Histological and histochemical comparative study of the skin of three different locations between gazelle and camel

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Abstract

This study was designed to describe and compare the histological features of camel (*Camelus dromedarius*) and gazelle *(Gazella subgutturosa*) skin. The study was carried out on skin samples collected from healthy adult local camels and gazelle (15 from each) immediately after slaughtering. Five specimens were taken from different skin regions upper lips, perineal, and thigh) fixed in 10% neutral buffered formalin for 72 hours. The sections were processed using routine histological technique and stained by Harris Hematoxylin and Eosin stain (H&E), Periodic Acid Schiff Reagent (PAS), Alcian Blue (AB), Combined Periodic Acid Schiff and Alcian Blue (AB-PAS), and Masson's trichrome stains. Skin sections revealed an epidermis thin outer layer composed of four strata: basal, spinosum, granulosum, and corneum. The mean total thickness of the epidermis in camel skin was greater than that in gazelle skin; however, the upper lips kin of gazelle and camel has the maximum thickness of the epidermis. The dermis consists of two layers; papillary and reticular, and contains primary and secondary hair follicles, sebaceous, and sweat glands. The dermis of the gazelle revealed a high thickness of papillary and reticular layers in comparison with the skin of the camel. The sebaceous glands were simple alveolar that present in small size of skin of each camel and gazelle. The tubule-coiled sweat glands were numerous and spread in the dermis. In conclusion, this study demonstrated the absence of differences in the general architectures skin in camel and gazelle except for variations in the thickness of skin strata.

Key words: Skin layers, Camel, Gazelle, Histological, Histochemical

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Introduction

Camels and gazelle live in a desert environment and are important source of human diet and milk (Kingdon, 1997; Ouajd and Kamel, 2009). The camel's body is covered with fawn-colored, sparse, paler hair on each of the abdomen and limbs, but very dense and darker hair on the neck and back (Ouajd and Kamel, 2009). In gazelles, the hairs are comparatively thick and

single, but occasionally, two-three hairs may emerge from a single follicle as short and long hair. During the cold weather, gazelles raise their hairs to form an insulator layer between the body and the environment. The short, stout, erect hairs are responsible for forming this insulation barrier, and the long hairs are for a protective outer cover (Al-Abbas *et al*., 1999). The skin or cutis covers the entire outer surface of the animal body (Dyce *et al*., 2010) and, in general, is considered the body's largest organ, making up 16 % of the body weight. The skin has multifunction like protection, sensation, metabolism, and thermoregulation. The skin consists of epidermis, dermis, and skin appendages (Al-Abbas *et al.,* 1999). Ali (2008) mentioned that the skin consists of two layers that differ in function, appearance, and embryological origin. The outer layer (epidermis) formed by epithelium, and it is of ectodermal origin. The underlying thicker layer, the dermis consists of dense irregular connective tissue and develops from the mesoderm (Aktaş and Daglıoglu, 2009; Macneill*et al.,* 2005). Beneath the two layers, the subcutaneous layer of the loose connective tissue, the hypodermis which binds the skin to underlying structures (Moradi and Sheibani, 2000). Hair, nails, sweat, and sebaceous glands are of epithelial origin and called the skin appendages. The skin and its appendages are called the integumentary system (Dyce *et al*., 2010). The epidermis of mammals consists of stratified squamous epithelium. It is regenerated continuously from the basal stratum at the basement membrane toward the skin surface with or without keratinized cells, melanocytes, Langerhans, and Merkel cells (Macneill*et al.,* 2005). The epidermis may be thick or thin (Moradi and Sheibani, 2000). The thin epidermis covers the largest part of the body, but the thick epidermis is found in animals' soles, and tails (Abdul Raheem and AL-Hety, 1997). The dermis is thicker than the epidermis and consists of connective tissue, hair follicles, sebaceous glands, and sweat glands (Mobini, 2012; Shabir *et al.,* 2011). The skin an important role in the body's temperature regulation (Abdul Raheem and AL- Hety, 1999). The hypodermis acts as an energy store and thermally insulating layer, protecting the body from external influence (Steinhagem *et al.,* 1986). Regulation of the body water content is one of the major problems of desert life. However, the animal skin plays an important role in regulating of body temperature through radiation, convection, and conduction and it influence in water uptake and the prevention of water loss (Pourlis and Chritoulpoulos, 2008; Nunes *et al.,* 2010).

