HISTOLOGICAL AND HISTOCHEMICAL STUDY OF EYELID OF NATIVE CATTLE (OX)

Naizak Subhi Ahmed

Department of Anatomy, College of Veterinary Medicine, University of Mosul

Mosul, Iraq

(Received 2 October 2012,Accepted 30 October 2012)

Key word: Zeis gland, Tarsal Gland , Cattle.

ABSTRACT

The aim of the present work is to study the skin eyelids of native cattle (ox) with special emphasis on the structures that constituted eyelids, detected types of carbohydrates present in these structures and to compare the results with other studied ruminant. Five pairs of eyelids of healthy local ox used for histological and histochemical study. The present works included four areas upper eyelids, lower eyelids , medial canthus and lateral canthus of each eyelid. Eyelids covered internally by palpebral conjunctiva with goblet cells and covered externally by the skin. There was a significant increase on thickness of epidermis and corneal layers of the lower eyelid areas. Dermis included tarsal plate (large amount of collagen fibers, moderate amount of elastic fibers) with many sections of orbicularis muscle and scattered bundle of Müllerś muscle. Eye lash follicle varies in extension and diameter, arranged in double or triple rows. The follicle of eyelashes devoid of arrector pili muscle. They were many types of glands accompanied eyelash follicle, *glands of Zeis (small sebaceous glands) that have a significant difference especially on lower eyelid areas ,** gland of moll; (apocrine sweat glands) that revealed a significant difference in their dimension between lateral canthus , upper eyelid and lower eyelid areas , these glands began near the basement membrane of the upper eyelids . There are modified multilobulated sebaceous glands; Tarsal glands or meibomian glands; that are well developed on the upper eyelids and near the surface of the skin in the same area. Each tarsal gland empty their secretion onto the surface of the eyelid through a duct lined with typical stratified squamous epithelium, ducts vary in extension, they were deeper in the upper eyelids than other studied areas. Histochemistry study revealed a moderate amount of carboxylated glycoprotein in ground substance of eyelids; eye

lashes follicle, tarsal gland and gland of moll, while goblet cells revealed strong reaction with carboxylated and neutral glycoproteins. Negative reaction for glycogen, sulphated glycoproteins , sulphated or carboxylated glycosaminoglycans in all studied areas.

INTRODUCTION

The eyelids provide a protective cover for the cranial surface of the cornea. Eyelids covered externally by skin and internally by conjunctiva. The presence of skeletal muscle like orbicularis muscle that play a role in eyelid closure , while the eyelids elevated by ; Müllerś muscle; (smooth muscle originates from the internal surface of Levator muscle and end under tarsal plate (1, 2) . Special hairs (eyelashes) are numerous in the upper eyelid of all species except in the cat (3,4) but in the lower eyelid the eyelashes are fewer in number in ruminant and horse absently in cat ,dog and pig . Tactile hairs may be present on or near the eyelids (4) .The most characteristic feature of eyelids is the tarsal glands, which were better developed in the upper eyelid .They are multilobular sebaceous glands with central ducts that are surrounded by a dense collagen plate (tarsal plate). Modified sweat glands (gland of moll) open anterior to tarsal gland and near the eyelashes or into the hair follicle of eyelashes. There is another type of gland present in the dermis of eyelid; modified sebaceous gland; connect with lash follicle (a gland of Zeis) (2 , 4).

The skin of the eyelids of native cattle received no attention. The aim of the present work is to study the skin of eyelids of the local ox with special emphasis on the structures that are described previously , detected types of carbohydrates present in these structures and to compare the results with other studied ruminant.

MATERIAL AND METHODS

Small pieces about 1.5 cm were cut from the healthy skin of upper eyelids , lower eyelids , medial canthus and lateral canthus areas from the right and left eye of five ox (1-2 years old) slaughtered at Mosul abattoir in Iraq Fig (1) . Specimens were promptly fixed in neutral formalin for 24-48 hours , and then transferred to 4% phenol for another 24 hours in order to soften the keratinized material of the skin. Specimens of each region processed routinely for paraffin sectioning , some

section were stained by Delafield ś Hematoxylin and Eosin for morphological study , Verhoeffś elastic stain and Masson Trichrome stain for distinguish between collagen fibers and muscle fibers (5, 6, 7) another section stained by PAS technique for explore of carbohydrate , Best carmine for glycogen granules , Alcian blue pH 2.5 after methlysation and sponification for carboxylated glycoproteins , Alcian blue pH 1 for sulphated glycoproteins , PAS Distase Alcian blue pH2.5 for neutral glycoproteins and toludine blue for carboxylated and sulphated glycosaminoglycans in different structures of eyelids of local ox (8) .

For statistics analysis, One-way analysis of Variance, ANOVA test was used. In cases of appearance of a significant difference ($P \leq 0.05$) between groups Duncan's Multiple Test was used (9) .

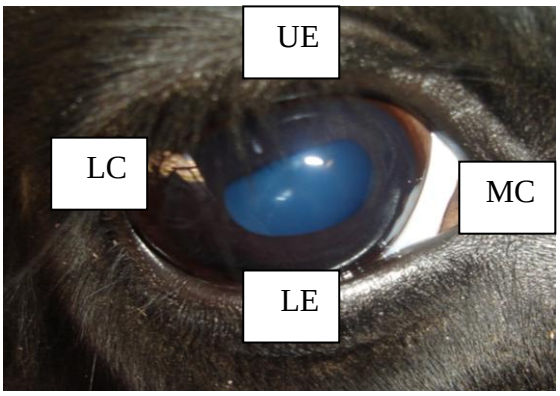

Fig (1): Explain the mode of tacking Specimens of eyelid of native cattle (ox)
UP: upper eyelid; LU: lower eyelid; MC: medial canthus; LC: lateral canthus.

