the third eye lid. Abbreviations: D: duct.

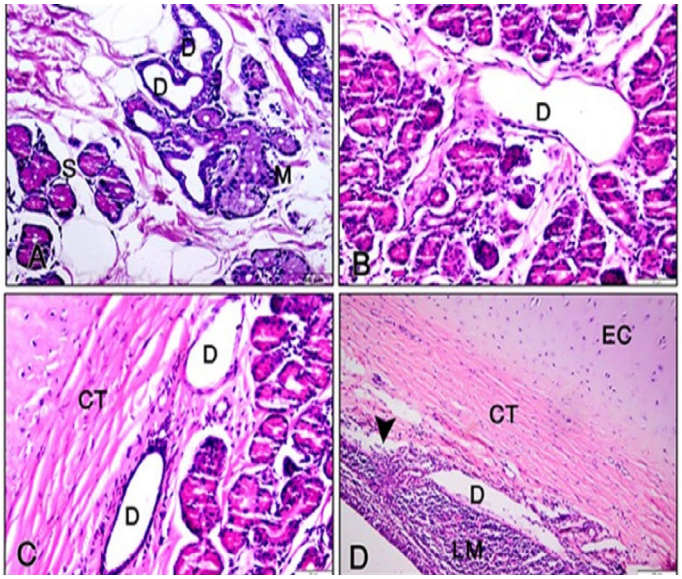

Fig. 6. Duct system of the third eyelid gland. A: Paraffin sections stained with Hx and E showing the intralobular duct (D) lined with low cuboidal cells. B, C: Interlobular duct lined with flat squamous cells. D: Finally, the interlobular duct opens into the bulbar conjunctiva (arrowhead). Abbreviations: CT: connective tissue, LN: Lymph nodule, EC: elastic cartilage, S: serous, M: mucous.

In group I, the eyelids, cornea, and conjunctiva were histologically examined in the operated eye. The palpebral conjunctiva coated the inner surface of the eyelids and bulbar areas, as

well as its adnexal structures, were entirely normal as opposed to the control (Fig. 6A). The lining epithelium of the palpebral conjunctiva ranged from stratified squamous epithelium to stratified columnar epithelium with interspersed goblet cells that reacted positively to PAS and AB (Fig. 8 B, C). Furthermore, the corneal epithelium and stroma were in perfect condition (Fig. 8 D). We did not demonstrate any signs of inflammation, degeneration, or ulcer in the corneal and conjunctival lining epithelium.