Materials and method

Fresh skin specimens from healthy Arabian camel (*Camelus dromedarius*) and black-tailed gazelle *(Gazella subgutturosa*), 15 from each, were collected immediately after slaughtering at AL Muthanna abattoir, 388 kilometers south of Baghdad. The hair of the skin was removed by the depilatory ointment for (7-10) minutes. The cleaned skin specimens were fixed in 10% neutral buffered formalin for 72 hours, processed by the routine histological technique, embedded in paraffin wax (58-60 ^{0}C), and sectioned at 5-7µm. The skin sections were stained using different stains:

1. Hematoxylin and Eosin (H&E) to demonstrate the histological architectures of the skin.

2. Alcian blue (AB) for the weak acidic polysaccharides pH=2.5).

3. Periodic Acid Schiff (PAS): to detect presence of carbohydrate, mucoprotein, glycoprotein; mucopolysaccharides, and basement membrane.

4. Combined Alcian blue (pH=2.5) and Periodic Acid Schiff stain (AB-PAS) for neutral mucopolysaccharides.

5. Masson's trichrome stain for collagen fibers and smooth muscle (Suvarna *et al*., 2018).

All skin sections were examined under a light microscope. The histological measurements that include the thickness of layers composing of epidermis (Corneum, Granulosum – Spinosum, Basal) and dermis (Papillary and Reticular layers) were done by calibrated ocular micrometer mounted on the objective ocular lens which is calibrated using a stage micrometer Measure of the total thickness of the epidermis and dermis layers were collected. Additionally, the thickness of each epidermal stratum (corneum, granulosum, spinosum, basal) as well as papillary and reticular layers of the dermis. The mean and standard error for five sections from each sample were calculated for each region of the camel and the gazelle skin (Al-Rawi and Kalaf-Allah, 1980). Statistical significance was assessed by (t-test) for comparison of parametric variances of skin of the gazelle and camel. The significance level set at $P<0.05$.

Results

The mean total thickness of the epidermis of the skin camel upper lip, perineal, and thigh regions was (36.3 ± 0.2, 32.2 ± 0.1, 34.7 ± 0.3 μm) respectively, which was greater than that in the same regions in the gazelle skin (28.1 ± 0.6, 22.8 ± 0.4, 24.4 ± 0.5 μm) respectively. The mean thickness of the upper lip region was greater than that in other examined regions. However, all studied animals revealed a lesser thickness in the perineal skin (Table 1).

The epidermis comprises thin outermost layer composed of avascular keratinized stratified squamous epithelium. It includes four strata arranged from inner to outer as basal, spinosum, granulosum, and corneum in all regions under study (Figure1).

In camel skin, the basal stratum consisted of a single row of columnar cells with elongated darkly stained nuclei which are closest to the dermis (Figure 1).

Table1. Measurements of thickness of the wall of the skin layers of camel and gazelle (μm).

Measurement / Parts	Epidermis				Dermis		
	Total	Corneum	Granulosum - Spinosum	Basal	Total	Papillary	Reticular
Upper lip in Camel	36.3 ± 0.2*	11.2±0.01*	22.8±0.02*	2.4±0.04	149.1±0.3	17.3±0.3	132.3± 2.1
Gazelle	28.1 ± 0.6	8.6 ± 0.07	17.6 ± 0.03	2 ± 0.02	185.1± 3.6*	22.1±0.4*	164.1± 3.6*
Perineal in Camel	32.2 ± 0.1*	10.4±0.04*	21.4±0.03*	1.2±0.01	174.6± 2.5	24.2±0.6	151.6± 2.5
Gazelle	22.8 ± 0.4	5.9±0.01	16.6 ± 0.04	1.4±0.03	208.6± 3.1*	27.1±0.5	182.5± 4.1*
Thigh in Camel	34.7 ± 0.3*	11.8±0.02*	22.1±0.05*	2.1±0.02	162.1± 4.2	19.4±0.2	142.6± 3.1
Gazelle	24.4±0.5	6.2±0.01	17.2±0.01	1.9±0.04	198.1± 2.5*	25.1±01*	172.5± 4.1*

Values are the means ± S.E ;* = $P<0.05$.

The basal layer in gazelle skin was one or two cell layer thick, cylindrical epithelial cells containing granules. It was stained darkly because of the melanin pigment

granules that extended downward around the hair follicle bulbs (Figure. 2). The mean thickness of the basal layer in the upper lip, perineal, and thigh regions was (2.4±0.04, 1.2±0.01, 2.1±0.02 µm) and (2±0.04, 1.4±0.03, 1.9±0.04 µm) for camel and gazelle respectively (Table 1).