RESULTS

General histology and cytology

The eyelids of all studied areas covered internally by pelpebral conjunctiva consist of a basal layer of cuboidal cells, one to three intermediate layers of polygonal cells , scattered among the epithelial cells goblet cells that are more numerous in the internal

parts of the conjunctiva. The eyelids covered externally with Skin , there was no a significant increase in the thickness of corneal layer of all studied areas , while the thickness of epidermis was significantly increase of the lower eyelids at the level $P \leq 0.05$ compared with others groups (Table1) .

Table (1) Total thickness of corneal layer and epidermis of eye lids of native cattle (ox) Micrometer (µm)

Areas (µm)	**MC**	**LC**	**UE**	**LE**
Thickness of corneal layer	24.97 ± 1.83 a	21.90 ± 0.83 a	23.83 ± 1.29 a	26.92 ± 1.84 a
Thickness of epidermis	111.36 ± 3.46 a	109.43 ± 3.1 a	104 ± 3.69 a	125.68 ± 8.3 b

Different letters (a, b) on horizontal rows refereed to a significant $P \leq 0.05$
MC =Medial Canthus, LC=Lateral Canthus

The epidermis became thinnest in upper eyelids especially at area of prescience of tarsal glands, the melanin granules distributed between the cells of stratum basale and stratum spinosum, these granules distributed within the hair follicles and dermal connective tissues. The basal membrane was less wavy in the upper eyelids , lateral and medial canthus of the eye , while more wavy in the lower eyelids . The dense irregular connective tissue of the dermis containing amounts of elastic fibers and large amount of collagen fibers in the tarsal plate with cross section of skeletal muscle of orbicularis muscle that penetrate the eyelids and scattered bundles of smooth muscle fibers (Müllerś muscles) present. The eye lashes follicle located at marginal part of skin that are much numerous especially in the upper eyelids areas. The eyelashes varying in extension and diameter. The eyelashes presence in double or triple rows in the upper and lower eyelids. The follicle of eye lashes lack of arrector pili muscle (Fig 2, 3).

Gland of Zeis smaller sebaceous gland that do empty their secretion into the follicle lashes (Fig 4) there was a significant difference in the longitudinal section of the gland of Zeis of the lower eyelids compared with other studied areas. These glands near the basement in lateral canthus, medial canthus, upper eyelids respectively while

became more deep in lower eyelid areas, there was no a significant difference of the level of extension of disappearance of gland of Zeis of all studied areas Table (2).

Table (2): Different measurements of Zeis's glands of eyelids of native cattle (ox).
Micrometer (µm)

Areas / Parameters(µm)		MC	LC	UE	LE
Dimension	Cross section	371.95 ± 21.4 a	415.12 ±19.6 a	497.72 ±45.59 a	422.5 ±45.9 a
	Longitudinal section	317.1 ±23 .6 a	274.92 ±16.84 a	282.15 ±18 .41 a	153.29 ±14.83 b
(Appearance)of gland of Zeis's from the basement membrane		244.56 ±15.62 a	210.88 ±8 a	246.69 ±13.43 a	338.13 ±28.66 b
(Disappearance) of gland of Zeis's from the basement membrane		594.26 ±13.79 a	595.12 ±38.93 a	573.53 ±33.24 a	579.48 ±46.96 a

.

Different letters (a , b) on horizontal referred to a significant $P \leq 0.05$.
MC= Medial Canthus, LC =Lateral Canthus
UE= Upper Eyelid, LE=Lower Eyelid

Modified apocrine sweat gland of moll; ciliary gland; that consist of two portions secretary parts and duct system, the height of secretary parts lined with flattened cuboidal to tall columnar depending on the secretary status or activity of the cells. Many of the latter cells formed an apical protrusion indicating an apocrine secretary mechanism toward the glandular lumen. This secretary material appeared as irregular granular mass, which were most common, or spherical drops spread between the granular masses. Myoepthelial cells located basally to the secretary unit (Fig 5). The duct of these gland empty near the hair follicle of the eye lashes. The dimension of these glands being smaller in lower eyelid, upper eyelid areas but became larger in medial canthus, lateral canthus areas progressively. These glands began nearly the basement membrane in lateral eyelids, lateral canthus, medial canthus, upper eyelids areas respectively, while extended far distance in medial canthus, upper eyelids, lateral canthus then lower eyelids (Table 3).

Table (3): Different measurements of glands of Moll eyelids of native cattle (ox).
Micrometer (µm)

Areas / Parameters (µm)		MC	LC	UE	LE
Dimension	Cross section	252.01 ±31.6 a	244.19 ± 16.86 a,c	189.38 ±13.01 b,c	156.4 ±12.25 b
	Longitudinal section	142.25 ±15.99 a	157.89 ±18.37 a	133.38 ±14.03 a	108.05 ±9.61 a
(Appearance) of gland of Moll from the basement membrane		659.63 ±37.01 a	657.86 ±28.6 a	848.71 ±84.52 b	682.76 ±15.1 a
(Disappearance) of gland of Moll from the basement membrane		1006.05 ±36.95 a	941.01 ±28.83 a	963.30 ±77.30 a	832.72 ±16.09 a

.

