COMPARATIVE HISTOLOGICAL STUDY OF THE INTEGUMENT IN BUFFALO AND COW

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ABSTRACT

This study was done to make a histological comparison between the epidermis of skin in buffaloes and cows. The samples were taken from six body areas (muzzle, neck, thorax, dorsum, abdomen and perineum). The present results revealed that, the skin in buffaloes was thicker and stronger than that of the cow in all regions of the body. Generally, the skin of buffalo and cow consist of two main layers, epidermis and dermis. The epidermis of the skin in both animals consists of four clear and prominent layers except the stratum lucidum which was not present. In buffalo, all layers of the epidermis presented high number of melanocytes in comparison with that of cow which showed few numbers in all studied regions except the muzzle region in which the number increased. The epidermis of the buffalo contained more number of epidermal ridges in comparison with that of the cow. The dermis of the skin in both animals subdivided into indistincted layers, the upper papillary and the inner reticular layers. There is no sharp demarcation between the two layers of the dermis.

Key words: skin, epidermis, corneum, dermis, basement membrane, Buffalo, Cow.

INTRODUCTION

The integument of an animal is not only the protective cover of its body, but also it acts as a medium through which the animal is in continuous contact with its surroundings that mean sensory organ. The role played by the skin in the adaptation of the animal to its environment is manifested through various modifications of this important tissue and its accessory structures in various species living in different habitat (Mahdi, 1979). The histological structure of the skin of both buffaloes and cows consists of two main layers; the epidermis was the outermost layer of the skin. The thickness of the epidermis varies with its location; in regions where there is a heavy protective coat of hair, the epidermis is thin but in non-hairy regions, the epidermis is thicker. The epidermis layer is devoid of blood supply (Di *et al*., 2001). The dermis, is the connective tissue layer that supports the epidermis.

MATERIALS AND METHODS

Samples Collection and Tissue processing

Skin specimens were collected immediately after slaughtering from adult males of buffaloes and cows from the local Shu'lla abattoir. The specimens were taken from six body areas (muzzle, neck, thorax, dorsum, abdomen and perineum). Samples were fixed in 10% neutral buffered formalin for 24 hours. Specimens were dehydrated using upgrading of alcohol 50%, 60%, 70%, 80%, 90% and 100%. Xylen was used as clearing reagent. Paraffin was used for infiltration and embedding. Paraffin blocks were cut about (5-7 µm), and then stained by many kinds of stains, Harris hematoxylin and eosin stain for appearance of the general structure of the tissue, Periodic-acid Schiff (PAS) reagents for coloring the basement membrane (Luna, 1968) and Van Gieson stain for appearance of the collagen fibers. Oculometer was used for measurement thickness of corneum, folded epidermis, smooth epidermis, total epidermis (smooth and folded), diameters of melanocytes, thickness of basement membrane, total dermis.

RESULTS AND DISCUSSION

Histological Study

The basic structure of skin ruminants (buffaloes and cows) was the same as that of mammals. A number of variations must be considered. The present study revealed that the skin of both buffaloes and cows consists of two main layers namely, the epidermis and dermis in addition to the skin appendages i.e. hair follicles, arrector pili muscle and the skin glands (sebaceous and sweat glands) (Figure 1 and 2).

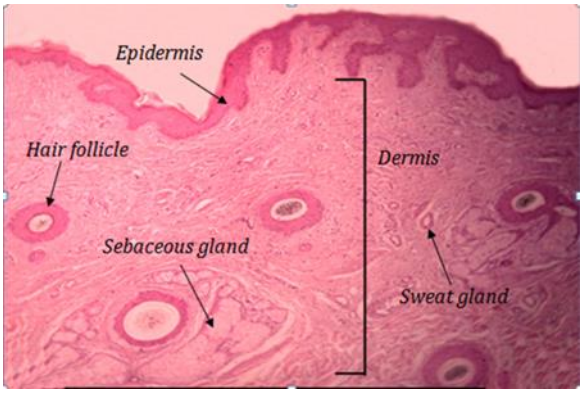

Figure 1. Showed genereal structure of the skin in the buffalo (⟶) (H and E, X40)