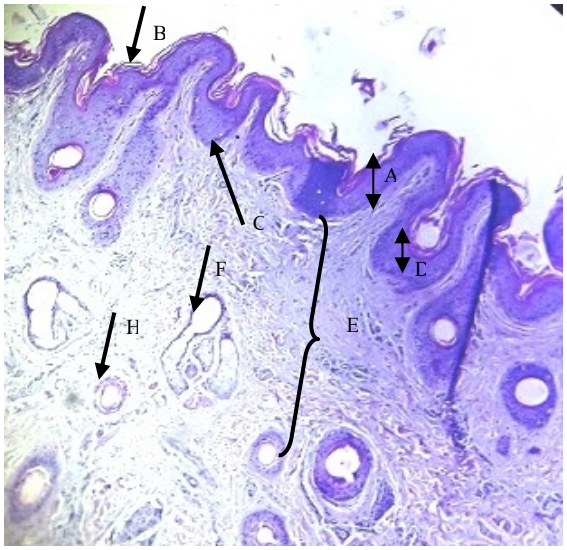

Figure 1. Cross section of the camel lip upper lip skin, epidermis (A), Corneum (B), basal (C), Granulosum - Spinosum(D), dermis (E), sweat gland (F), hair follicle (H), H&E X100.

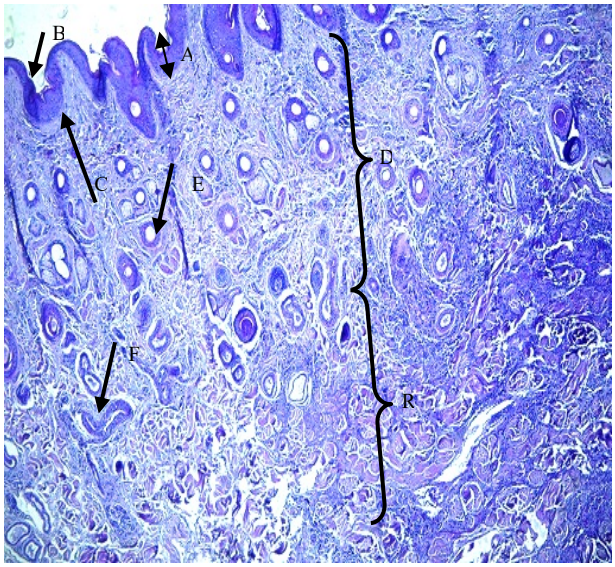

Figure 2. Cross section of the gazelle upper lip skin, epidermis (A), corneal (B), basal (C), papillary of dermis (D), hair follicles (E), sweat gland (F), reticular (R), H&E X100.

granulosum layer appeared as broken patches of cells. The granulosum layer was located under the corneum layer as a prominent continuous layer (Figures 3, 4). The mean thickness of the granulosum and spinosum layers in the upper lip, perineal, and thigh skin regions were (22.8±0.02, 21.4±0.03, 22.1±0.05 µm) and (17.6±0.03, 16.6±0.04, 17.2±0.01 µm) for camel and gazelle

of several cornified dead cells (Figures 1, 4). Its cells were scaly flattened or squamous and has network appearance filled with densely packed keratin (Figure 4). The mean thickness of the corneum layer in the upper lip, perineal, and thigh skin regions was (11.2±0.01, 10.4±0.04, 11.8±0.02µm), and (8.6±0.07, 5.9±0.01, 6.2±0.01 µm) for camel and gazelle respectively (Table 1).