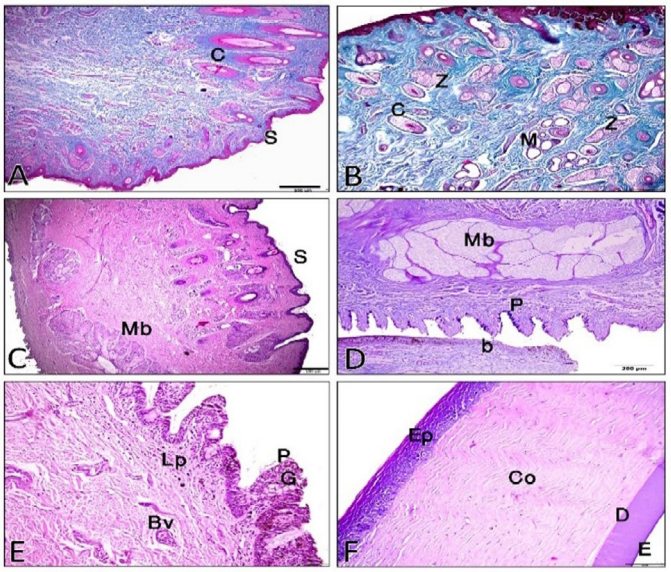

Fig. 7. Conjunctiva and cornea in the control group. A, B: Paraffin section stained with Crossman's trichrome showing the outer surface of the eyelids is bordered by the skin (S); however, the underlying dermis (green) is made up of a fibrous connective tissue B; there are also a variety of adnexal specializations, such as cilia (C), the glands of Moll (M) and zeis glands (Z). C: Hx and E stained section showing the meibomian glands (Mb). D, E: PAS/ AB and Hx and E stained section showing the bulbar (b) and the palpebral conjunctiva (P) consisted of stratified epithelium with goblet cells (G). E: Goblet cells showed positive reaction for both AB/ PAS (purple color). F: The cornea of donkeys consisted of four layers: the stratified squamous epithelium (EP), thick layer of collagen fibers (Co), descemet's membrane (D) and endothelium (E). Abbreviations: BV; blood vessel, LP; lamina propria.

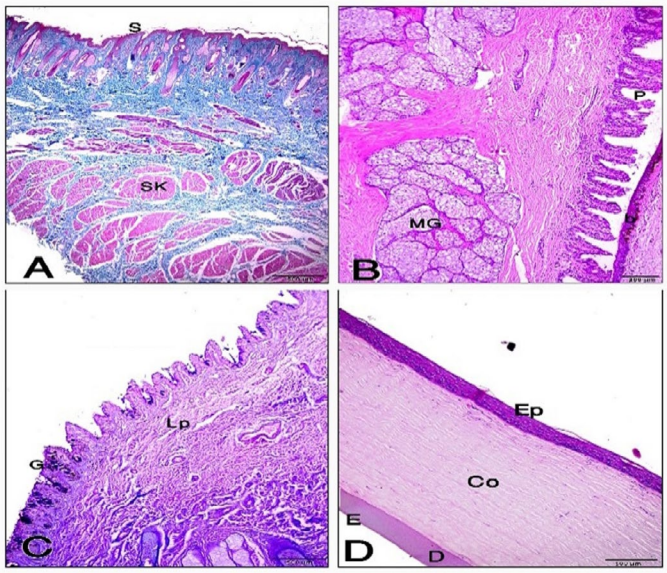

Fig. 8. Eyelids, cornea and conjunctiva in group-I operated eyes. A, B: Paraffin section-stained Crossman's trichrome and Hx and E showing the palpebral conjunctiva (P) lining the inner aspect of the eyelids skin (S), and bulbar regions (b) was completely normal. C: Paraffin section-stained PAS/AB showing the lining epithelium of the palpebral conjunctiva ranged from stratified squamous epithelium to stratified columnar epithelium with interspersed goblet cells with purple color. D: The corneal epithelium (Ep) and collagen fibers (Co) were in perfect condition. No signs of inflammation, degeneration or ulcer in the corneal and conjunctival lining epithelium were observed. Abbreviations: G; goblet cells; E; Endothelium; Sk: skeletal muscle; MG: meiboiman gland.

Specimens were taken from the third eyelid, eyelids, cornea, and conjunctiva in the operated eye in group II. We found that the palpebral and bulbar conjunctiva, as well as the cornea, is fully normal and free from lesions, degeneration, or ulcers (Fig. 9 A-D). However, acinar atrophy, degeneration, and periacinar fibrosis were visible in the glandular tissue of the third eyelid (Fig. 10 A-D). Cystic dilation and periductal fibrosis were visible in the nictitans gland's duct system (Fig. 10 D- F). Although the glandular tissue near the bottom of the third eyelid was normal, the duct system displayed dilatation and periductal fibrosis (Fig. 10 F).