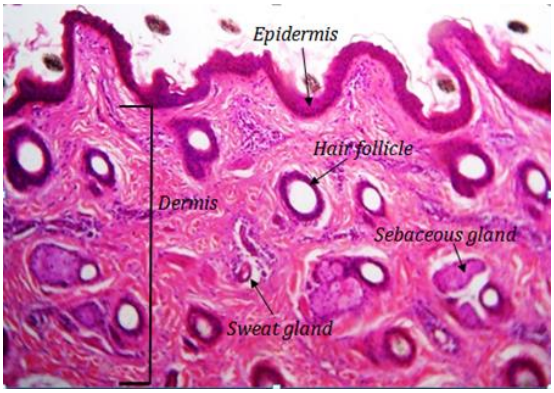

Figure 2. Showed genereal structure of the skin in the cow (⟶) (H and E, X40)

EPIDERMIS

The epidermis in both buffaloes and cows formed the external layer of the skin which consist of stratified squamous keratinized epithelium. It consist of four layers from bottom to top was stratum basale, stratum spinosum, stratum granulosum and stratum corneum, this was similar to the results of (Abdul Raheem *et al*., 2006 ; Abbasi, 2008). Stratum lucidum was not observed in both buffaloes and cows, this finding was in agreement with the findings of (Hafez *et al*, 1955). The deepest layer, the stratum basale, was a single layer of crowded basophilic cuboidal to columnar cells, resting on a basement membrane. This layer was characterized by high mitotic activity and was responsible for renewal of epidermis, in addition to the initial portion of stratum spinosum. Both the stratum basale and the initial portion of stratum spinosum called stratum germinativum. The cells of this layer possessed darkly stained nuclei, which may be covered by high concentration of melanocytes granules in most studied regions in buffaloes and in the muzzle region of cow (Figure 3 and 4).

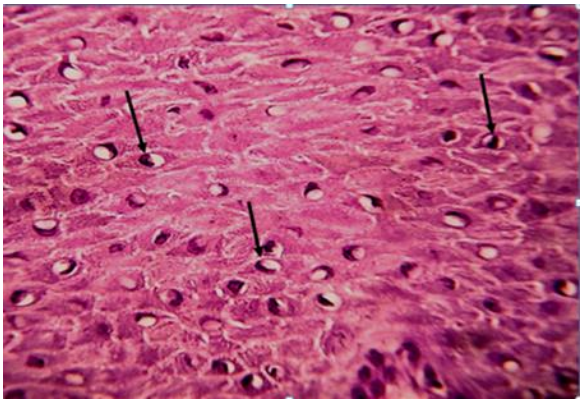

Figure 3. Showed melanocytes in muzzle of the buffalo (⟶) (H and E, X400)

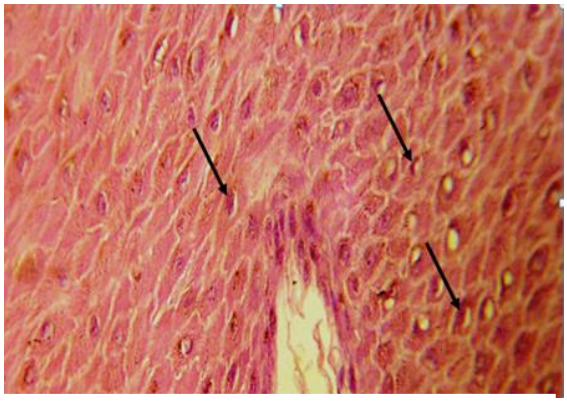

Figure 4. Showed melanocytes in muzzleof the cow (⟶) (H and E, X400).