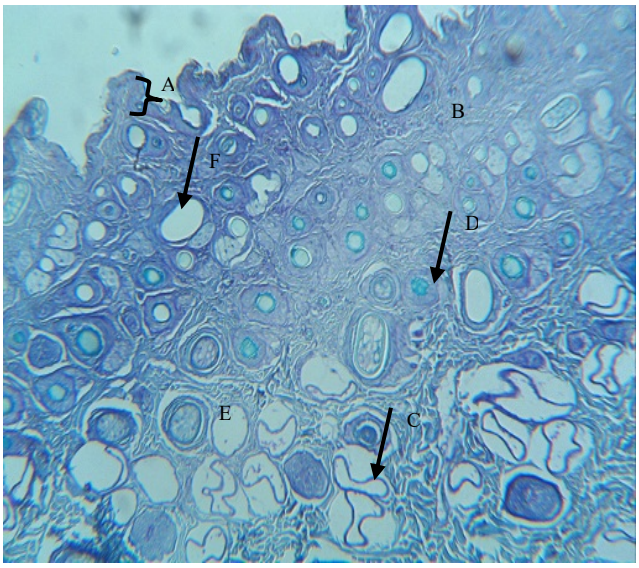

Figure 3. Cross section of the gazelle perineal skin, epidermis (A), dermis (B), sweat gland (C), hair follicle (D), Sebaceous gland (E), Adipose tissue (F), AB-PAS, X100.

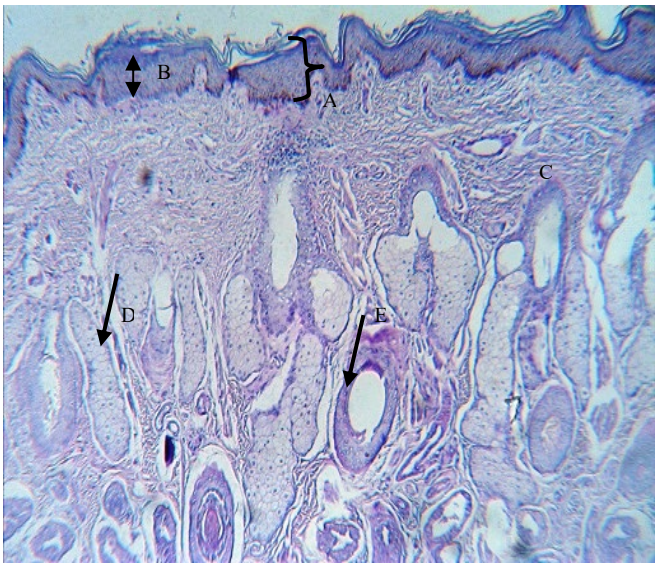

Figure 4. Cross section of the camel thigh skin, epidermis (A), Granulosum – Spinosum (B), dermis (C), sebaceous gland (D), hair follicle (E), PASX100.

The mean total thickness of the dermis in gazelle skin was greater than that in camel skin in all examined regions (the upper lip, perineal, and thigh); the measurements were (85.1±3.6, 208.6±3.1, 198.1±2.5 µm) and (149.1±0.3, 174.6±2.5, 162.1±4.2 µm) for gazelle and camel respectively. Moreover, the mean thickness of the perineal region was greater than that in other regions but less in the upper lip region (Table 1).

The dermis revealed a thick inner layer composed of dense irregular connective tissue consisting of elastic, collagen, and reticular fibres. It has blood vessels, nerves, sebaceous glands, and sweat glands (Figures 5-8). The dermis revealed two layers the papillary layer (superficial) that was located beneath the epidermis, and the mean of its thickness in the upper lip, perineal, and thigh regions were (22.1±0.4, 27.1±0.5, 25.1±01) and (17.3±0.3, 24.2±0.6, 19.4±0.2) for gazelle and camel respectively (Table 1). The second was the reticular layer which was thicker than the papillary layer in the gazelle and camel. It was in the depth of the dermis and extended to the hypodermis and contained large hair primary follicles (Figures 9-12).

The reticular layer comprised compact, dense collagen fibers and many striated muscle fibers (Figure 5). It was supplied by large blood vessels associated with the hair follicles (Figure 10). The mean of its thickness in the upper lip, perineal, and thigh regions was (132.3±2.1, 151.6±2.5, 142.6±3.1 µm) and (164.1±3.6, 182.5±4.1, 172.5±4.1 µm) for the camel and gazelle, respectively (Table 1). Sebaceous and sweat glands were observed in the dermis. The sebaceous glands appeared as small unilobular

alveolar gland around the hair follicles. The sweat glands were simple coiled structure consisting of the straight canal and coiled secretory portion. These glands are situated deep in the dermis between the layers of fine and coarse collagen fibers (Figures 9, 10). Most examined sections revealed differences in the density of glands with numerous aggregations in the dermis of camel skin (Figure 5). In camel, the reticular layer of the dermis was seen as compact and comprised two layers; the superficial layer consisted of thin collagen fibers, which have hair follicles, blood vessels, sebaceous gland and was located superficially and surrounded by the simple cuboidal epithelium, the arrector pili muscles, and ducts of the sweat glands (Figures7, 8). The underlying layer was not compact and comprised thick collagen fibers that have blood vessels and large tubular sweat glands beneath the hair tufts and their ducts open near the hair pores (Figures 5, 7, 8). The invaginations of the epidermis during embryonal time form the hair follicles in the dermis. The smooth muscles cells from the hair are erector muscle attach the hair follicle to the dermal layer (Figures 6, 10). The hypodermis was located under the dermis and consisted of loose connective tissue, collagen, elastic and reticular fibers. The spaces in this layer were filled with adipose tissue and no difference between the camel and gazelle in the hypodermis were observed (Figures 5, 12).

