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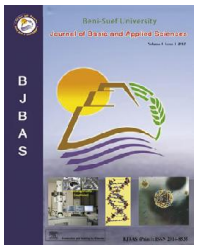

Full Length Article

Morphohistological and surgical anatomy of the sinus interdigitalis in Egyptian native breeds of sheep

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ABSTRACT

The current study aimed to characterize the gross and microscopic structures of the interdigital sinus in Egyptian native breeds of sheep and goats (Baladi sheep and goats) to aid in conducting farther studies on such sinus and its surgical importance. The study was carried out on the distal fore and hind limb specimens (below the fetlock joint) obtained from healthy mature ten Baladi sheep and ten Baladi goats of both sexes. For sheep, thirty specimens were subjected to gross anatomical investigation and the other ten specimens were used for histological examination. While, the forty goats fore and hind feet specimens were examined grossly. The results obtained revealed the presence of a well-developed interdigital sinus in Baladi sheep, but there was no evidence for the existence of this unique structure in all examined specimens of Baladi goats. Such sinus appeared grossly as a pipe-like, composed of two parts; body (corpus) and neck (column) which opened in the skin of the interdigital area with an external orifice. The body consisted of three distinct parts in the forefeet and a single part in the hind feet. Histologically, the wall in body and neck regions of the interdigital sinus composed of three distinct layers; epidermis, dermis and a fibrous capsule. Many sebaceous glands, hair follicles and sweat glands in addition to the glandular apocrine gland were distributed within the dermis. The secretory adenomers of the interdigital sinus arranged in different lobules and lined with cuboidal cells with bleb-like projections. It reacted positively with Periodic Acid Schiff (PAS), Sudan black and Sudan III stains, on the other hand it negatively reacted with Alcian blue stain (AB).

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1. Introduction

In ruminants, there is a unique anatomical structure termed interdigital sinus. It has been mentioned as a foot skin organ (Raesfeld, 1978), a growing organ (Sivachelven et al., 1992), interdigital sinus (Bavdek, 1981; Schaller, 2007; Konig and Liebich, 2007; Aslan et al., 2010; Pourlis, 2010), interdigital pouch (Konig and Liebich, 2007; Aslan et al., 2010; Dyce et al., 2010) and also it is termed interdigital gland (Atoji et al., 1988; Janicki et al., 2003; Demiraslan et al., 2014). This sinus is a socket-like skin invagination with a characteristic structure and special functions (Pourlis, 2010). Many other species belonging to the Artiodactyla order have well-developed interdigital glands, but they differed morphologically from species to another (Janicki et al., 2003). They are found on the forelimbs and hind limbs of sheep of both sexes (Dyce et al., 2010). It is a tubular invagination of the skin composed mainly of sebaceous and apocrine type glandular structure (interdigital glands) that are varied in morphology, described in many Artiodactyla including; Tuj sheep (Aslan et al., 2010), deer (Wood, 2003; Reiter et al., 2003), Japanese serow, *Capricorniscrispus* (Atoji et al., 1988) and Asian elephant, *Elephasmaximus* (Lamps et al., 2001).

Many researchers reported that the interdigital sinuses were located in both fore and hind feet of many breeds of sheep as; Lori's sheep, Douchke sheep, Japanese serow, Akkaraman sheep, antelope and moufflon (Calislar, 1971; Raesfeld, 1978; Atoji et al., 1988; Abbasi et al., 2009; Avdic et al., 2013). Even though, Calislar (1971) reported that the hair and Angora goats have no interdigital sinus in their feet, other researchers observed that the interdigital gland was found only in the hind feet of the roebuck (Janicki et al., 2003) or rudiment in the native hair goats (Bahadır and Yakışık, 1988).

Regards their function, the interdigital sinus is a specialized skin pouch found in sheep in which the sloughed surface cells combined with the secretion of their glands to form a pungent mixture, which is used for marking of the animals (Konig and Liebich, 2007). These sinuses are considered scent glands produced odorous signals and pheromones that play important biological roles in the non-specific chemical communication, as active territorial demarcation and also in the expression of social behavior (Epple et al., 1993; Parillo and Diverio, 2009). The secretion of the interdigital gland may be related to reproductive activities (Konig and Liebich, 2007; Demiraslan et al., 2014). Moreover, (Abbasi et al., 2009) mentioned that the gland has fungicidal and bactericidal effects, and protects against ultra-violet radiation.

Regarding the surgical importance of the interdigital sinus comes from its peculiar position that makes it prone to injury (Sivachelvan et al., 1992). Although the relationship between lameness and the interdigital sinus is not yet defined but about 16% from lameness cases were accompanied with its inflammation (Bokko et al., 2003). Surgical removal of the interdigital sinus is an easy operation (Misk and Misk, 2013) but will cause lameness in young lambs (Sivachelvan et al., 1992; Egwn et al., 1994) and it is not described for control infection in foot rot (Wassink et al., 2010).

The current study highlighted the anatomical and the histological structure of the interdigital sinus in Egyptian native breeds of sheep aimed to clarify its role and surgical importance by farther studies.

2. Materials and methods

2.1. Animal specimens

The four feet (below the fetlock joint) of ten Baladi sheep (n = 40) and ten Baladi goats (n = 40) of both sexes were collected from the slaughterhouse at Beni-Suef governorate, Egypt from apparently healthy animals with age about 9–18 months.

2.2. Gross examination

Gross study was conducted on fresh thirty feet of sheep and forty feet of goats which were carefully dissected to show the gross structures, topography and relations of the interdigital sinuses.