Different letters (a, b, c) on horizontal referred to a significant $P \leq 0.005$.
MC= Medial Canthus, LC=Lateral Canthus, UE =Upper Eyelid , LE =Lower Eyelid

There are another type of gland ; Tarsal gland; meibomian gland are modified multilobular sebaceous gland embedded in the tarsal plate , with central duct lined with keratinized epithelium extending throughout the meibomian gland , measured about (1650.7± 84µm) in the upper eye lids (1600.8±45 µm) in the lower eye lids (1180.6±27 µm) in the medial canthus while in lateral canthus measured (1186.3±33µm) . They do not communicate with the hair follicle of the eyelashes, these duct varied in extension being shallow in medial canthus, lateral canthus, deep in lower eyelids and very deep in upper eyelids Fig (6). Tarsal gland are better developed in upper eyelid area then other studied areas their dimension 5796 x 2346µm ,the gland is very near the surface of the skin specially in the upper and lower eye lid while, fare distance from the surface of the skin in the medial and lateral canthus of the eye (Table4) , Fig (7).

Table (4): Different measurements of Tarsal glands of eyelids of native cattle (ox).
Micrometer (µm)

Areas / Parameter (mµ)	MC	LC	UE	LE
Dimension: Longitudinal section	2706.17 ±65.24 a	2642.33 ±63.67 a	4986.15 ±83 .87 b	3100.71 ±119.12 c
Cross section	1896.44 ±45.82 a	2060.01 ±58.52 a,c	2757.70 ±26.24 b	2142.46 ±129.58 c
(Appearance) of Tarsal gland from the basement membrane	453.42 ±13.47 a	466.35 ±11.65 a	296.43 ±19.06 b	432.74 ±15.05 a
(Disappearance) of Tarsal gland from the basement membrane	2769.75 ± 65.24 a	2739.4 ±101.20 a	3067.75 ±108.37 a	3462.20 ±159 .73 b

.

Different letters (a, b, c) on horizontal referred to a significant $P \leq 0.05$.
MC = Medial Canthus, LC= Lateral Canthus
UE = Upper Eyelid, LE= Lower Eyelid

Histochemistry

Many structure like Tarsal gland ,gland of Moll, gland of Zeis, ground substance of eye lids and goblet cell in conjunctiva reveled positive reaction for PAS and negative reaction for Best carmine this assure the presence of glycoprotein . Alcian blue technique pH 1 reveled negative reaction in all previously structures so this reveled absent of sulphated glycoprotein. Alcian blue pH 2.5 after methylation and sapofication reveled a moderate reaction in ground substance , basement membrane of cell lined tarsal gland, gland of moll and glassy membrane of eyelashes follicle while reaction was strong in goblet cells conjunctiva this assure the presence different amount of carboxylated glycoprotein. The technique PAS-D-Alcian blue pH 2.5 reveled a moderate reaction in the basal part of some cells of Tarsal gland , duct of the same gland, cortex of hair shaft of eyelashes , while the reaction was strong in goblet cells conjunctiva this reveled the presence of alternative concentration of neutral glycoprotein Fig (8 , 9) . No reaction reveled when using toludine blue so this revealed absence of carboxylated or sulphated glycosaminoglycans.