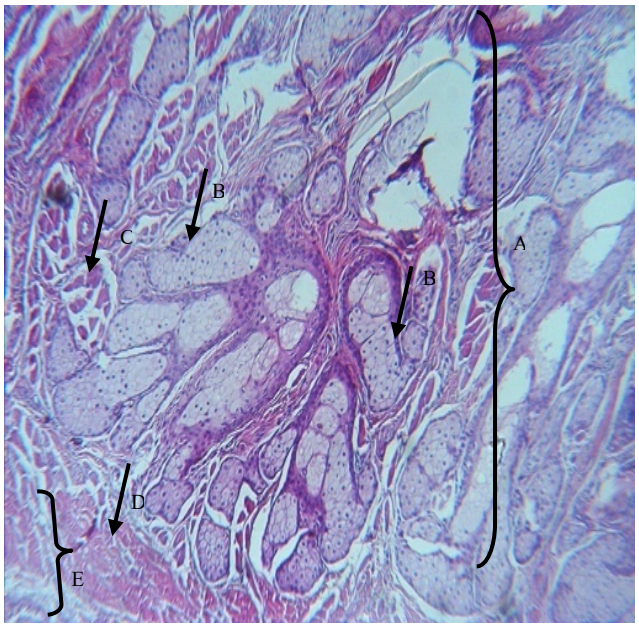

Figure 5. Cross section of the camel perineal skin, dermis (A), sebaceous gland (B), skeletal muscles (C), smooth muscles (D), Hypodermis (E), H&EX100.

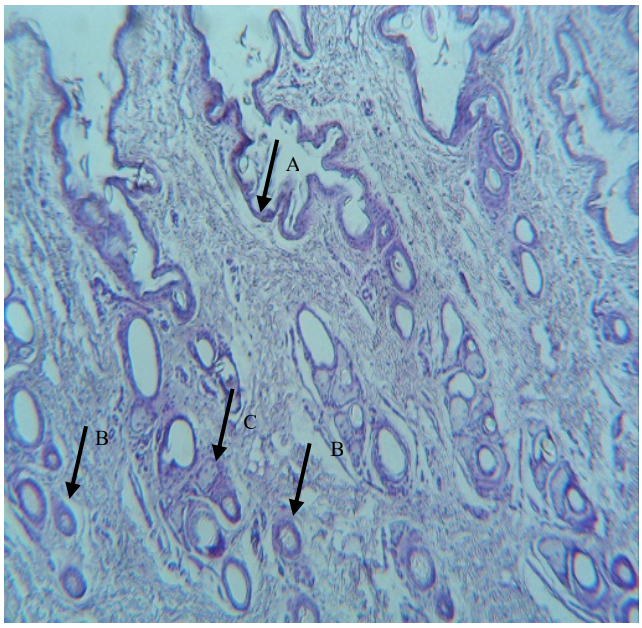

Figure 6. Cross section of the gazelle thigh skin, epidermis (A), hair follicles (B), sebaceous gland (C), PASX100.