2.3. Microscopic investigation

The microscopic examination was done on the whole interdigital sinus in distal limbs of ten sheep and it was carried out by careful removal of the whole interdigital sinuses with its glandular structures which were immediately obtained after slaughtering. The specimens were fixed by immersion in Bouin's fluid for 24 h and subjected to routine dehydration in ascending grades of alcohol, cleared in xylene, consequently embedded in Paraplast blocks and 5 μm thick sections were cut using rotary microtome. The obtained sections were then stained using Haematoxyline and Eosine (H&E), Crossmon's Trichrome stain, Alcian blue (AB) and Periodic acid Schiff (PAS). Fresh samples were cut using a freezing microtome and stained by Suddan black and Sudan III stains. These stains were used as outlined by Bancroft and Gamble (2008).

3. Results

3.1. Gross anatomical examination

In all examined fore and hind feet of Egyptian native breeds of sheep, it was detected that the interdigital sinus was located in the space between the two digits. From its lateral view; the interdigital sinus appeared as pipe-like in shape. It was formed of body (corpus or glandular part) and neck connected to the exterior by external orifice (Figs. 2–4).

3.1.1. The external orifice (Fig. 1)

The external orifice was determined on the dorsal aspect of the fore and hind feet between the axial sides of the two digits opposite to the proximal inter-phalangeal articulation, nearly in the center of a skin triangle in the hind feet (Fig. 1b) or deviated to the medial digit in the fore feet (Fig. 1a). The external orifice appeared clearly when the two digits

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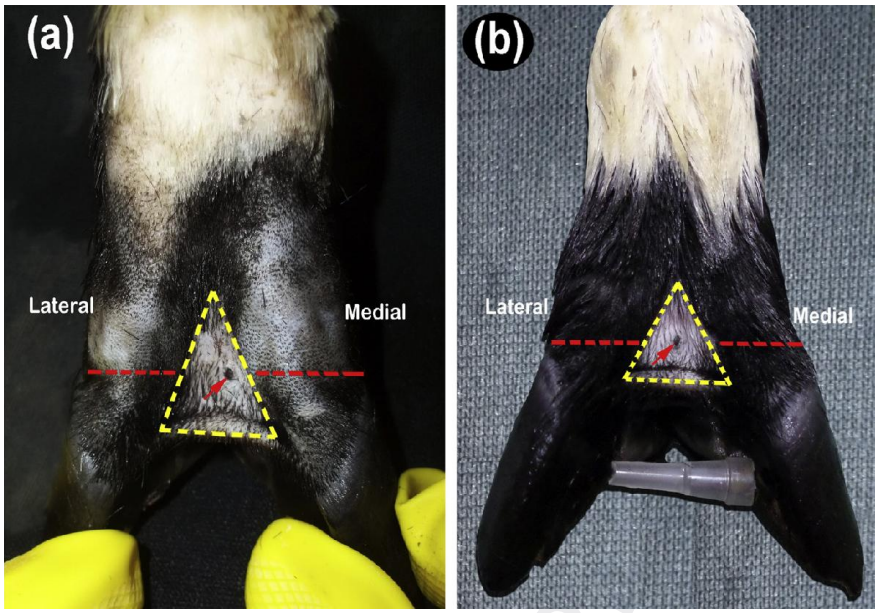

Fig. 1 – Gross dorsal view of the interdigital region of baladi sheep at the right forefoot (a) and right hind foot (b) showing the position of the external orifice of the interdigital sinus (red arrows) at the level of the proximal inter-phalangeal articulation (red lines) and the anatomical boundaries (triangles) used as a guide for surgical interferences.

diverged away from each other. A clear skin fold with about 0.2 cm thickness in both feet, about 2 cm in length in the fore feet and 1.3 cm in length in the hind feet and extended transversely between the two digits to form the base of this triangle. The lateral borders of this triangle measured about 3 cm in length in the fore feet and about 2 cm in the hind feet. The apex of the triangle located at the level of middle of the proximal phalanx.

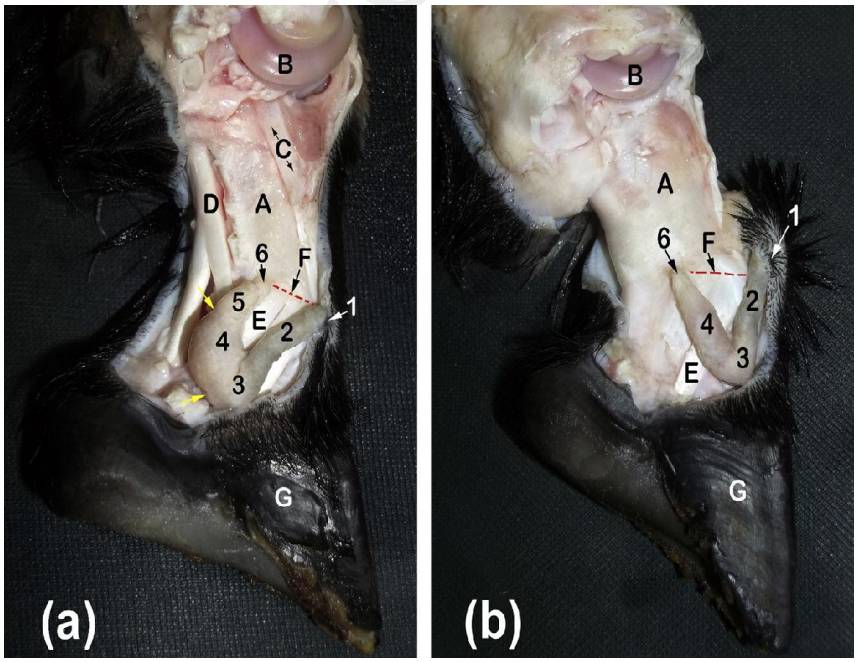

Fig. 2 – Medial view of the interdigital region in Baladi sheep at forefoot (a) and hind foot (b); A. Proximal phalanx, B- Distal end of the metacarpus (a) and metatarsus (b) after removal of the lateral digit, C-Axial extensor branch of the suspensory ligament, D-Superficial and deep flexor tendons, E– Axial collateral ligament of the pastern joint (interdigital ligament), F-level of the proximal inter phalangeal articulation (red lines), G-axial aspect of the claw. 1- External orifice, 2- neck, 3-Flexure (distal part of the body), 4- Body of the sinus, 5- Proximal part of the body, 6- Attachment to the proximal inter phalangeal articulation, yellow arrow indicate the constriction in the body of the sinus in forefoot.

