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BESTAMİ YILMAZ

RAHŞAN YILMAZ

İSMAİL DEMİRCİOĞLU

İLKER ARICAN

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Morphological and histological structure of the interdigital gland in Awassi sheep (*Ovis aries*)

Bestami YILMAZ^{1*}, Raşan YILMAZ², İsmail DEMİRCİOĞLU¹, İlker ARICAN³

¹Department of Anatomy, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey

²Department of Pathology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey

³Department of Anatomy, Faculty of Veterinary Medicine, Uludağ University, Bursa, Turkey

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Abstract: We investigated the effects of sex and breeding season on the morphological and histological structures of the interdigital gland in Awassi sheep (*Ovis aries*). Forelimbs and hind limbs were collected from 10 males and 10 females in both the breeding (June–July) and nonbreeding (November–December) seasons. The interdigital glands were located between the first and second phalanges and were present on all of the limbs. The mean weight of the gland was 1.03 ± 0.03 g. Mean body length, body thickness, duct length, duct diameter, and duct opening diameter were 17.42 ± 0.23 mm, 8.12 ± 0.08 mm, 26.81 ± 0.31 mm, 4.65 ± 0.05 mm, and 3.53 ± 0.03 mm, respectively. All of the parameters had higher values in males than in females. In the breeding season, values were generally higher than those in the nonbreeding season. Gland secretions were pink and necrotic cell debris was observed in the gland lumen. The morphometric and histological data from this study indicate that the structure of the interdigital gland in Awassi sheep is similar to that in other sheep breeds. The results also suggest that this gland may play a role in sexual communication in sheep.

Key words: Anatomy, Awassi sheep, breeding, histology, interdigital gland

1. Introduction

Interdigital glands in ruminants are defined as skin invaginations located in the region between the digits (1–3). They are specialized skin structures that include all of the skin layers and have both sebaceous and apocrine sweat glands (4,5). The prenatal development of the pipe-shaped structure is complete by approximately the 90th–100th day (6). These glands are considered scent glands, and are responsible for sexual communication with the contents being secreted (7–11). Chemical secretions from the gland reportedly play an important role in the social and biological behaviors of ruminants, and are also useful for regional determination (12). The interdigital gland contributes to elasticity of the skin in the region where it is located, plays a role in protection from ultraviolet rays, and has fungicidal and bactericidal effects (13). In addition, its location and structure contribute to its clinical importance. Because of its location, the gland is subject to infections, such as interdigital sinusitis, that may be due to poor hygienic conditions and high humidity levels, particularly in the spring, as well as to mechanical injury by foreign bodies entering the gland (14). In sheep, interdigital sinusitis frequently occurs due to microorganisms and

foreign bodies entering the interdigital gland (4). Its incidence among foot diseases in sheep is 1.6% during pasture and 25.3% in the corral period (15).

The anatomical position and histological structure of the interdigital gland have been reported in various breeds of sheep (7,12,16), goat (17,18), serow (19), and roebuck (20). This is the first study on the morphological and histological structures of the forelimb and hind limb interdigital glands of male and female Awassi sheep (*Ovis aries*) during and outside of the breeding season.

2. Materials and methods

2.1. Sample collection

The interdigital gland samples used in the study were collected from Awassi sheep that were slaughtered in a private slaughterhouse in Şanlıurfa, Turkey. Samples were taken during both the breeding (June–July) and nonbreeding (November–December) seasons. For each season, the forelimbs and hind limbs from 10 males (average body weight: 65 kg; age: 12 months–2 years old) and 10 females (average body weight: 55 kg; age: 18 months–3 years old) were collected.

* Correspondence: byilmaz@harran.edu.tr

2.2. Radiography

Gland secretions were evacuated by massaging the feet in an in situ position. Then the gland sinus was lavaged using a saline solution with a plastic cannula placed within the orifice of the excretory duct, after which a contrasting agent containing barium sulfate (R-X Suspension 100%, Yenişehir Lab, Turkey) was injected. X-ray images were taken from both the dorsopalmar/dorsoplantar and mediolateral aspects.