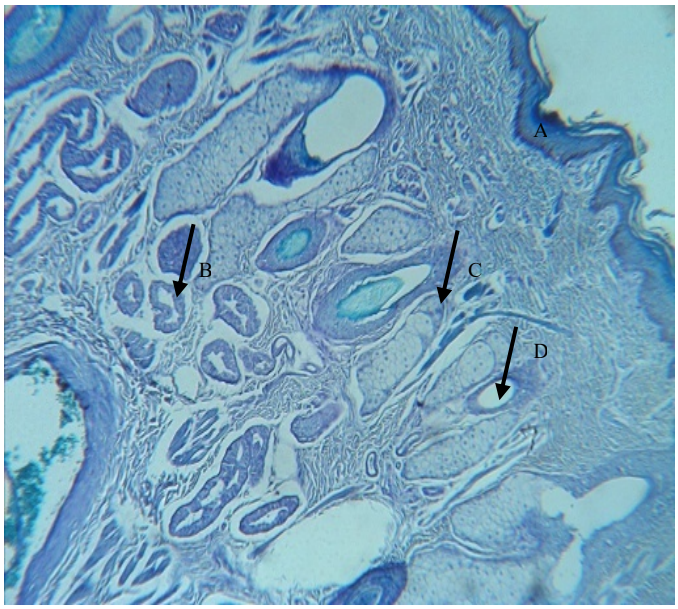

Figure 7. Cross section of the camel thigh skin, epidermis (A), sweat gland (B), sebaceous gland (C), Hair follicle (D), Masson X100.

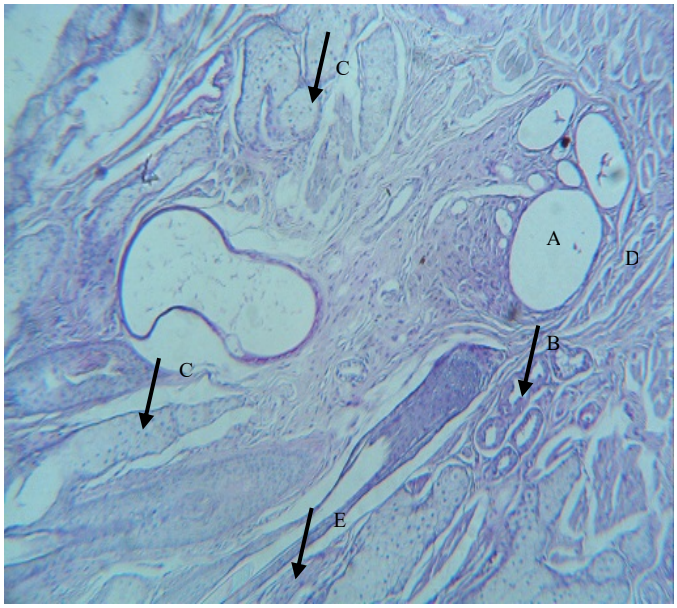

Figure 8. Cross section of the camel perineal skin, sweat gland (A), sweat gland ducts (B), sebaceous gland (C), Connective tissue (D), hair erector (E), PASX100.

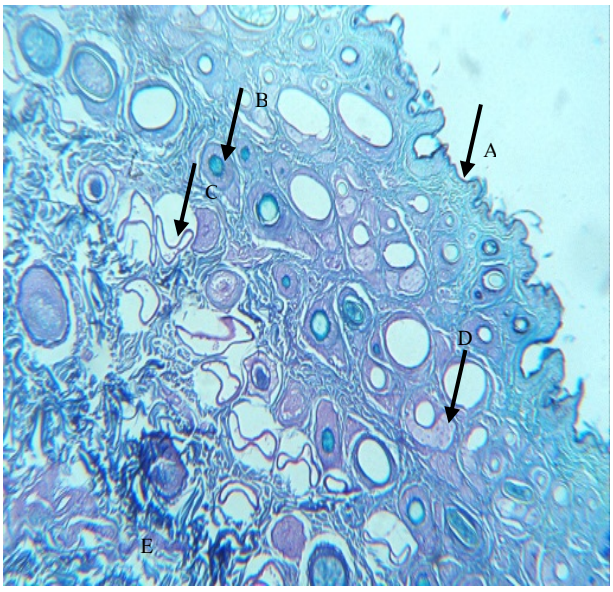

Figure 9. Cross section of the gazelle upper lip skin, epidermis (A), hair follicles (B), sweat gland (C), sebaceous gland (D), connective tissue (E) AB-PASX100.