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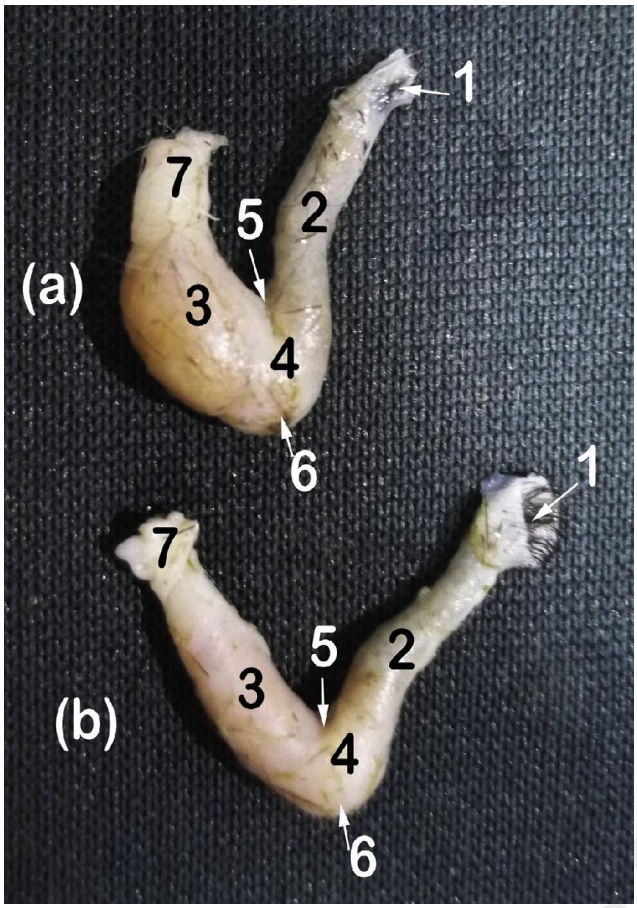

Fig. 3 – Gross view of the interdigital sinus in baladi sheep at forefoot (a) and hind foot (b); 1- External orifice, 2- Neck, 3- Body of the sinus, 4- Flexure (distal part of the body), 5- internal angle, 6- External angle, 7- fibrous attachment to the proximal part of the gland.

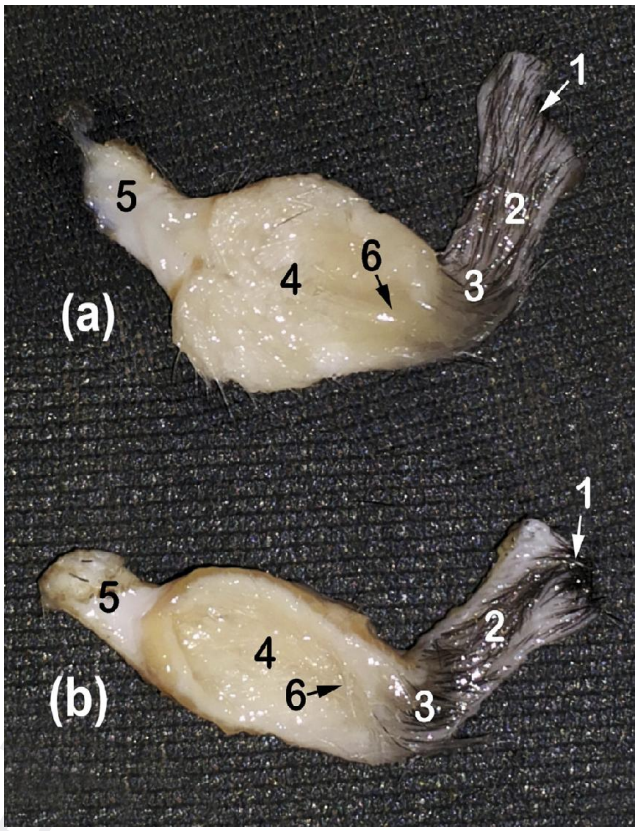

Fig. 4 – Gross view of the interior surface of interdigital sinus in baladi sheep at forefoot (a) and hind foot (b); 1- External orifice, 2- Neck, 3- Flexure, 4- Body of the sinus 5- fibrous attachment of the proximal part of the gland, 6- secretion of the interdigital gland.

3.1.2. *The neck* (Figs. 2–4)
The neck appeared darker in color, directed obliquely from the corpus of the interdigital sinus to open on the dorsal aspect of the foot with an external orifice. In fore and hind feet, its length was about 1.5 cm with 0.2 cm diameter. It traversed the second phalanx, extended from the pastern joint to the distal extremity of the middle phalanx and related axially to the interdigital ligament (axial collateral ligament of the pastern joint).

3.1.3. *The body (corpus)* (Figs. 2–4)
The body of the interdigital sinus extended in a dorsopalmar (dorsoplantar) direction. It was located behind the neck to which it was connected with a flexure with proximal and distal border.