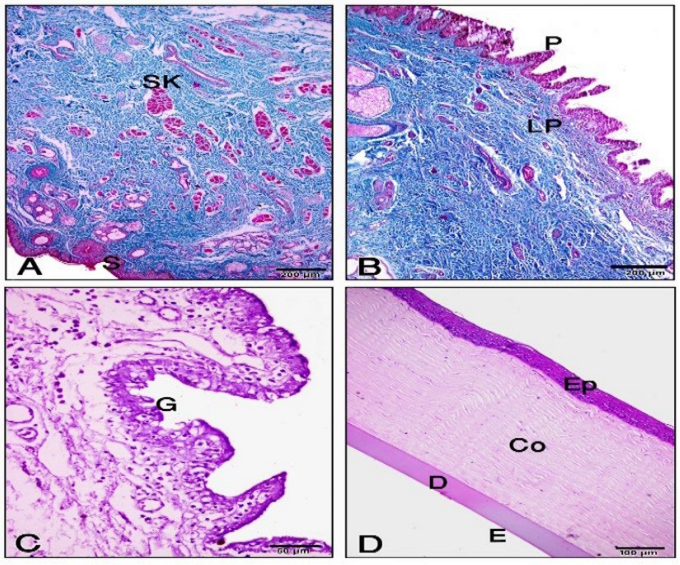

Fig. 9. Eyelids, conjunctiva and cornea in group-II operated eyes. A, B: Paraffin section-stained Crossman's trichrome showing the eye lid and the palpebral conjunctiva (P). C: Showing the goblet cells (G) in the palpebral conjunctiva. D: Showing the cornea layers and it was free form any degenerations or ulcers. Abbreviations: S; skin, SK; skeletal muscle, LP; lamina propria, Ep; epithelium, Co; collagen fibers, D; descemet's membrane, E; endothelium.

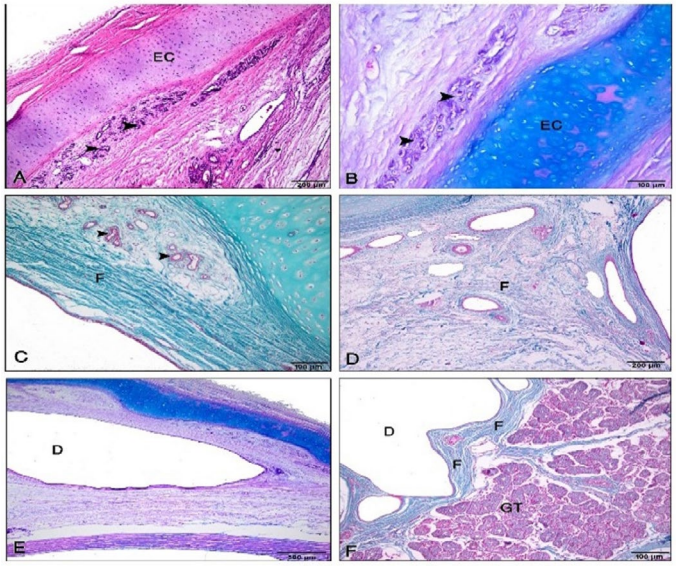

Fig. 10. The glandular tissue of the gland in in group-II operated eyes. A, B: Paraffin sections stained with PAS/AB showing a piece of third eyelid elastic cartilage (EC) and atrophy in the glandular tissue (arrowheads). C, D: Sections stained with Crossman's trichrome showing the fibrosis (F) in the glandular tissue and the glandular tissue atrophy (arrowheads). E: PAS/AB stained section showing the dilated duct (D). F: Sections stained with Crossman's trichrome showing the glandular tissue near the base of the gland was normal however, the ducts (D) showed dilation and periductal fibrosis (F).

DISCUSSION

Nictitating membrane's diseases in donkeys include inversion, eversion, hypertrophy, protrusion, prolapse of the gland, laceration trauma, and neoplasia particularly squamous cell carcinoma (SCC) as the third eyelid is the most common site in horses so surgical resection of the nictitating membrane has been

regarded as an effective treatment for the majority of affections (Lavach and Severin, 1977; Lavach, 1990). A big debate in literature was discovered about the removal of the third eyelid. Therefore, investigating the potential of partial or complete resection of the third eyelid as a suitable option for specific third eyelid diseases SCC is required. The current study aimed to compare complete and partial resection of the third eyelid and its effect on the ocular surface.