2.3. Macroanatomic and morphometric examinations

The interdigital gland was dissected from the area between the digits. A precision scale (Vibra HT 224, Shinko Denshi Co., Ltd, Japan) was used to weigh the gland. Morphometric values (body length, duct length, body width, diameters of the duct and opening) from the gland were measured using digital calipers (Mitutuyo Corporation, Japan). Images of the gland were taken with a digital camera (Canon EOS 650D).

2.4. Histological techniques

Gland tissues were embedded in paraffin according to a routine procedure after fixation in a 10% neutral formaldehyde solution. Then 4- μ m-thick sections were stained with hematoxylin and eosin. Histological examinations were performed using a microscope (Olympus BX51) equipped with a digital camera (Olympus DP71).

2.5. Statistical analysis

The effects of sex (male or female) and season (breeding or nonbreeding) on the morphometric measurements of the interdigital gland were analyzed using two-way analysis of variance. Differences between morphometric measurements of the glands located on the forelimbs and hind limbs as well as on the right limbs and left limbs were tested using an independent t-test. Statistical analyses were performed using SPSS version 22. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Radiographic observations

The interdigital gland was located in the space between the digits at the first and second phalanges (Figures 1 and 2).

3.2. Macroanatomic and morphometric observations

The interdigital gland was present in all of the limbs. The orifice was located between the medial and lateral digits at the level of the anterior part of the proximal interphalangeal joint (Figures 3A and 3B). Hairs were observed dangling from the tip of the orifice. The gland resembled a tobacco pipe, with an orifice at the proximal tip of a long and narrow neck, a wide body, and a bend connecting the two structures (Figure 3C). The duct of the gland was oriented in a distopalmar/distoplantar direction, starting from the orifice to the middle of the medial phalanx. The gland formed a bend by curling up



Figure 1. Dorsopalmar (A) and mediolateral (B) radiographic appearance of the interdigital gland (forelimb).

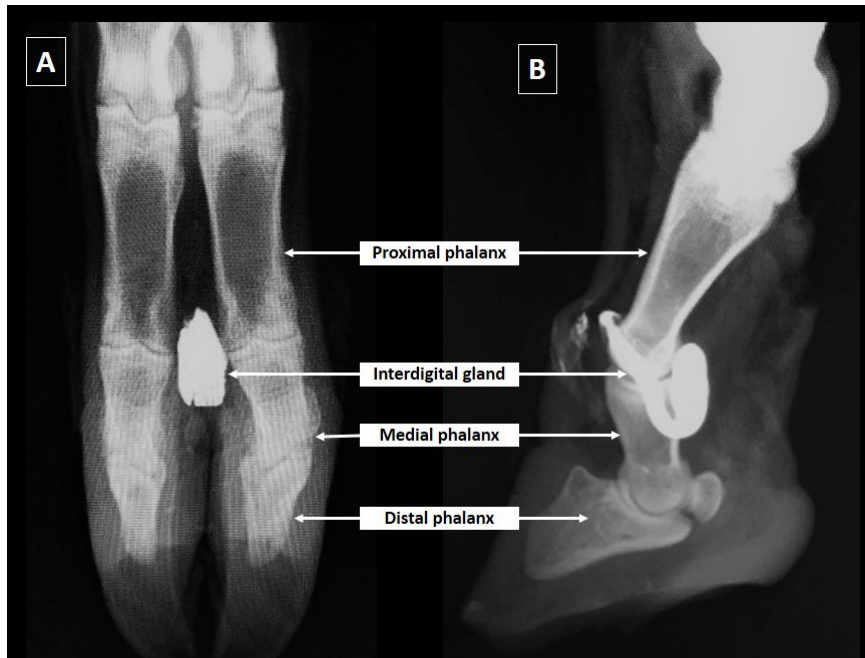


Figure 2. Dorsoplantar (A) and mediolateral (B) radiographic appearance of the interdigital gland (hind limb).