The diameter of melanocytes in buffalo ranges between (10-10.5 µm, ±0.2) and is larger than that of the cow (8-8.5 µm, ±0.1). The increase in concentration of melanin pigment in buffaloes leads to increase absorption of sun light which was the main reason that the buffaloes was regarded as a less tolerant animals to the heat (poor thermo regulator animal) in comparison to the cow, this was in accordance with the finding of Ali (2008) in buffalo, and Ali *et al*., (2002) in Awassi sheep, who stated that the exposure to ultraviolet light particularly the sun's rays stimulates a more rapid rate of melanin production and thus acts as a protection against ultraviolet radiation effects because our explanation declaired that the dark color of the buffalo skin absorbs the sun light. The cell bodies of

melanocytes also appeared in the supra basale layers of epidermis showing upward migration to the upper most layers. The stratum spinosum formed several cells thickness. The cells were larger and lighter than those of the stratum basale. This layer consisted of polyhedral cells containing spherical or oval nuclei toward the upper part of this layer. They exhibit numerous cytoplasmic processes or spines, which gives this layer its name. Melanin granules were showed in their cytoplasm, this was similar to the observation of Banks (1993) in sheep. The stratum spinosum had many cell layers in epidermis was varied with the epidermal thickness and species of the animal. The present study found that the number of cell layers in buffaloes ranged between 6-8 cell layers in the dorsum, 16-19 cell layers in the muzzle and in other regions it ranged between 10-12 cell layers, while in cows the number of cell layers ranged between 3-5 cell layers in the dorsum, 14-17 cell layers in the muzzle and in other regions ranged between 7-9 cell layers, this was in agreement with the findings of Mulling (2000) in cows whereas in variance with the result of Sultan (2006) in local black goat, who referred that the number of cell layers of stratum spinosum ranged between 2-4 cell layers in most of skin regions while the number of cell layers ranged between 10-16 cell layers in the muzzle. In buffaloes, the cells of the stratum spinosum firmly bound together by the cytoplasmic spines (desmosomes) giving a spine- studded appearance (Fig. 5). The spine cellular processes that extend to every adjacent cell that strongly bind the cells of this layer together and provide the principle morphological feature of this region of the epidermis. Each process ends as a desmosome, making this region of the skin highly resistance to the mechanical effects of stretching and pressing and resist abrasion, this was similar to results of Junqueria and Carneiro (2005). The inter-cellular spaces between these processes may provide conduits for substances to be secreted eventually by the keratinocytes as they approach full development while in cow these cytoplasmic spines were smaller and not obvious (Fig. 6).

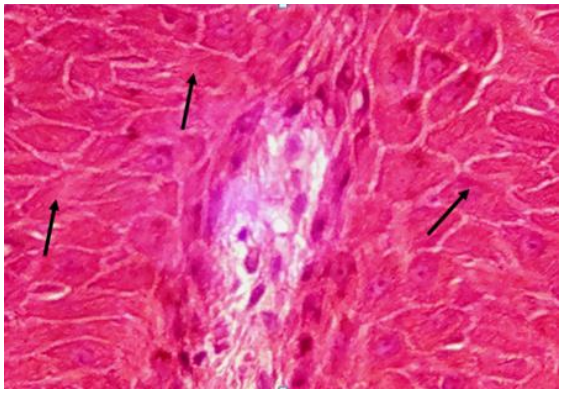

Figure 5. Showed stratum spinosum in the buffalo (⟶) (H and E, X400)

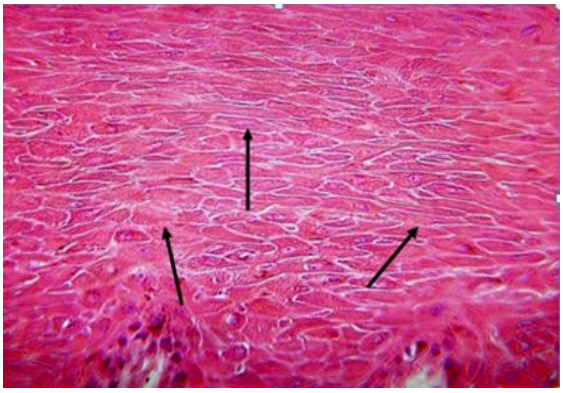

Figure 6. Showed stratum spinosum in the cow (⟶) (H and E, X400).