Minor ophthalmic surgical operations involving adnexal tissues including the globe, conjunctiva, third eyelid, and eyelids can be safely performed on the standing horse (Harper, 2009). In the present investigation, sedation, auriculopalpebral nerve block, local infiltration analgesia at the base of the third eyelid, and surface analgesia of the corneal surface were used to conduct complete and partial surgical resection of the nictitating membrane in donkeys. This agreed with Harper (2009) and Labelle and Clark-Price (2013) who noted that the nictitating membrane resection can be completed under sedation in a standing position, using nerve block, local anesthesia, and topical analgesia as the routine standing surgical resection of the third eyelid does not appear to have any deleterious effects to the remaining functional eye structures. However, Vestre *et al*. (1979) and Payne *et al*. (2009) reported that the general anesthesia for surgical removal of the third eyelid in animals induced a reduction in tear production in addition to the risk and high cost of a general anesthetic.

In the present study, after partial or total excision of the third eyelid, the wound was left without suture but the artery forceps was left in place for hemostasis and coaptation of excised margins to each other. This was in accordance with (Harper, 2009) who reported that the placement of sutures near the globe causes irritation to the cornea so is a strong reason to avoid suturing the wound and let the surgical site heal by second intention. In contrast, Millichamp (2006) has hypothesized that suturing the inferomedial conjunctival wound may prevent the protrusion of the cartilage of the nictitating membrane or herniation of orbital fat after resection of the nictitating membrane.

The Schirmer tear test (STT) is a semi-quantitative technique for calculating the amount of the aqueous component of the tear film (Verboven *et al*., 2014; Dias *et al*., 2020). According to the statistical analysis of the current study, there were non-significant changes in the values of STT I after total or partial surgical excision of the nictitating membrane this could be attributed to the essential function of the orbital lacrimal gland (OLG) which compensates for the tear production and is the main source of tear's aqueous part in donkeys. These findings were in agreement with Cooley (1992) who noted that the lacrimal gland has thus far been responsible for the production of aqueous tears in equine.

Williams *et al*. (1979) reported that despite lacrimal and nictitans glands playing a part in the formation of the aqueous tears in horses, no signs of dry eye was seen in horses exposed to the third eyelid gland excision. The same results were obtained in the present study that indicated the nictitans gland played just a minimal part in tear production as described (Carastro, 2004). However, in dogs, there was a significant reduction in STT I after the total excision of the third eyelid (Hussein *et al*., 2022). As a result, the nictitans gland in donkeys is small in size surrounded by a large amount of fatty tissue in comparison with dogs so the contribution of the nictitans gland is minor in tear production in donkeys compared to in dogs.

In the current study, there were non-significant changes in the values of pH of both groups of donkeys this could be attributed to the tear originating from the orbital lacrimal gland and other accessory glands including the 3rd eyelid gland so removal of the 3rd eyelid has no effect on pH because of the minor role of the 3rd eyelid gland in tear production in donkeys. These results were contrary to Saito *et al*. (2001) who noted that there was a significant increase in tear pH after resection of the nictitans gland in dogs as a result of decreased tear secretion. In our study the mean values of tear pH of donkeys of both groups were alkaline (7.5-7.6). These results were comparable with Beckwith Cohen *et al*. (2014) who observed that the tear pH of the equine was alkaline so the pH of tears in cattle, dogs, and horses was 8.32±0.14, 8.05±0.26, and 7.84±0.30, respectively. Tear pH was considerably higher in cattle compared to dogs and horses and in dogs compared to horses. Additionally, misuse of human ophthalmic drugs in equines by veterinarians may increase the alkalinization of tear pH which is usually associated with various ophthalmic disruptions, particularly keratitis (Norn, 1988; Ibrahim and Ahmed, 2021).