3.1.3.1. *In the forefeet*. The body of the interdigital sinus (Figs. 2a and 3aa) was divided by two visible outer constrictions into three parts; proximal, middle and distal. The proximal part was narrow and pale in color about 0.5 cm in length and 0.3 cm diameter and intimately attached with the fibrous capsule of the pastern joint giving the gland some sort of proximal fixation (Fig. 2a, b/6). The middle glandular part was light brown in color, oval in shape and had two borders; dorsal and palmar. The dorsal border was about 0.3 cm in length while the palmar one was about 1 cm in length with a diameter about 0.6 cm in its middle. The distal part of the corpus was found about 1 cm proximal to the corona region of the toe. It had a distal border measured about 1.3 cm in length, while the proximal border formed an acute angle between the middle part of the corpus and the neck.

3.1.3.2. *In hind feet*. The body of the interdigital sinus (Figs. 2b and 3b) was smaller in size than that in the forefeet, with no clear outer constrictions. The proximoplanter part of the corpus was tubular in shape about 1.2 cm in length and 0.3 cm in diameter. Each dorsal and planter borders was about 1.3 cm in length. The distal part of the corpus had two borders; the distal one was about 0.9 cm in length, while the proximal one showed a wider angle than those in the forefeet.

The interior of the interdigital sinus (Fig. 4) was covered by hair in the direction of secretion; dark colored hairs covered the lumen of the excretory duct and the distal part of the corpus, while the rest of the corpus contained pale colored non-pigmented hair and its lumen was filled with colorless viscous secretions.

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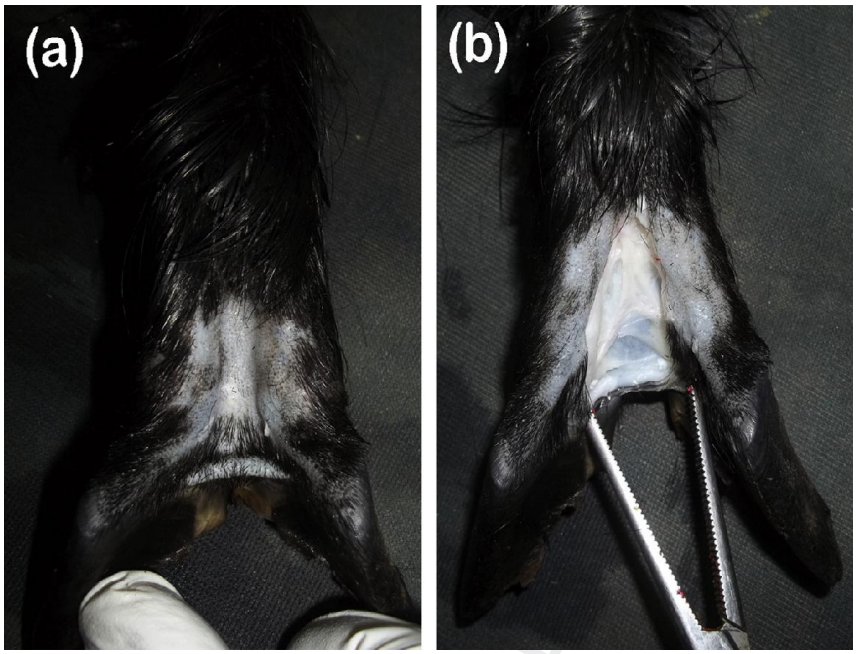

Fig. 5 – Gross dorsal view of the interdigital region of baladi goats at the forefoot (a) and the hind foot (b) showing no evidence for the presence of the interdigial sinus.

In all grossly examined forefeet and hind feet of male and female Egyptian native breeds of goats, there was no evidence for the existence of the interdigital sinus or external orifices between the digits in this animal species (Fig. 5).

3.2. *Histological examination*

Histologically, the examination of the prepared sections of the interdigital sinuses obtained from both fore and hind feet of Baladi sheep revealed that the wall of the interdigital sinus had three clearly distinguishable layers; epidermis, dermis and fibrous capsule. Stratified squamous epithelium with a prominent keratin layer, epidermis, was noticed facing the dense luminal content of the gland (Fig. 6). Sebaceous gland, hair follicles with different sizes and sweat glands were clearly visible within the dermis (Fig. 7).

The glandular structure of the sinus appeared composed of closely packed secretory units arranged into lobules divided by extensive dense collagen fibers and individual fine collagenic fibers were also noticed supported the secretory acini (Fig. 8). The secretory units composed of a single layer of cuboidal cells that rested directly on a basement membrane and were surrounded externally by myo-epithelial cells (Fig. 9). The cuboidal cells lined the secretory acini had acidophilic vacuolated cytoplasm, spherical centrally situated nuclei and often exhibited apical protrusions (Fig. 10). The

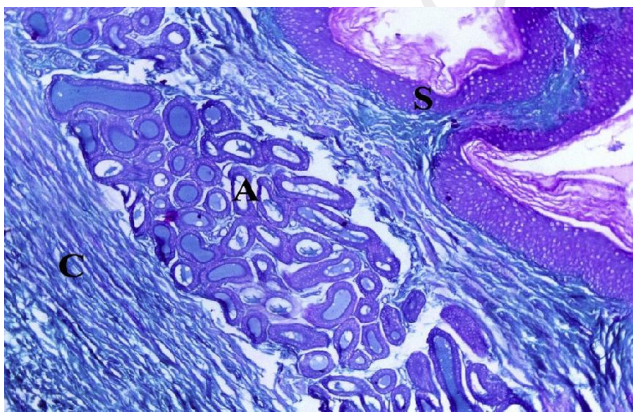

Fig. 6 – A photomicrograph of the interdigital sinus wall of sheep; epidrmis, dermis and capsule. Note stratified squamous epithelium keratinized (S) line the gland, secretory acini (A) and fibrous capsule (C) encircles the gland. Crossmon's trichrome stain X40.