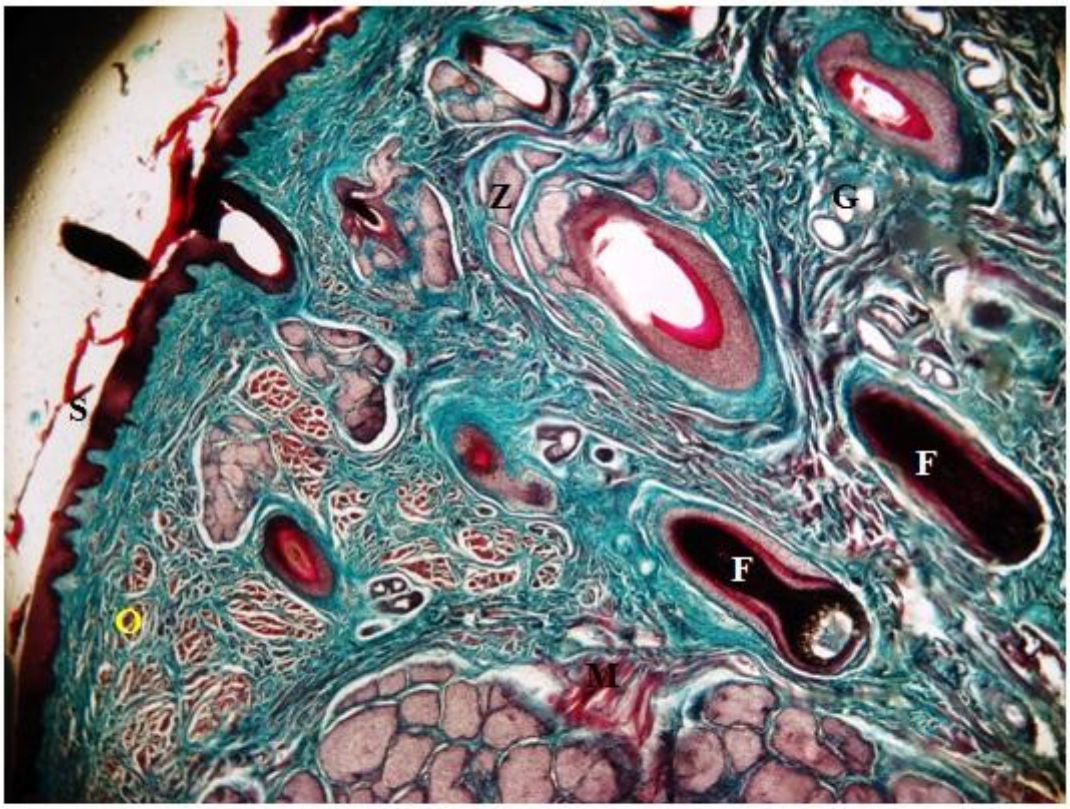

Fig 2 : upper eyelid of native cattle ,external surface (skin)S, eyelashes follicle F, gland of Zeis Z, gland of Moll G, Tarsal gland T, Tarsal plate with Collage fibers P, orbicularis muscle O, Muller muscle M,(Masson Trichrome stain, 35X)

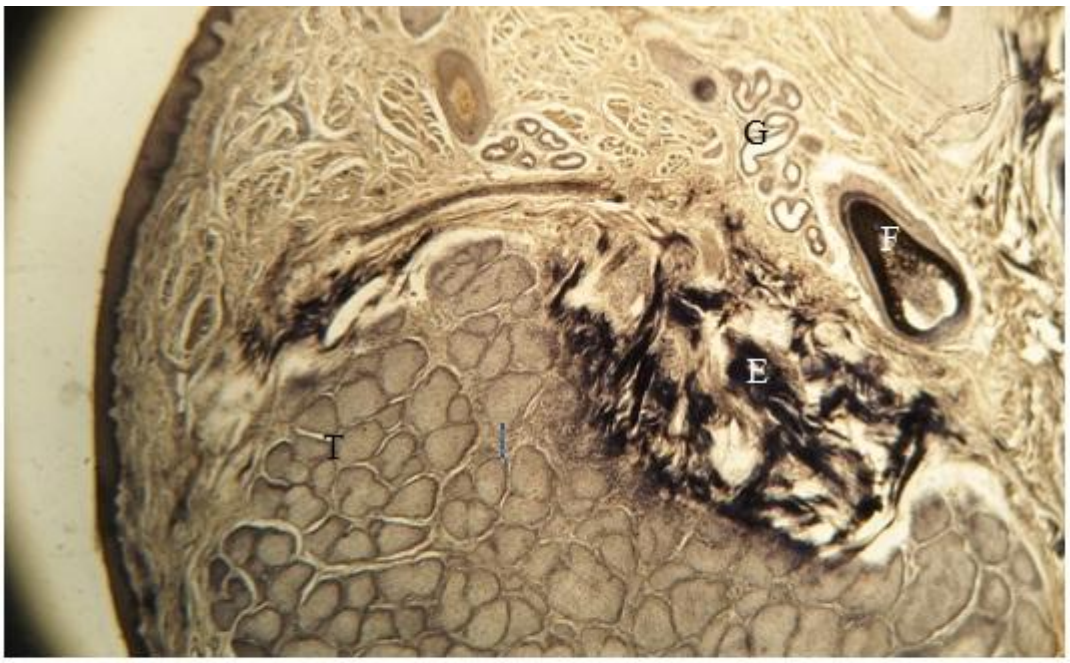

Fig3:Tarsal gland of upper eyelid T, elastic fiber E, gland of Moll G, eyelashes follicle F, verhoeff's stain, 24 X