Fluorescein dye is used to reveal corneal and conjunctival abnormalities as well as defects in the precorneal tear film (mucin and lipid deficiencies). When corneal epithelial abnormalities (ulcers) are evident, fluorescein dye, a hydrophilic chemical that dyes intercellular spaces but does not cling to the intact healthy corneal epithelium or descemet's membrane, is consumed by visible corneal stroma (Strubbe and Gelatt, 1999). In the present study, the fluorescein dye was negative in both groups of donkeys during the period of study, which indicates the absence of keratoconjunctivitis sicca (KCS) or "dry eye" in animals of both groups after surgical interference up to 3 months post-surgery. These findings agreed with Gelatt *et al*. (1977); Williams *et al*. (1979) and Harling (1988).

The microscopic examination of the eyelids, cornea, and conjunctiva in both groups compared to the control group confirmed our observations by showing that all of the tissues were in perfect condition, with no evidence of degeneration or corneal ulcers. However, in group II, the nictitans gland's duct system displayed cystic dilatation and peritubular fibrosis. As a result, the nictitans gland revealed acinar atrophy and degeneration. These changes could happen due to handling of the nictitating membrane during the resection as described before in dogs (Hussein *et al*., 2022).

CONCLUSION

The partial or complete surgical excision of the third eyelid in donkeys did not induce significant changes in tear production, tear pH, and the integrity of the ocular surface (corneal and conjunctival structures). Therefore, the partial or total excision of the third eyelid has been recommended for various surgical affections in the third eyelid without any postoperative complications on the integrity of the ocular surface.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

REFERENCES

Abdel-Maksoud, F.M., Hussein, M.T., Attaai, A., 2019. Seasonal variation of the intraepithelial gland in camel epididymis with special reference to autophagosome. Microsc. Microanal. 25, 1052-1060.

Beckwith-Cohen, B., Elad, D., Bdolah-Abram, T., Ofri, R., 2014. Comparison of tear pH in dogs, horses, and cattle. Am. J. Vet. Res. 75, 494–499.

Carastro S.M., 2004. Equine ocular anatomy and ophthalmic examination. Vet. Clin. North Am. Equine Pract. 20, 285–299.

Cooley P.L., 1992. Normal equine ocular anatomy and eye examination. Vet. Clin. North Am. Equine Pract. 8, 427–449.

Cooper, S., 2010. The canine third eyelid. UK Vet Companion Animal. 15, 52-57.

Craig, J., 2002. Structure and function of the preocular tear film. The Tear Film: structure, function and clinical examination. 1, 18-50.

Crispin, S.M., 2000. Tear-deficient and evaporative dry eye syndromes of the horse. Vet. Ophthal. 3, 87-92.

Dias, F.G.G., Jorge, A.T., Cintra, C.A., Pires, V.M., de Freitas Pereira, L., dos Anjos, D.S., dos Santos Honsho, C., 2020. Schirmer tear test, a useful diagnosis tool for the clinician. Braz. J. Dev. 6, 40354-40363.

Gelatt, K.N., Peiffer, R.L., Gwin, R.M., Williams, L.W., 1977. The status of equine ophthalmology. Journal of Equine Medicine and Surgery. 1, 9-13.

Harling, D.E., 1988. Epiphora and lacrimal system dysfunction in the horse. Equine Pract. 10, 27-38.

Harper, J.S., 2009. How to surgically remove the third eyelid in the standing horse. In Proc. Am. Ass. equine Practnrs. 55, 380-385.

Hussein, K., Hussein, M.T., Attaai, A., Ragab, L., Semieka, M., 2022. Effect of Nictitans gland and third eyelid excisions on ocular surface integrity, pH, and tear production in dogs. J. Adv. Vet. Res. 12, 90-98.

Hussein, M.T., Abdel-Maksoud, F.M., 2020. Structural investigation of epididymal microvasculature and its relation to telocytes and immune cells in camel. Microsc. Microanal. 26, 1024-1034.