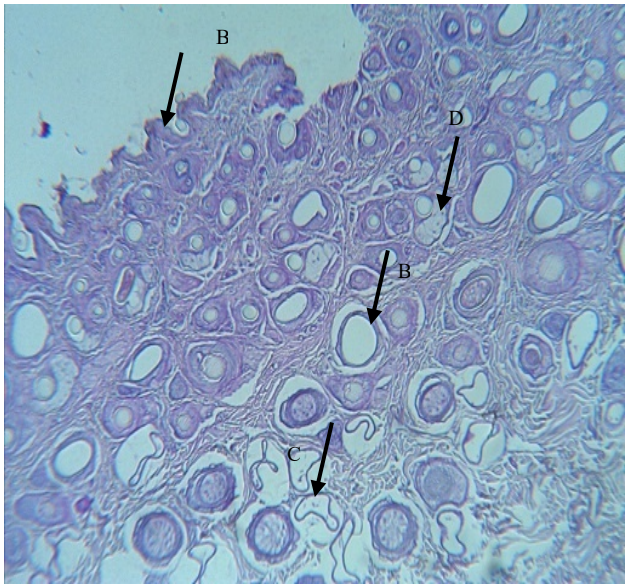

Figure 10. Cross section of the gazelle thighskin, epidermis (A), hair follicles (B), sweat gland (C), sebaceous gland (D), PASX100.

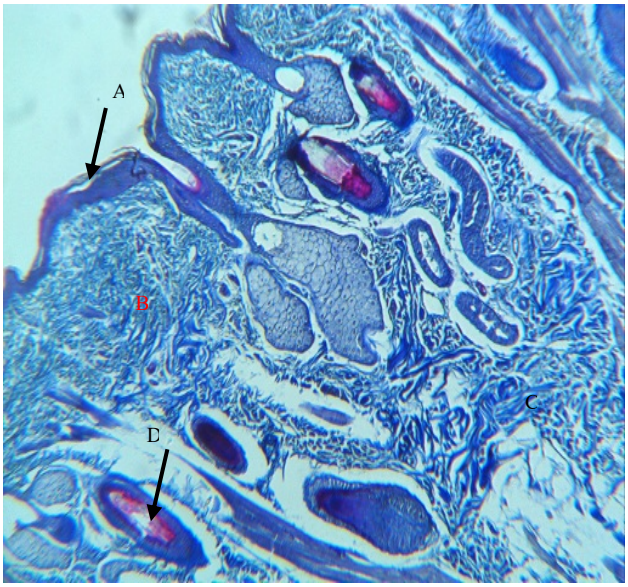

Figure 11. Cross section of the camel upper lip skin, epidermis (A), dermis (B), connective tissue (C), hair follicle (D), AB X100.

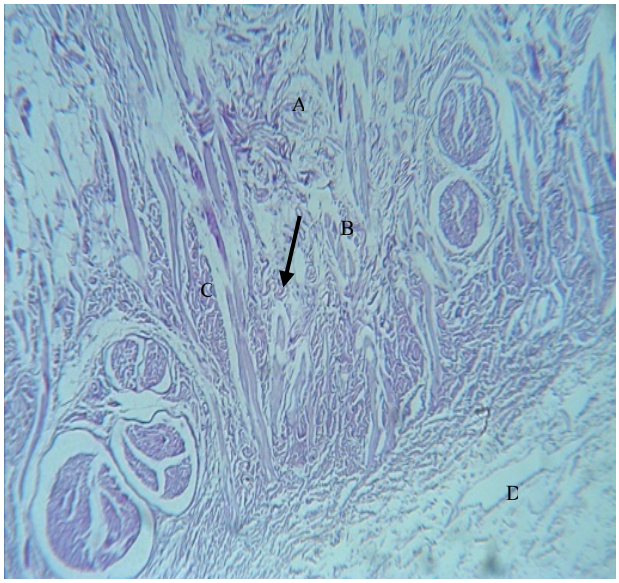

Figure 12. Cross section of the camel perineal skin, dermis (A), connective tissue (B), smooth muscle (C), hypodermis (D), PAS X100.

Discussion

The epidermis and its layers in camel skin are comparatively thicker significantly than the gazelle, even though these two animals live in a desert environment. These differences in the epidermis thickness permit considerable water loss by perspiration in the gazelle's dry air environment. The results of the current study revealed a greater thickness of skin in the upper lip but thinner in perineal skin. These results agree with findings of previous research (Abdul Raheem and AL-Hety, 1997) on black goats. The variations in skin thickness might be due to animals' species and other related factors, such as age and environment. The epidermal strata act as a protective barrier between the animal body and the outside environment against infection and chemical and thermal insults (Ouajd and Kamel, 2009; Saxena *et al*., 1994). The basal layer consists of a single row of columnar cells.