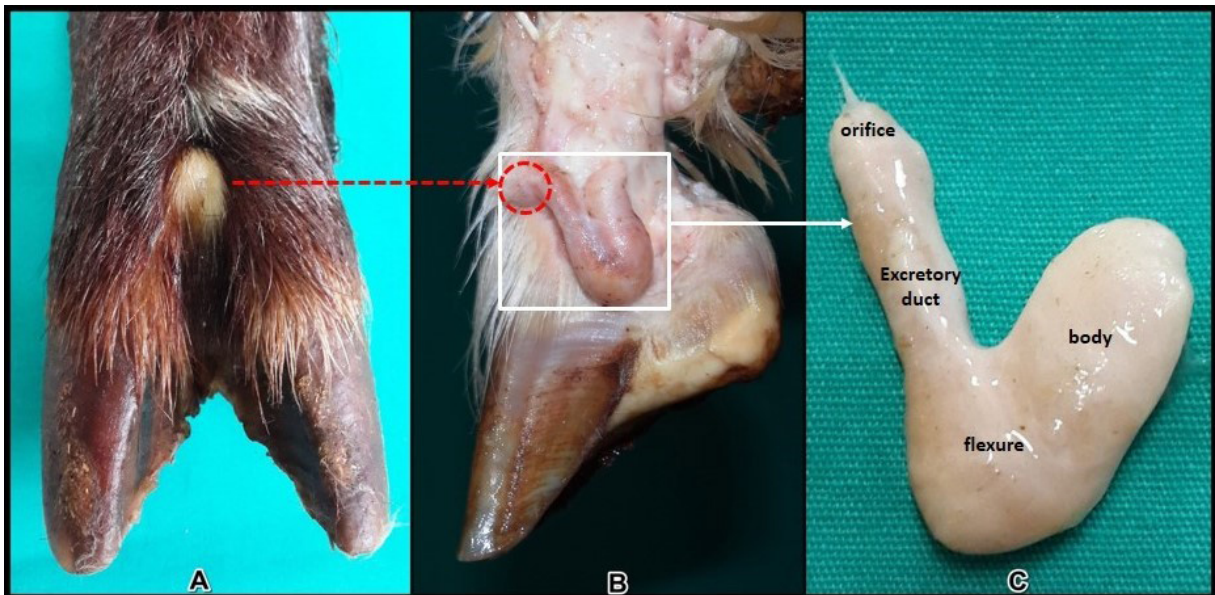


Figure 3. Cranial (A) and lateral (B and C) appearance of the interdigital gland.

at this level. The body, which formed a large part of the gland, reached the distal 1/3 of the proximal phalanx after the bend. The gland was attached to neighboring tissues by connective tissue. Hairs were observed within both the duct and the body. Morphometric measurements from the interdigital gland are shown in Figure 4. The mean weight

of the gland was 1.03 ± 0.03 g and was higher in males than in females ($P < 0.05$), as well as in the breeding season compared to the nonbreeding season ($P < 0.05$) (Table 1). Mean body length, body width, duct length, duct diameter, and duct opening diameter were 17.42 ± 0.23 mm, 8.12 ± 0.08 mm, 26.81 ± 0.31 mm, 4.65 ± 0.05 mm, and $3.53 \pm$