Ibrahim, A., Ahmed, A.F., 2021. The Impact of Surgical Excision of the Orbital Lacrimal Gland on the Aqueous Tear Production and Ocular Surface Health in Donkeys (*Equus asinus*). J. Equine.Vet. Sci. 97, 103344.

Knottenbelt, D.C., Malalana, F., 2014. Saunders Equine Formulary E-Book. Elsevier Health Sciences.

Labelle, A.L., Clark-Price, S.C., 2013. Anesthesia for ophthalmic procedures in the standing horse. The Veterinary clinics of North America. Equine practice, 29(1), 179–191.

Lamberts, D.W., 1994. Physiology of the tear film In: The Cornea. (eds Smolin G, Thoft RA.) Little Brown & Co, New York, pp. 439–455.

Lavach, J.D., 1990. Large animal ophthalmology. Publisher: St. Louis : Mosby. Vol I., United States.

Lavach, J.D., Severin, G.A., 1977. Neoplasia of the equine eye, adnexa, and orbit: a review of 68 cases. J. Am. Vet. Med. Assoc. 170, 202–203.

Martin, C.L., Pickett, J.P., Spiess, B.M., 2005. Ophthalmic disease in veterinary medicine. London: Manson, pp. 340-341.

Millichamp, N.J., 2006. Third Eyelid. In: Equine Surgery (Third Edition). Chapter 58, Jörg A. Auer, John A. Stick, Eds., W.B. Saunders, pp. 723-726.

Misk, N.A., 2020. Veterinary ophthalmology. 2nd ed. Faculty of veterinary medicine, Assiut university, Egypt.

Norn M.S., 1988. Tear fluid pH in normals, contact lens wearers, and pathological cases. Acta ophthalmolo. 66, 485–489.

Ollivier F.J., 2004. The precorneal tear film in horses: its importance and disorders. Vet. Clin. North Am. Equine Pract. 20, 301–318.

Payne, R.J., Lean, M.S., Greet, T.R.C., 2009. Third eyelid resection as a treatment for suspected squamous cell carcinoma in 24 horses. Vet. Rec. 165, 740-743.

Prince, J.H., Diesem, C.D., Eglitis, I., Ruskell, G.L., 1960. Anatomy and histology of the eye and orbit in domestic animals. Charles C Thomas, Publisher, E. Lawrence ave., Springfield, III, 1960. pp. 301-327.

Saito, A., Izumisawa, Y., Yamashita, K., Kotani, T., 2001. The effect of third eyelid gland removal on the ocular surface of dogs. Vet. Ophthalmol. 4, 13–18.

Samuelson, D.A., 1999. Ophthalmic embryology and anatomy. In: Veterinary Ophthalmology, 3rd ed. (Gelatt, K.N. ed.), Lippincott Williams and Wilkins, Philadelphia.

Strubbe, T.D., Gelatt, K.N., 1999. Ophthalmic examination and diagnostic procedures. in Gelatt KN (ed): Veterinary Ophthalmology (ed 3). Philadelphia, PA, Lippincott. pp. 427-466.

Verboven, C.A., Djajadiningrat-Laanen, S.C., Teske, E., Boevé, M.H., 2014. Development of tear production and intraocular pressure in healthy canine neonates. Vet. Ophthalmol. 17, 426–431.

Vestre, W.A., Brightman 2nd, A.H., Helper, L.C., Lowery, J.C., 1979. Decreased tear production associated with general anesthesia in the dog. J. Am. Vet. Med. Assoc. 174, 1006-1007.

Williams, R.D., Manning, J.P., Peiffer, R.L., 1979. The Schirmer tear test in the equine: normal values and the contribution of the gland of the nictitating membrane (Diagnosis of keratoconjunctivitis sicca). Journal of Equine Medicine and Surgery 3, 117-119.