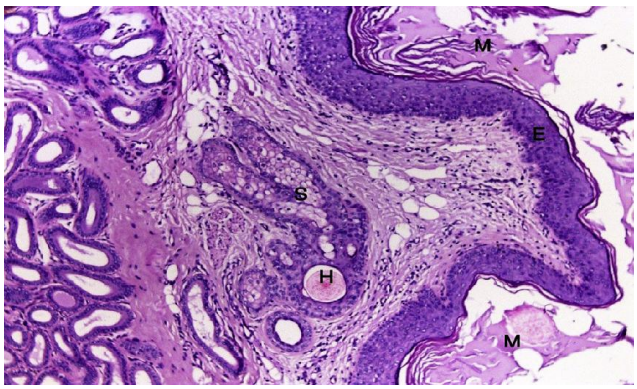

Fig. 7 – A photomicrograph of the interdigital sinus of sheep showing epidermis (E), Sebaceous gland (S) and hair follicle (H) within the dermis. Note, presence of secretory material (M) in the lumen of the interdigital sinus (M). H&E stain, X100.

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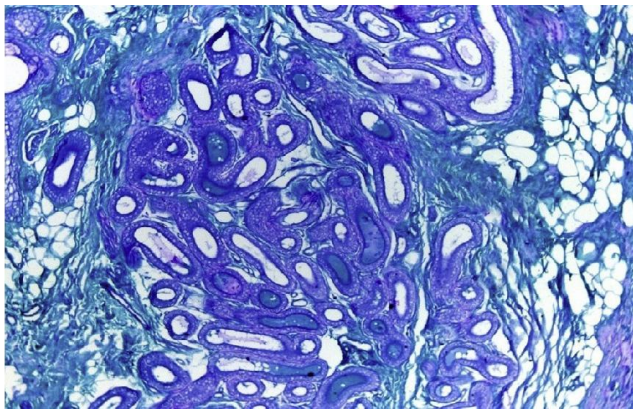

Fig. 8 – A photomicrograph of the interdigital sinus showing dense collagenic bundles separates the glandular lobules. Note, fine collagen fibers support the secretory acini. Crossmon's trichrome stain, X 100.

lumen of the secretory acini was occupied by acidophilic homogenous secreted contents. On the other hand, the lumen of the interdigital sinus was often occupied by secretory contents of sebaceous gland, sweat gland and the interdigital gland itself in addition to several wool fibers. The secretion of the apocrine gland, basal membranes of the epithelium cells, and the apocrine blebs showed positive PAS reaction (Fig. 11). At the same time, the glandular cells showed high concentration of sudanophilic substances (Figs. 12 and 13) and negatively reacted with Alcian blue stain. The fibrous capsule that represented the outermost layer of the gland appeared composed of several parallel bundles of collagen fibers and blood vessels. It connected externally with the skin surface.

Concerning the neck region of the interdigital sinus, it appeared microscopically has nearly the same structure of the body region. Our histological examination of all prepared specimens of the neck region revealed that it composed of few secretory adenomers covered internally with skin and

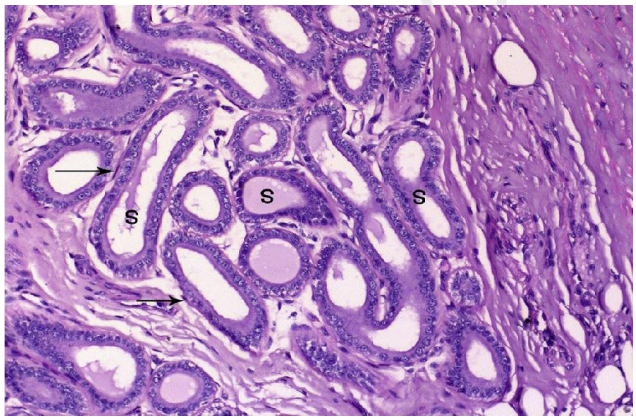

Fig. 9 – A photomicrograph of the interdigital sinus showing the secretory acini (S) lined with cuboidal cells and surrounded externally with myoepithelial cells (arrows). Note, some secretory acini filled with secreted contents. H&E, X200.

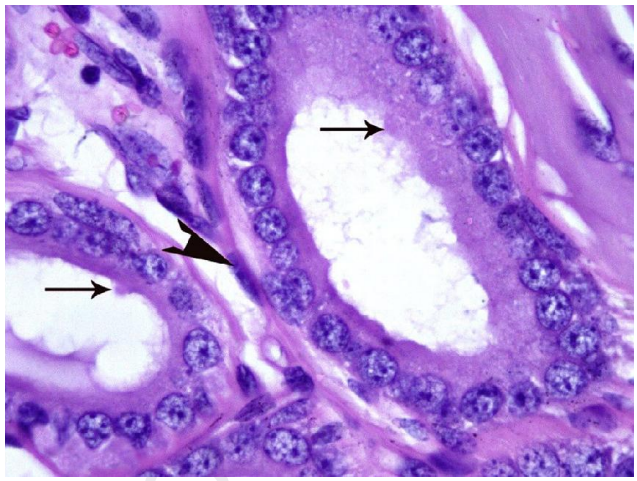

Fig. 10 – A higher magnification of Fig. 4. Showing the secretory acini lined with cuboidal cells contain vacuolated acidophilic cytoplasm and central spherical nuclei with bleb-like projections (arrows). Note, myoepithelial cell (arrow head), H&E, X1000.

enclosed externally with thin capsule. The epidermis consists of thin stratified squamous epithelium keratinized. Numerous sebaceous glands, hair follicles and sweat glands in addition to the glandular structures of the gland itself appeared close to the capsule within the dermis (Fig. 14).