The cells of stratum granulosum were flattened, fusiform and contain basophilic keratohyalin granules, the nuclei of this layer showed progressive signs of degeneration towards the stratum corneum, this was similar to the results of Bagi and Vtas (1983) in buffaloes. Stratum granulosum consist of 3-5 cell layers in buffaloes and 1-3 cell layers in cows, this was in variance with the findings of Al-Bideri (1995) in sheep, who stated that the stratum granulosum consist of one row of cells in the ear epidermis. Stratum granulosum was present in all body regions examined in buffalo and cow, this layer was also observed by Ali *et al.*, (2003) in local goat and in variance with Hafez *et al*., (1955) who stated that the stratum granulosum appeared faintly in some skin regions in buffalo and was absent in cattle. The last layer, the upper most stratum corneum consisted of dead, horny, scale–like cells, the cytoplasm had been replaced by a protein keratin, this layer was represented by strips of highly acidophilic keratin which breaks into weakly acidophilic filaments towards the free surface, and this was in agreement with the results of Onet and Kelleher (2005) in cow. The principle difference between the epidermis of thick and thin skin was as follow, in all regions of the body, the thickness of the stratum corneum in buffalo was more than in the cow (Table 1).

Table 1. The thickness of corneum in buffaloes and cows (micrometer)

Region	Buffaloes Mean ± SE μm	Cows Mean ± SE μm
Muzzle*	124.4±1.7	81.6±1.2
Neck*	20.5±0.5	9.9±0.1
Chest*	55.5±0.9	25.5±0.9
Abdomen*	22.47±0.3	10.3±0.2
Perineum*	25.2±0.9	12.5±0.2
Dorsum*	18.9±0.2	8.1±0.02

* - Represent significant differences at (P<0.05) between horizontal rows.

Stratum corneum in buffalo was dense and compact especially in the muzzle (Fig. 7) in comparison with the cow where it was loose and separate (Fig. 8).

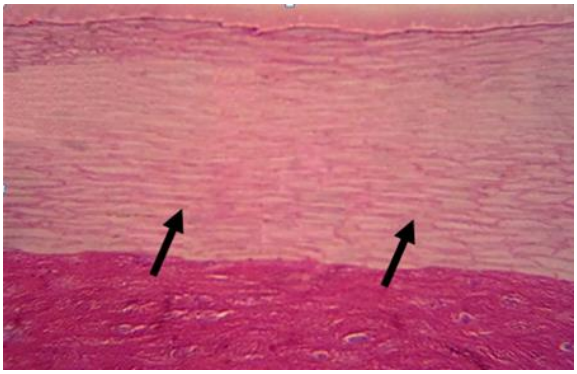

Figure 7. Showed stratum corneum in buffalo was dense and compact (⟶) (H and E, X400)

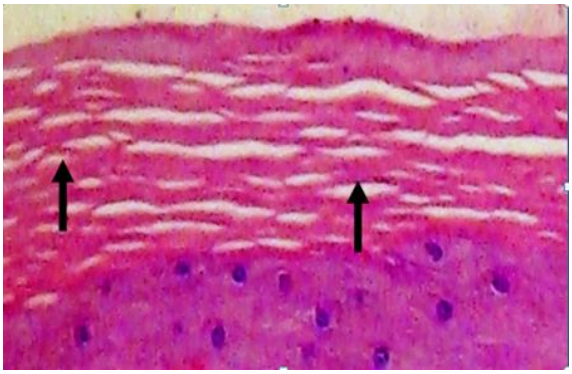

Figure 8. Showed stratum corneum in cow was loosely attach (⟶) (H and E, X400)

The current study declared that the highest thickness of the stratum corneum in buffalo and cow was in the muzzle region while the lowest thickness was in the dorsum; the chest, perineum, abdomen and neck comes in between these two readings because on the muzzle, where there was considerable abrasive action, the stratum corneum was thickened. The present study found that the thickness of epidermis (smooth and folded) varies in the different body regions (Table 2).