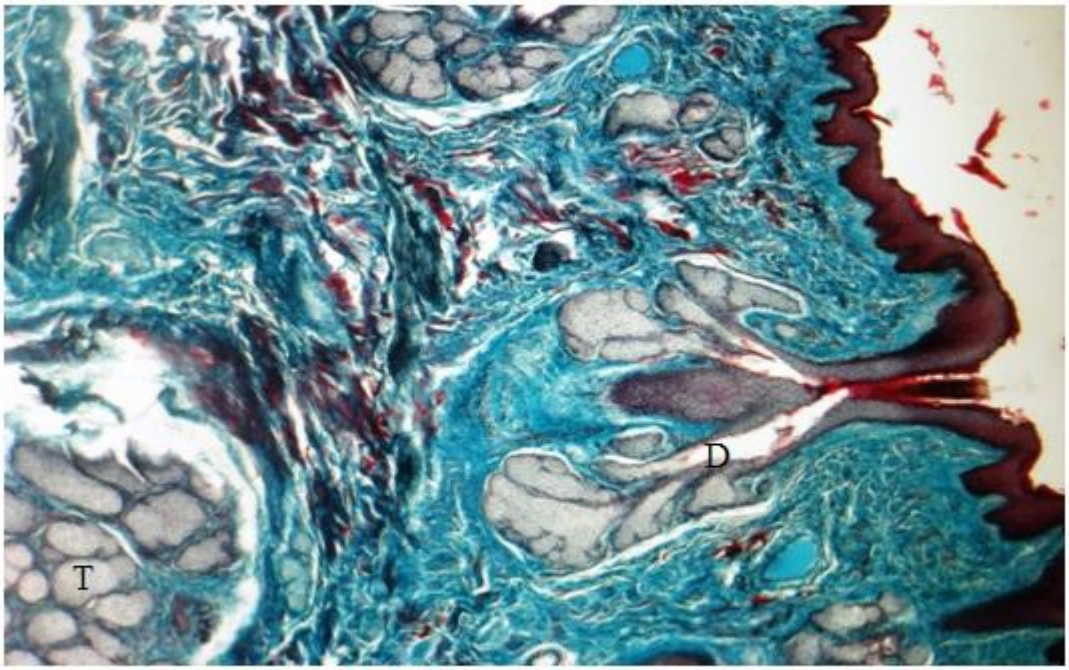

Fig4:lateral canthus of eyelid of native cattle, duct of gland of Zeis open to eyelashes follicle D, Tarsal gland T, ,(Masson Trichrome stain, 27X)

Fig5: A: medial canthus of eyelid of native cattle, activity of gland of Moll G, Hematoxylin and Eosin,115X B: apocrine secretion of gland of Moll a, Myoepthelial cell( ) (Masson Trichrome stain, 370X)

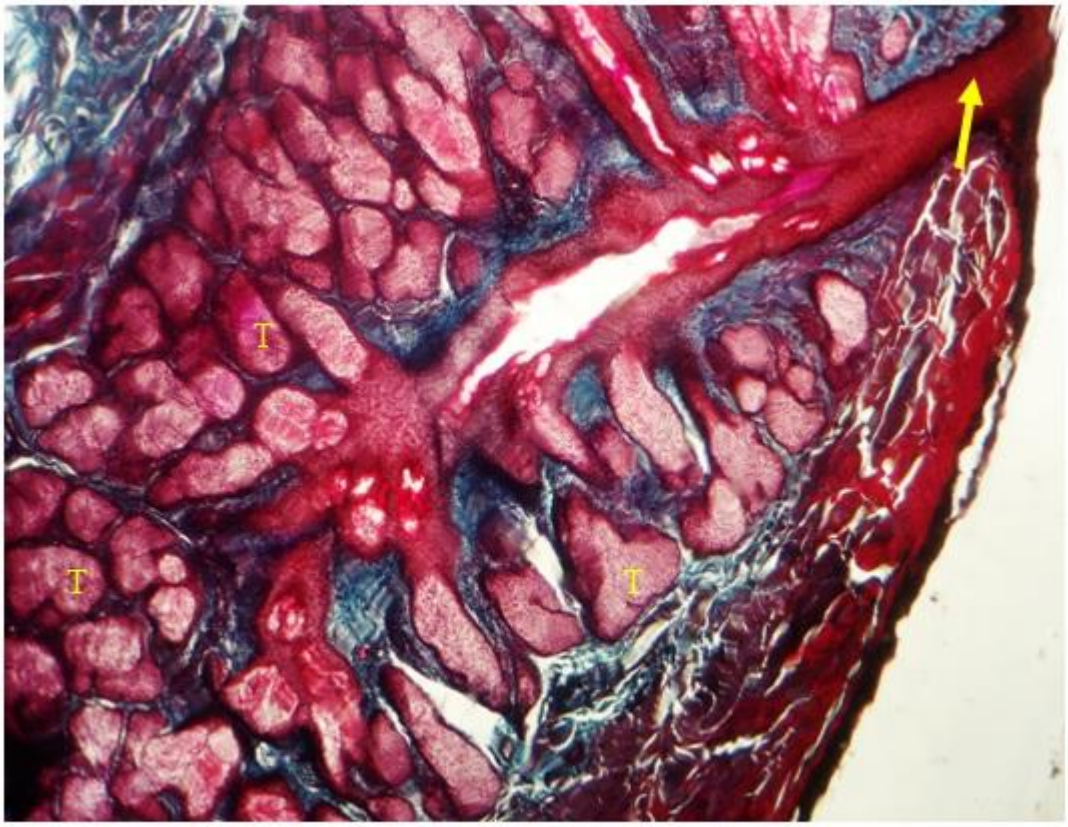

Fig6:lower eyelid of native cattle pilosebaceous duct (⟵) Tarsal gland T , Masson Trichrome stain , 26X