The secretory apocrine adenomeres appeared few in number and small in size in comparison with that observed in the body region. The adenomeres lined with cuboidal cells with spherical nuclei and the lumen contained acidophilic vacuolated materials (Fig. 15). Collagen bundles were clearly seen surrounded the acini (Fig. 16).

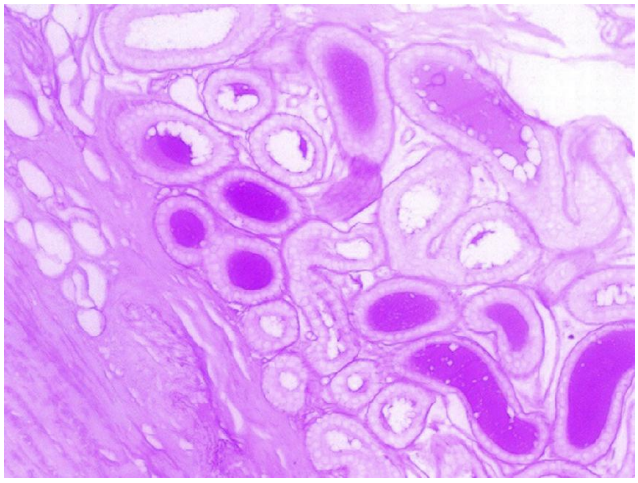

Fig. 11 – A photomicrograph of the interdigital sinus showing different degrees of PAS reaction; strong reaction in the secretion of secretory acini and basement membrane moderate in connective tissue and apical border of the acinar cells and weak in the acinar cells. PAS reaction, X200.

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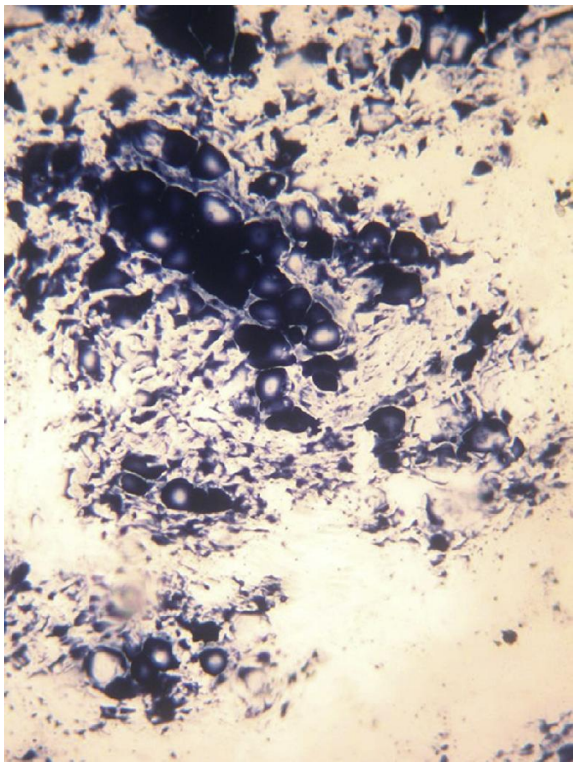

Fig. 12 – A photomicrograph of the interdigital sinus showing sudanophilic substances in the secretory acini. Sudan black stain, X100.

The secretory materials within the lumen of some adenomeres were reacted strongly with the PAS stain, while others in addition to the basal surface of the secretory cells reacted moderately. The glandular epithelium showed weak reaction (Fig. 17).

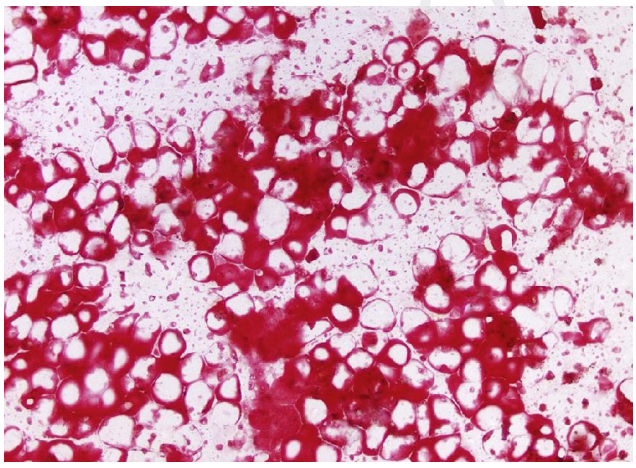

Fig. 13 – A photomicrograph of the body region of the interdigital sinus showing intensive sudanophilic substances filled the lumens of the secretory adenomeres. Sudan III stain, X100.

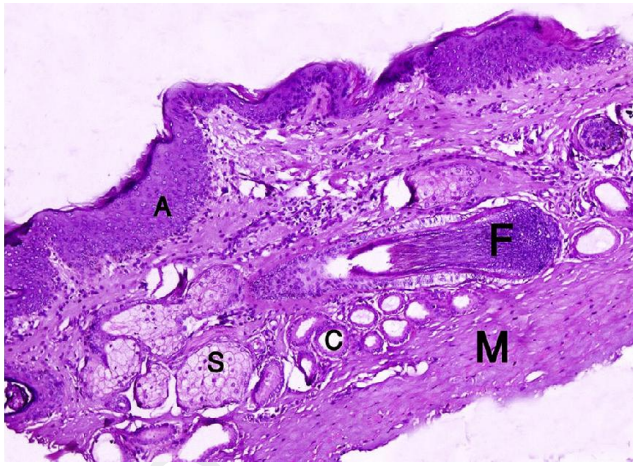

Fig. 14 – A photomicrograph of the neck region of the interdigital sinus showing that it covered internally by skin; epidermis (A) and dermis contains numerous sebaceous glands (S) associated with hair follicles (F) few secretory acini (c). Note, the neck region enclosed by thin fibrous capsule (M). H&E X100.