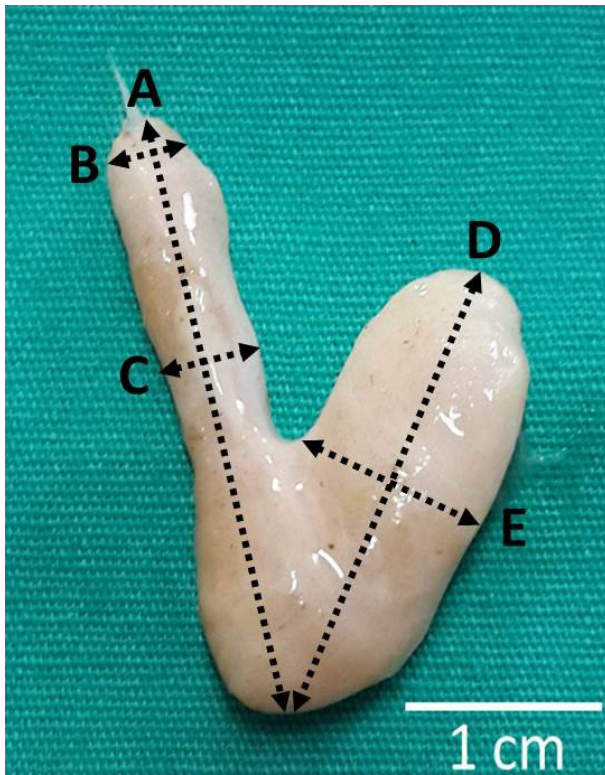


Figure 4. The interdigital gland and its parts (lateral appearance). A: duct length, B: duct opening diameter, C: duct diameter, D: body length, E: body width.

0.03 mm, respectively (Table 1). Males had higher values for gland weight ($P < 0.05$), body length ($P < 0.01$), body width ($P < 0.01$), duct length ($P < 0.001$), duct diameter ($P < 0.001$), and duct opening diameter ($P < 0.001$).

Measurements for gland weight ($P < 0.05$), body width ($P < 0.001$), duct length ($P < 0.001$), duct diameter ($P < 0.05$), duct opening diameter ($P < 0.05$), and body length were higher in the breeding season than in the nonbreeding season. All the morphometric parameters of the gland in the forelimb were higher than those in the hind limb ($P < 0.001$) (Table 2). No statistically significant differences in the measurements of the gland between right and left limbs were observed ($P > 0.05$) (Table 2).

3.3. Histological observations

Microscopic examination of the interdigital gland showed a similar structure in the neck and body of the gland, except that the body had a larger lumen compared to the neck. The lumen of both parts was filled with secretions of the apocrine sweat glands and sebaceous glands that were dark pink. Indeed, there were keratin debris, fragmented wool fibers, and desquamated cells of epithelium within the secretion. The secretion in the lumen of the body was more viscous than that in the neck. The sinuses of both the neck and body were covered by three layers of skin consisting of the epidermis, dermis, and a fibrous capsule (Figure 5). The epidermis was covered with a multilayered, keratinized, flat epithelium. In the dermis, hair follicles were scattered in the loose connective tissue with sebaceous and apocrine sweat glands and erector pili muscles. The sebaceous glands were located closer to the surface around the hair follicles. Apocrine sweat glands were located in clusters in the lower part of the dermis. The apocrine sweat gland epithelium was composed of a single row of cubic cells on the basal layer (Figure 6). The lumen of the glands was particularly large in samples collected during the breeding season and the presence of light pink secretions was noted. The fibrous capsule around the gland was composed of a dense connective tissue that included blood vessels, nerves, and fat cells.

Table 1. The effect of season and sex on the morphometric measurements of the interdigital gland analyzed by two way analysis of variance.

Parameter	Variable	Gland weight (g)	Body length (mm)	Body width (mm)	Duct length (mm)	Duct diameter (mm)	Duct opening diameter (mm)
Season	Breeding	1.101	17.601	8.488	27.899	4.761	3.596
	Nonbreeding	0.967	17.24	7.755	25.723	4.536	3.465
	P	*	n.s.	***	***	*	*
Sex	Male	1.097	18.151	8.394	28.266	4.892	3.738
	Female	0.971	16.691	7.849	25.356	4.405	3.324
	P	*	**	**	***	***	***
Grand mean		1.034	17.421	8.122	26.811	4.648	3.531
SEM		0.031	0.232	0.085	0.312	0.050	0.031

* ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$); n.s.: not significant

Table 2. The effect of direction and site on the morphometric measurements of the interdigital gland analyzed by t-test (mean \pm S.E.M.).