In contrast, the spinosum layer consists of several layers of the polyhedral cells, which have large number of desmosomes. These desmosomes anchor the cells to each other, and it is joined by intermediate filament cytoskeleton (Al-Abbas *et al*., 1999). When these cells shrink slightly during fixation, the desmosomes from the neighboring cells remain tightly bound to each other, and these connections look like 'prickles', so the name prickle cells (Ozfliz *et al.,* 2002). Razvi *et al.,* (2014) mentioned that the granular layer cells move up into this layer and lose their nuclei and cytoplasmic organelles turning into keratohyaline granules to gradually change into the keratinized squamous of the next layer. The granules contain lipid-rich secretion and act as a water sealant (Ouajd and Kamel, 2009). The dermis was thicker, more compact, and contained dense collagen fibers and many striated muscles in the gazelle but thinner in the camel. These results are like the observations reported previously by (Al-

Abbas *et al.,* 1999; Abdul Raheem and AL-Hety, 1997). They may differ because they consist of dense irregular reticular and papillary layers. The dermis can be divided into a superficial zone with fine collagen fibers and a deeper layer with thick collagen fibers. The dermis housed the sensory receptors for pain, temperature, pressure, touch. (Dyce *et al.,* 2010). It contains blood vessels, nerve, lymphatic vessels, and sweat glands that open onto the skin's surface (Ershad *et al.,* 2016).

The adipose tissue of the hypodermis has metabolic functions which are responsible for producing vitamin D and triglycerides. The dermis exhibits seasonal variations. Moreover, Morais *et al*. (2001) showed the variation in skin thickness in different areas of some animals, such as the hedgehog, and the distribution of the sebaceous and sweat glands because of their need for water retention and avoidance of energy loss through heat (Al-Abbas *et al.,* 1999).

The seasonal variations significantly affect the structure of skin layers. For example, at the beginning of the winter, the dermis forms adipose tissues that gradually transform into fibrous connective tissue; as the stored fat is used up and so in early summer, the dermis is largely devoid of the fat (Dyce *et al*., 2010; Fourneau *et al.,* 2020). Therefore, during the cold season, the layer of the fatty tissue gives insulation against the heat loss through the skin and provides the energy store on which the animal depends on vegetation is scarce (Fourneau *et al.,* 2020). Short, stout, and erect hairs are responsible for forming the insulation barrier, and the long hairs probably form a protective outer cover (Aktaș and Daglıoglu, 2009). The undercoat humidity is relatively high due to the sweat secretion and insensible perspiration. The presence of the humid barrier may reduce the total evaporation and enable gazelles to live in the desert despite a thin epidermis (Al-Abbas *et al.,* 1999). The sebaceous glands are individually present in the dermis of the gazelle and camel skin. These glands produce oily sebum by holocrine mode of secretion (Dyce *et al.,* 2010). The sebum helps the skin to remain moist, soft, acts as a barrier, antibacterial and antifungal. It also reduces friction, contributes to thermal insulation, contributes to vitamin D formation, and prevents water entry into the hair and the skin (Ali, 2008). The presence of sebaceous glands depends on the animal's environment, gender, and season (Kingdon, 1997). These glands are altered by animal age, and the diet which may affect the level of the sex hormones and play a role in the number and distribution of these glands (Al-Abbas *et al.,* 1999). The current study showed that the sweat glands were simple tubular glands, which agrees with other studies (Al-Abbas *et al.,* 1999; Dyce *et al.,* 2010) who showed the occurrence of sweat glands in groups under clusters of hairs. In gazelles, sweat glands distribute singly all over the body and start to sweat when the environmental temperature reaches about 22°C and body temperature 38°C (Kingdon, 1997). However, Ouajd and Kamel (2009) mentioned that the camel sweats when its body temperature exceeds 35°C. The sweat glands secret fluid that consists of water and salts to regulate body temperature (Ali, 2008). The hair on the body is numerous, which helps exchange between the water and the animal's body (Aktaș and Daglıoglu, 2009). The elastic fibers in the thigh skin are present compared with other skin regions (lip and perineum). Most of the blood vessels in the upper lip skin region can indirectly serve to help the sense of touch and regulate mucosal secretions (Al-Abbas *et al.,* 1999). The hair follicles were numerous in the upper lip skin area and extended to the reticular layer of the dermis. They were used in breathing, sense of smell in mammals and tactile sensation (Kingdon, 1997). A layer of adipose tissue was also observed in the animal perineal skin, and this layer was not well developed in the upper lip skin (Ouajd and Kamel, 2009).

In conclusion, the results of this study showed the differences in the skin layers thickness between the camels and gazelle that live in the same environment. These observations indicate more adaptation of the camel than that of the gazelle to its surrounding environment.

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