4. Discussion

The current study tried to give accurate anatomical details on the interdigital sinuses in the Egyptian native breed of sheep and goats; their existence, histomorphological structure and surgical boundaries. In this concern, the gross examination of the interdigital areas in the present investigation revealed that the glandular or ductal structures of the interdigital sinuses could not be seen by the naked eye in the forefeet and hind feet of the Baladi goats, this comes in agreement with Calislar (1971) in the hair and Angora goats. On the other hand, Bahadır and Yakışık (1988), Karahan et al. (2007), Parillo and Diverio (2009), detected rudimentary interdigital sinuses in

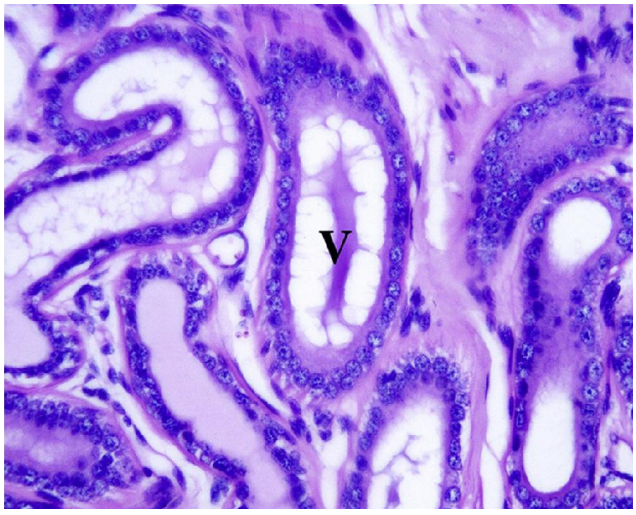

Fig. 15 – A photomicrograph of the secretory acini of the neck portion of the interdigital sinus showing different stages of secretory activity. Note, vacuolated secretory materials (V) in the acinar lumen. H&E X400.

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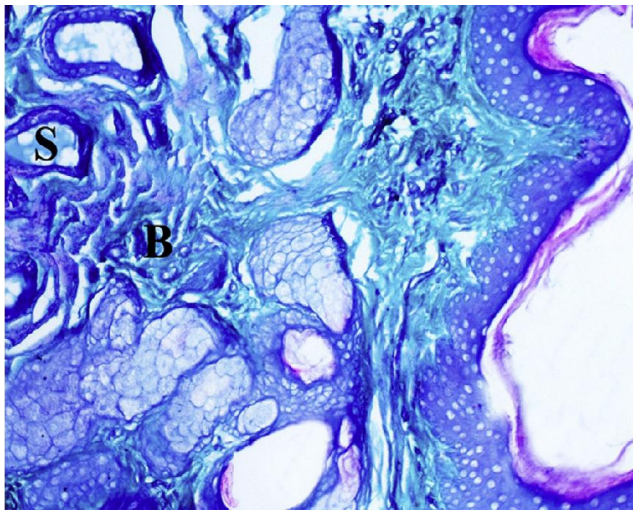

Fig. 16 – A photomicrograph of the neck region of the interdigital sinus showing collagen bundles (B) surrounded both the sebaceous glands and the secretory acini (S). Crossmon's trichrome stain, X200.

goats. Moreover, Janicki et al. (2003) detected the gland in the hind feet only in the Roebuck.

In Egyptian native breeds of sheep, the current investigation declared that the interdigital sinuses of the forefeet and hind feet were detected in the interdigital space, the same observations were reported in many breeds of sheep as; Lori's sheep, Douchke sheep, Japanese serow, Akkaraman sheep, antelope, and moufflon (Calislar, 1971; Raesfeld, 1978; Atoji et al., 1988; Abbasi et al., 2009; Avdic et al., 2013).

The current investigation in male and female native breeds of sheep revealed that there was no sex difference of the interdigital sinuses as mentioned by Misk and Misk (2013). On the other hand our findings met with (Atoji et al., 1988) in

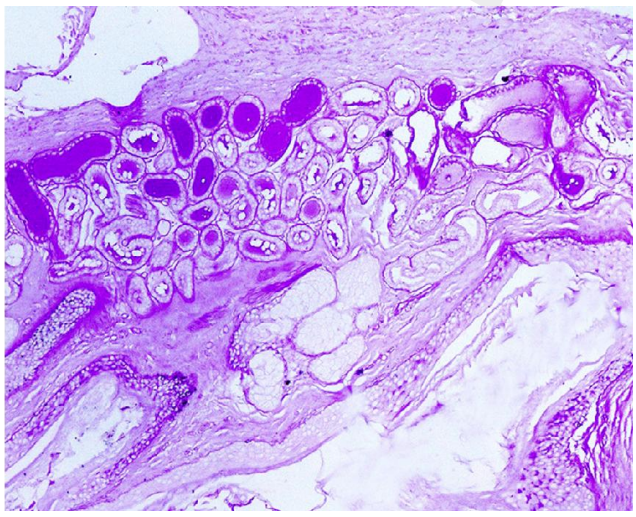

Fig. 17 – A photomicrograph of the neck region shows strong fucshinophilic reaction in secretory material of some secretory acini, others showing moderate reaction while the acinar cells showed weak reaction. PAS, X100.

Japanese serow and (Abbasi et al., 2009) in Lori's sheep who observed that, the size of interdigital glands in forefeet are larger than in the hind feet. Such observations were in contrast with most of the available literature who stated that there were no differences between the size and shape of the interdigital sinus in fore and hind feet of sheep. Moreover two visible outer constrictions were observed in the corpus of the interdigital sinuses in the forefeet of animals under investigation leading to its division into three parts; proximal, middle and distal. These constrictions were not clear in that of the hind feet so the proximo-planter part of the corpus appeared tubular.