Table 2. The thickness of (folded and smooth) epidermis in buffaloes and cows (micrometer)

Areas	Folded epidermis		Smooth epidermis	
	Buffaloes Mean ± SE µm	Cows Mean ± SE µm	Buffaloes Mean ± SE µm	Cows Mean ± SE µm
Muzzle*	1123.3±5.7	1055.4±5.1	746.52±3.1	726±2.4
Neck*	187.04±1.4	146.4±0.7	65.3±0.3	51.42±0.4
Chest*	227.5±1.5	193.2±0.2	105.9±0.9	78.97±0.5
Abdomen*	193.7±0.7	159.6±0.6	71.9±0.9	60.7±0.9
Perineum*	198.5±1.1	170.5±0.3	85.7±0.8	63.4±0.4
Dorsum*	137.3±1.2	103.5±0.4	54.1±0.6	41.4±0.6

* - Represent significant differences at (P<0.05) between horizontal rows.

Also, the present study revealed that the epidermis and dermis in buffaloes was thicker than that of the cows in all regions of the body (Table 3).

Table 3. The thickness of total (epidermis and dermis) in buffaloes and cows (micrometer)

Areas	Total Epidermis		Total Dermis	
	Buffaloes Mean ± SE µm	Cows Mean ± SE µm	Buffaloes Mean ± SE µm	Cows Mean ± SE µm
Muzzle*	934.91±2.03	890.9±3.3	7922.5±1.8	7785.3±2.1
Neck*	126.17±0.8	99.4±0.5	8195.5±0.7	8023.9±2.7
Chest*	166.63±1.1	136.08±0.4	6846.3±2.6	6521.8±2.8
Abdomen*	132.8±0.9	110.15±0.7	6582.2±3.4	6371.8±1.9
Perineum*	142.1±0.9	116.95±0.4	7843.4±2.03	7459.3±2.1
Dorsum*	95.7±0.8	72.45±0.5	8168.3±4.3	7914.6±2

* Represent significant differences at (P<0.05) between horizontal rows.

The muzzle region was the thickest in comparison with other body regions in spite of the presence of a few amount of hair follicles, this was due to the presence of interpapillary pegs which extend deeply into the dermis. Table 4 showed that the epidermal ridges has direct proportion with the thickness of the skin as it was increased in the thick skin and decreased in the thin skin so that the number of the epidermal ridges in buffalo was more than the cow. The table 4 also showed that the muzzle and abdomenal regions were registered the highest results as the muzzle region undergoes abrasive action and the abdomen holds the weight and pressure of the bowels, this was similar to the results of Abdul Raheem and Al-Hety (1999) in one humped camel.

Table 4. The density of the epidermal ridges and hair follicles/mm2 in buffaloes and cows (micrometer)

Areas	Density of epidermal ridges		Density of hair follicles	
	Buffaloes Mean ± SE µm	Cows Mean ± SE µm	Buffaloes Mean ± SE µm	Cows Mean ± SE µm
Muzzle*	11.6±0.2	9.2±0.1	0.33±0.1	0.44±0.03
Neck*	8.4±0.1	5.7±0.2	4.3±0.2	13.5±0.2
Chest*	9.4±0.1	6.3±0.2	2.6±0.1	9.3±0.2
Abdomen*	10.2±0.1	7.8±0.1	3.8±0.1	12.6±0.1
Perineum*	6.5±0.1	4.3±0.1	3.2±0.1	11.1±0.3
Dorsum*	9.8±0.1	7.2±0.2	4.8±0.2	14.4±0.4

* -Represent significant differences at (P<0.05) between horizontal rows.