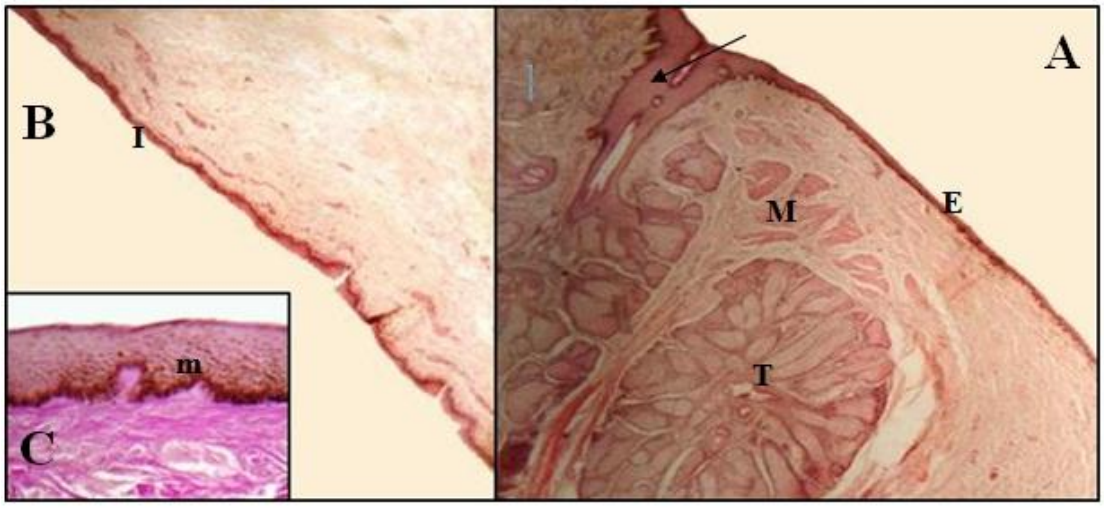

Fig7: lateral canthus of eyelid ,A & B : external or skin surface E, internal or conjunctival surface I, Tarsal gland T, pilosebaceous duct (⟵) ,skeletal muscle M, Hematoxylin and Eosin stain , 26X C: melanin granule within stratum Basle and stratum spinosum m,PAS stain,

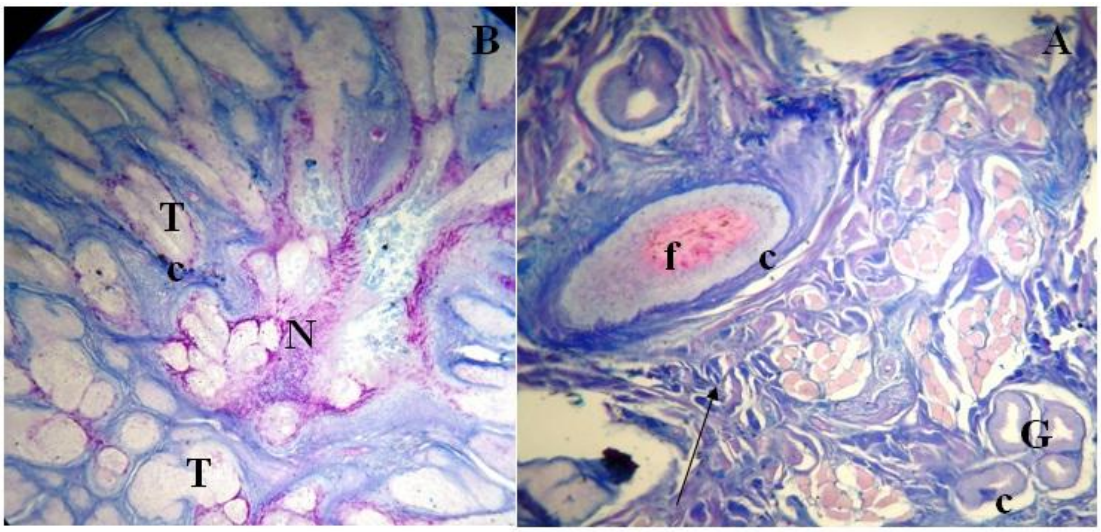

Fig 8 : upper eyelid of native cattle ,A: Carboxylated glycoprotein C in eyelashes follicle F ,gland of Moll G and Tarsal plate (↗) PAS-D-alcian blue ph 2.5 stain, 90X)
B: Carboxylated glycoprotein C and neutral glycoprotein N in Tarsal gland T (PAS-D-alcian blue ph 2.5 stain 60X)

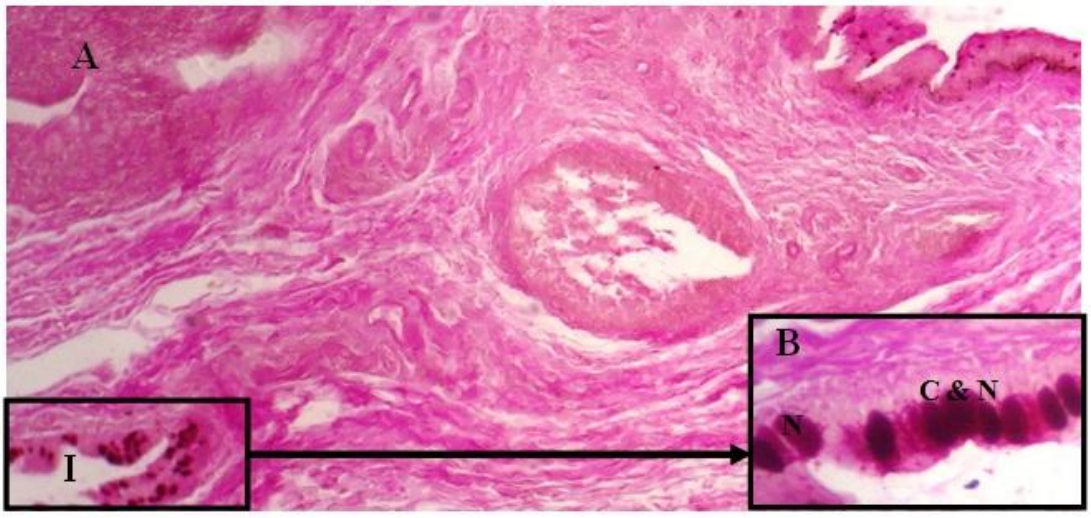

Fig9: lateral canthus of eyelid ,A & B : internal or conjunctival surface I, Carboxylated glycoprotein C and neutral glycoprotein N in goblet cell (PAS-D-alcian blue ph 2.5 stain, 165X)