The gross examination of the interdigital sinus in the current study came in agreement with Karahan et al. (2007), where it was divided into body, neck and external orifice. While, Demiraslan et al. (2014) named it as interdigital gland and divided into orifice, excretory duct, and corpus. Moreover, the later author considered the distal part of the gland as a flexure with external and internal angles. Other available literature have different divisions of the interdigital sinus as Avdic et al. (2013) divided the interdigital gland into the corpus, collum, and fundus, Abbasi et al. (2009) defined the interdigital gland as excretory and secretory systems. Meanwhile, Janicki et al. (2003) divided the interdigital gland into deep, middle, and excretory sections.

In all available literature about the interdigital area, there is no such literature explain the topography of the external orifice on the dorsal aspect of the interdigital cleft opposite to the proximal inter-phalangeal articulation as reported in the present study. When the two digits diverged away from each other, a clear skin triangle could be seen with clear margins lodging the external orifice of the interdigital sinus. In hind feet, the orifice was found in the center of this skin triangle. While in the forefeet it was slightly deviated toward the axial side of the medial digit. Such observations in all examined feet could be a valuable guide for postmortem identification of the two limbs. The measurements stated in this study about the interdigital skin triangle in the forefeet and hind feet of sheep and the position of the external orifice of the interdigital sinus could be used as a guide for its surgical interference.

Janicki et al. (2003) in roebuck stated that the interdigital space at the level of the second phalanx acts as a pump for the gland through the movement of lifting, gathering, sagging and interspacing of hoofs which mechanically discharges the secretion of the gland. Moreover, Gosling (1985); Dyce et al. (2010), reported that the position of the orifice of interdigital gland on the dorsal surface is of great advantage to active marking of sheep during locomotion.

Regarding the neck of the interdigital sinus in our work, it appeared darker in color, directed obliquely from the corpus of the interdigital sinus to open on the dorsal aspect of the foot with external orifice. In forefeet and hind feet, its length was about 1.5 cm and its diameter was about 0.2 cm. The length of the neck in sheep is 2.6 cm and 0.49 cm in diameter (Abbasi et al., 2009), while it is about1.8–2 cm in length and 0.2–0.4 cm diameter (Nickel et al., 1981) and 0.1 cm in a Japanese serow (Atoji et al., 1988).

The histological examination of the interdigital sinus in this study revealed the presence of hair follicles, sebaceous glands and sweat glands; this indicated that the interdigital

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sinuses containing the interdigital glands were indeed peculiar tubular invaginations of the integument (Eurell et al., 2006).

Histochemical staining of the interdigital gland showed that the secretory acini secret neutral glycoproteins, visualized with positive reaction to the PAS procedure. On the other hand, acidic glycocoproteins did not seem to be present as demonstrated by the negativity of AB. These results were in agreement with Parillo and Diverio (2009) in the fallow deer (*Damadama*). The myoepithelial cells surround the secretory acini through their contraction help in gland discharge (Abbasi et al., 2009).

The interdigital gland varies among species; in the elephant, the interdigital glands are exclusively eccrine, discharging their secretion directly onto the skin surface (Lamps et al., 2001). On the other hand, the ovine interdigital glands have glandular secretory acini mainly of sebaceous and apocrine types, emptying their secretory content into hair channels (Kozlowski and Calhoun, 1969). Many histologists reported that, the ovine apocrine interdigital glands open into the hair channel; however, the glandular secretory acini do not function like apocrine glands as they empty their cytoplasmic content enveloped in granules via exocytosis process (Sokolov and Stepanova, 1988).

In accordance with previous studies, (Karahan et al., 2007; Abbasi et al., 2009), the lumen of the gland appeared filled with dense secretory materials of sebaceous, sweat and the interdigital gland itself along with groups of wool fibers which embedded in the luminal contents. Unlike, Janicki et al. (2003) who reported that the interdigital gland of the roebuck (*Capreolus capreolus* L.) devoid of connective tissue capsule and sweat glands, the interdigital gland under investigation surrounded by a capsule and also has sweat glands.

In conclusion, there is an obvious relation between the interdigital area (seat of the orifice of the interdigital sinus) and lameness in sheep. The main causes of lameness in sheep include foot rot, interdigital dermatitis and contagious ovine digital dermatitis (Wassink et al., 2003; Olechnowicz and Jaśkowski, 2011), and all of them accompanied with disorder in the interdigital space. Our study demonstrated the external orifice of the interdigital sinus on the dorsal aspect of the interdigital cleft opposite to the proximal inter-phalangeal joint. This orifice may acts as a port for entrance of Fusobacterium necrophorum subcutaneously developing foot rot in sheep (Bennett et al., 2009; Hodgkinson, 2010). At the same time, studies of Christodoulopoulos (2009), Olechnowicz and Jaśkowski (2011) did not mentioned the interdigital disorders as the main causes of lameness in goats but reported instead the cracks and erosion on the horn of the bulbs of the heel that extended along the internal side of the axial hoof wall. The correlation between absence of the interdigital sinus in goats and exclusion of the interdigital problems as main causes of lameness in these animals is something questionable about the role of such sinus and lameness. This relationship between the interdigital sinus inflammation and lameness is not yet defined, but correlation of lameness and the inflammation of such sinus is reported in about 16% from clinically lame cases in sheep (Bokko et al., 2003), that may support the positive opinion about this relation. On the other hand, it was found that prevalence of lameness is less in sheep (8–10%) than in goats (9–15%) (Olechnowicz and Jaśkowski, 2011) in spite of absence of interdigital sinus in goats that may reveals presence of additional lameness causes in goats related to their anatomical features and nature of activity.

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