The present results revealed that the dermis and epidermis were attached by the underlying basement membrane which adapt to the interdigitations between the two layers and appear as smooth in skin protected by dense coat of hair (thin

skin) and undulant in areas subjected to mechanical stress (thick skin), this was similar with the findings of Dellmann and Brown (1976) in different animals; Al-Bideri (2003) in adult Iraqi mammals, who said that according to the thickness of the skin there was two shapes of basement membrane (Figures 9 and 10).

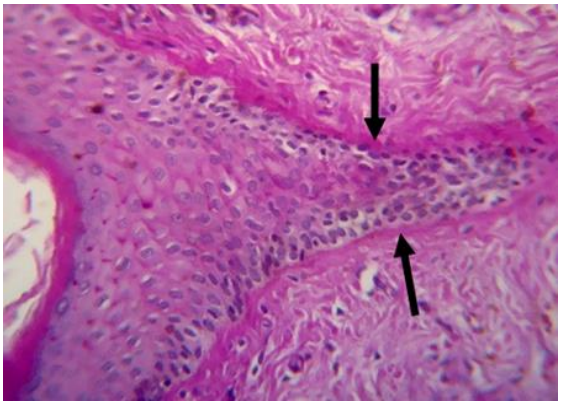

Figure 9. Showed basement membrane in abdominal region of the buffalo (⟶) (PAS, X400)

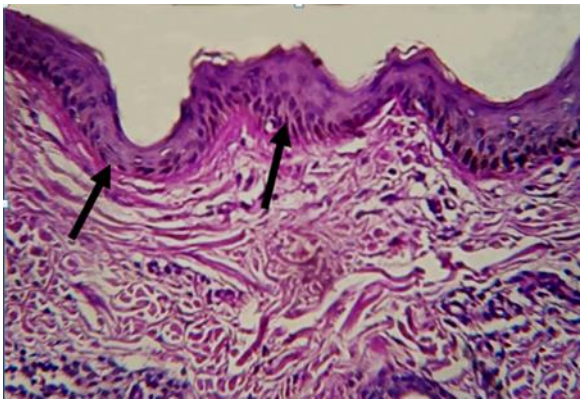

Figure 10. Showed basement membrane in abdominal region of the cow (⟶) (PAS, X400)

This study firstly stated that the basement membrane in buffaloes was thicker than in the cows, the thickness of the basement membrane in buffaloes ranges between (3.3-5µm, ±0.3SE) and in the cow range between 2-2.5 µm, ±0.1SE).

DERMIS

Beneath the epidermis, the dermis was a layer of connective tissue was separated from the epidermis by a basement membrane. The surface of the dermis was very irregular and has many projections (dermal papillae) that interdigite with the projections (epidermal pegs or ridges) of the epidermis. In this study, the dermis was composed of connective tissue containing collagen, elastic and reticular fibers, hair follicles, sebaceous glands, sweat glands, arrector pili muscle, blood vessels. The dermis divided into two layers, the superficial papillary and the deep reticular layers. There is no sharp demarcation between the two layers of the dermis, this was similar to the findings of Panchal *et al*., (1999) in indian buffalo. The papillary layer consisted of dense connective tissue and fine, delicately arranged collagenous fibers with elastic fibers. The cells of the dermis were similar to those found within other connective tissue of the body. The fibroblast, plasma cells, mast cells and macrophage are predominate, leukocytes may be found, this was in agreement with the findings of Ownby (2004) in ruminant skin. Reticular layer was the deeper and thicker layer of the dermis and composed of coarse bundles of collagenous fibers which

usually parallel to the skin surface. Elastic fibers were thick and less abundant in the deeper part of the skin, this layer have fewer connective tissue cells than does the papillary layer ,this was similar to the finding of Sultan (2006) in local black goat. The thickness of the dermis varies between buffaloes and cows. The dermis was thick in both buffaloes and cows. Table 3 showed that the thickness of the dermis in buffaloes was more than in the cows. The thickest dermal region in both of the two animals was in the neck and dorsum, the thinnest dermis was in the abdomen and chest; Muzzle and perineum come in between. The thickest dermis was the neck and dorsum due to its contain large number of connective tissue and highly vascularized, this was in accordance with the findings of Abdul Raheem *et al*., (2006) in cow. Generally, the current results demonstrated that in buffaloes there were numerous wide short bands of collagen fibers oriented in different directions (Figure 11), whereas in cows, the collagen fibers were less abundant in comparison with buffaloes (Figure 12).