DISCUSSION

Thickness of epidermis of skin eyelids of ox varied in studied areas , the epidermis of lower eyelids is thick compared with other areas, conclusion more wavy basement membrane , so this increase surface area that assist nutrition , the same results found in the skin of black goat (10) . The presence of melanocyte granule in stratum basale and hair follicle give color to eyelid skin and hair. Prescience orbicularis muscle with Müller muscle within tarsal plate that consist of large amount of collagen fibers and moderate amount of elastic fiber , the same finding appear in the eyelids of

human(11) that recorded these structures support eye lid traction , reinforcement structure and neutral barrier for the upper eyelid from conjunctivitis . (12) Referred that the superior tarsus is a plate of tissue that stiffens the upper eyelid of human gives it support and determines its form. The eyelashes different in extension, its taller in upper eyelids of ox then other area, no investigators reported that, but the prescience of long eyelashes give protection for the eyes. The eyelashes arranged in double or triple rows, the same finding were be reported in mammals (3, 4). The present study revealed that the eyelashes devoid from arrector pili muscle, the same results reported in the eyelids of human (13). Marked differences in cell height and the formation of tall cell protrusion in gland of moll, represent typical feature of an apocrine secretary cell (sweat gland) the same finding was be reported in skin of cow with (14) and human eyelids (15). (15) Referred that the presence of sweat gland with different epithelial types in same section suggest that the glands are either not in the same stage of secretion or that they may not be all activated at the same time . (16) Suggest that gland of moll play an important role in immune defense against bacteria and other pathogens in eyelid shaft and on the other ocular surface. The meibomian gland (Tarsal gland) of the local ox in the upper eyelid are larger and near the epidermis compared with other studied areas. The same result was observed in Philippine buffalo (17) that recorded these glands were better developed in upper eyelids than lower eyelids . Tarsal gland better developed in upper eyelid of ox than other studied area , its diameter more than 5.79 x 2.34 mm , (4) were reported that volume of sebaceous gland (0.2- 2) mm in mammals and these glands have many function , like diminished microbes that enter skin , decrease loss of water , sebum contain vit D so the skin and hair remain smooth and pliable , while (3) reported that the secretion of these glands produced the oily tear film that retarded evaporation . The previous factors assure that tarsal gland in upper eyelids more active in previous function than other studied areas. Gland of Zeis accompanied with eyelashes follicle, which appear and disappear in lateral canthus areas compared with another studied areas, the present of these gland assist the function of tarsal gland. The percentage of goblet cells was high specially in internal part of conjunctiva , (2) was reported that the inner most layer of tear film produced by goblet cells of the conjunctival epithelium and lead to adherence of the precorneal film to the corneal surface . Histochemistry results revealed the presence many types of carbohydrate in the eyelids of ox, like neutral and carboxylated glycoproteins in the goblet cells of

conjunctiva. Carboxylated glycoprotein also found in the ground substance of the eyelids, in the basement membrane of the acini of tarsal gland and in the acini of gland of moll . There were a moderate amount of neutral glycoprotein in some acini of tarsal gland and duct of the gland , while the reaction was weak in apical cell of gland of moll in all studied areas in eyelids of ox . (15) reported when he studied gland of moll in eyelids of human was positive reaction with alcian blue that observed on the secretary epithelium of gland of moll , they suggest considerable capacity of transepithelial ion and fluid transport , while (18) referred that gland of moll of human detected moderate amount of Carboxylated glycoprotein this complex contain material eliminate pathomicroorganis . The histochemical techniques revealed no reaction for glycogen, sulphated glycoprotein, carboxylated and sulphated glycosaminoglycans in all studied area of eyelids of local ox, no reports referred to these results . So the differentiation between the present results with others reports could be due to used of different fixation, staining purity and histochemical technique for manufacturing histological slides .

ACKNOWLEDGMENT

The author thanks College of Veterinary Medicine. University of Mosul , for support this study.

دراسة نسيجية ونسيجية كيميائية للجفون في ذكور الأبقار المحلية

نيزك صبحي احمد

فرع التشريح ، كلية الطب لبيطري ، جامعة الموصل . الموصل ، العراق

الخلاصة

تهدف الدراسة الحالية التعرف على التراكيب المتواجدة في جلد أجفان الأبقار المحلية (الثيران) والكشف عن أنواع الكاربوهيدرات وتوزيعها ضمن التراكيب المختلفة للجفن ومقارنه نتائج الدراسة مع نتائج دراسات مماثله لأنواع أخرى من المجترات .استخدم للدراستين النسيجية والنسيجية الكيميائية خمسه أزواج من أجفان ثيران بالغه سليمة. اخذ من كل جفن أربعه مناطق وهي منطقه الجفن العلوي ، منطقه الجفن السفلي ، منطقه زاوية العين الانسيه ومنطقه زاوية العين الوحشية . تبطن أجفان جميع المناطق المدروسة من الداخل بالملتحمه والتي تضم أعدادا من الخلايا الكاسية بينما يغطيها من الخارج الجلد. لوحظ زيادة معنوية في سمك البشرة والطبقة المتقرنه لمناطق الجفن السفلي . تضمنت أدمة الأجفان الصفيحة الجفنية (نسبة كبيرة من الألياف الغراوية مع بعض الألياف المرنة)، واحتوت الصفيحة مقاطع متعددة من عضلة المحجر الدائرية مع حزم مبعثرة من عضلة مولر. تباينت جريبات شعر الرموش بامتداداتها وأقطارها وانتظمت على شكل صفوف ثنائية أو ثلاثية