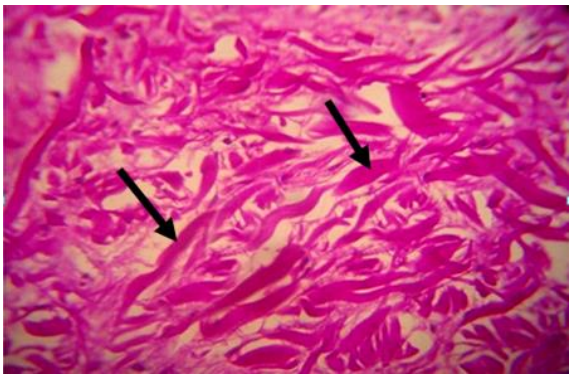

Figure 11. Showed the thicker bands of collagen fibers in the buffalo (⟶) (Van Gisien, X400)

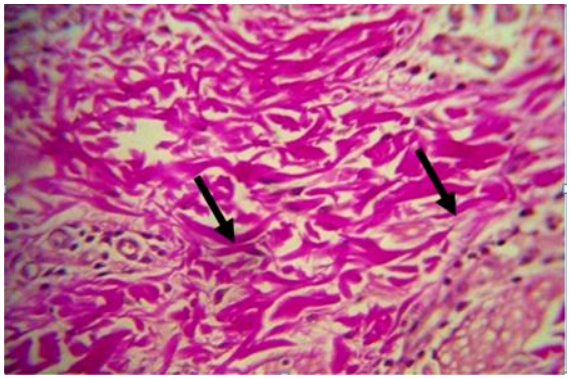

Figure 12. Showed the thinner collagen fibers in the cow(⟶) (Van Gisien, X400)

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دراسة نسجية مقارنة لجلد الجاموس والأبقار

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المستخلص

أجريت هذه الدراسة للمقارنة بين جلود الأبقار والجاموس من الناحية النسجية. جمعت ست عينات من مناطق الخطم، والرقبة، والصدر، والظهر، والبطن، والعجانية. أظهرت اهم النتائج ان سمك الجلد في الجاموس يكون اسمك واقوى من الأبقار ولجميع مناطق الجسم التي خضعت للدراسة. بصورة عامة جلد الجاموس والأبقار يتكون من طبقتين رئيسيتين هما البشرة والأدمة. تتكون بشرة الجلد في كلا الحيوانيين من اربع طبقات كانت جميعها واضحة ومميزة، الطبقة الصافية (Stratum lucidum) لم تلاحظ في نتائجنا. احتوت جميع طبقات البشرة (Epidermis) في الجاموس على أعداد كبيرة من خلايا الميلانين (Melanocytes) مقارنة بالأبقار التي ظهرت بأعداد قليلة في جميع المناطق التي درست باستثناء منطقة الخطم (Muzzle) إذ ازدادت اعدادها. احتوت طبقة البشرة في الجاموس على أعداد أكثر من البروزات البشروية (Epidermal ridges) مقارنة بالأبقار. انقسمت طبقة الأدمة (Dermis) في كلا الحيوانيين إلى جزئيين رئيسين هما، الطبقة الخارجية الحليمية والطبقة الداخلية الشبكية وكانت الحدود الفاصلة بينهما غير واضحة.

الكلمات المفتاحية: الجلد، البشرة، الطبقة المتقرنة، الأدمة، الغشاء القاعدي، الجاموس، الابقار.