وخلت تلك الجريبات من العضلة المقفة للشعر . تواجدت أنواع متعددة من الغدد المرافقة لجريبات شعر الرموش منها غدد زيبس (غدد زهمية صغيرة) ، حيث أظهرت فروقات معنوية بين المناطق المدروسة مقارنة مع مناطق الأجفان السفلية لوحظ تواجد نوع ثان من الغدد (غدد مول) وهي غدد عرقية ذات إفراز قمي والتي أظهرت فروقات معنوية في أبعادها مابين المناطق المدروسة ، فكانت تلك الغدد قريبة من الغشاء القاعدي لمناطق الأجفان العلوية . تواجد نو ع أخر من الغدد وهي الغدد الجفنية (غدد زهمية مفصصة محوره) واضحة المعالم وقريبة من سطح الجلد خاصة في مناطق الأجفان العلوية، تفرغ محتوياتها على سطح الجلد من خلال قناة مبطنة بخلايا ظهارية حرشفية مطبقة غير متقرنه ، تباينت في امتداداتها اعتمادا على تطور الغدد الجفنية .

أظهرت الدراسة النسيجية الكيميائية تواجد كميات متوسطة من البروتينات السكرية الكاربوكسيلية في المادة الأرضية للأجفان وجريبات الرموش وفي الغدد الجفنية وغدد مول ، بينما أظهرت الخلايا الكاسية احتوائه ا تراكيز كبيرة من البروتينات السكرية الكاربوكسيلية والمتعادلة. لم يلاحظ تواجد الكلايكوجين والبروتينات السكرية الكبريتاتيتية والسكريدات السكرية بنوعيها في جميع المناطق المدروسة.

REFERENCES

1. Henrikson RC, Key GT, Mazurkiewicz J 1997. Histology.1st ed. Baltimore: Williams & Wilkins Waverly Co; 422 – 423.
2. Samuelson DA . , 2007. Textbook of Veterinary Histology. 9th ed. China: Saunders Elsevier Inc; 511-512
3. Bank WJ. 1986. Applied veterinary histology. 2nd ed. Baltimore: Williams & Wilkins,; 543 -544..
4. Dellman HD, Brown EM. 1976. Text of Veterinary Histology .2nd; ed. Philadelphia: Lea & Febiger,; 475 - 476.
5. Luna LG. 1968. Manual of histological staining method 3rd ed. New York: Mac Grew Hill Book Co,; 164-173.
6. Drury RA, Wallington EA, Cameron SR, 1980. Carlton's histological technique .5th ed. London: Oxford University press; 312 -313.
7. Humason L. 1972. Animal tissue technique. 3rd ed. San Francisco: W. H. freeman Co,; 426 - 427.
8. Kiernan JA. 2010. Histological and histochemical methods theory and practice, 4th ed. London: Scion Publishing Ltd ; 282- 286.
9. Petrie A, Watson P. 2006. Statistics for veterinary and animal science. 2nd ed. Oxford: Blackwell Publishing Ltd,; 99.

10. Ghada AS. 2007. Comparative Histological, Morphometrical & Topographical study of the skin of local males black goat. [Master thesis] Mosul: Mosul University,; 3-10

11. Folia O. 2005. Fibrous connective tissue between Muller's muscle, the pelpebral conjunctiva reinforcement structure & a neutral barrier for the upper eye lid. Anat jpn ; 82:79-82.

12. Milz S, Jorge N, Ichiro H, Reinhardt P, and Michael B. 2006. An immunohistochemical study of the extra cellular matrix of the tarsal plate in the upper eyelid in human beings. J Anat; 37–45.

13. Gartner LP, Hiatt JL. 2007. Color textbook of histology. 3rd ed. Philadelphia: Saunders an imprint of Elsevier Inc,.

14. Amakiri SF Adepoju JJ. 1979. Changes in sweat glands morphology in cattle before & during heat stimulation. Acta Anat; 105:140-150.

15. Stoeckelhuber M, Stoeckelhuber BM , Welsh U . 2003. Human of Moll: Histochemical and Ultrastructure characterization of the gland of Moll in the human eyelid. J of Investigative dermatology; 121: 28-36.

16. Fukuo Y, Takeda N, Hirata H, Kato T, Kadoi C, Katayama T, and Kubota Y. 1994. Immunohistological studies of an cocytoma. Ophthalmologica; 208: 267-269.

17. Maala CP, DeOcampo G, Veridiane AA. 2009. The anatomy of eyelid of the Philippine Buffalo (Bulbus Bulbalis). Phillip Vet J; 46:73-81.

18. Stoeckelhuber M, Sliwa A, Welsch U. 2000. Histo – physiology of the scent – marking glands of the penile pad, anal pouch, and the forefoot in the aardwolf (*Proteles cristatus*). *Anat Rec*; 259: 312–326.