Parameter	Variable	Gland weight (g)	Body length (mm)	Body width (mm)	Duct length (mm)	Duct diameter (mm)	Duct opening diameter (mm)
Direction	Fore	1.254 \pm 0.050	19.877 \pm 0.398	8.919 \pm 0.150	29.476 \pm 0.586	5.144 \pm 0.093	3.947 \pm 0.059
	Hind	0.814 \pm 0.036	14.965 \pm 0.266	7.324 \pm 0.111	24.146 \pm 0.349	4.153 \pm 0.054	3.114 \pm 0.044
	P	***	***	***	***	***	***
Site	Right	1.035 \pm 0.050	17.648 \pm 0.464	8.270 \pm 0.156	26.880 \pm 0.650	4.638 \pm 0.087	3.538 \pm 0.069
	Left	1.032 \pm 0.050	17.194 \pm 0.406	7.974 \pm 0.160	26.742 \pm 0.472	4.659 \pm 0.101	3.524 \pm 0.070
	P	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*** (P < 0.001); n.s.: not significant

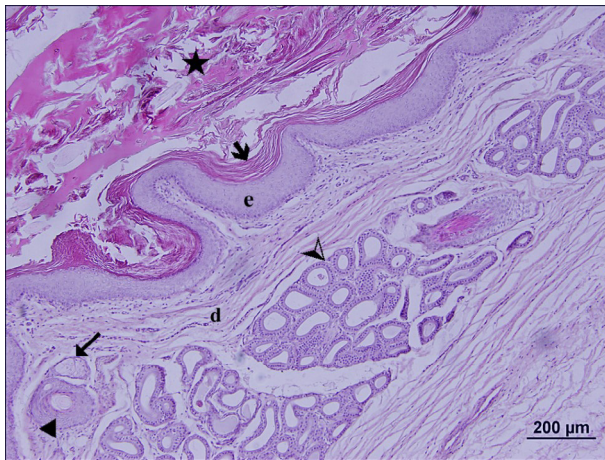


Figure 5. Microscopic appearance of the interdigital gland, 100 \times , H&E. Star: mass in the lumen; thick arrow: keratin layer; e: epidermis; d: dermis; arrowhead: cluster of apocrine glands; thin arrow: sebaceous gland; filled triangle: hair follicle.



Figure 6. Microscopic appearance of the apocrine sweat gland, 400 \times , H&E. Arrowhead: Apocrine sweat gland; thick arrow: excretory duct, thin arrow: capillary, L: the lumen of apocrine sweat gland and excretory duct.

4. Discussion

This study presents the topographic, morphometric, and histologic characteristics of the interdigital gland in Awassi sheep. Many studies have examined the interdigital gland. Its absence (17,21) and the existence of a rudimentary gland (18) were reported in goats. It was reported that the sheep (12,22,23), antelope (24), Japanese serow (5), and fallow deer (23) have the gland in all four limbs, whereas it is present only in the hind limbs in the deer (1) and roebuck (20). It is also present in all four limbs in Awassi sheep, and is located between the proximal and distal interphalangeal joints of the two main digits (7,11,12,14,16,17,25). The location of the interdigital gland in our study was consistent with the literature.

The shape of the interdigital gland reportedly resembles a pipe (7, 16) or sock (19). Awaad et al. (21) reported that the interdigital gland consists of a body with a neck, whereas Abbasi et al. (13) described it as a narrow channel

with a large pouch. Avdic et al. (14) defined the sections of the gland as the fundus, body, and column, whereas Szer et al. (7) described the parts of the gland as a blind proximal end with a large body and a long, narrow neck. Demiraslan et al. (16) described the gland as having four sections: mouth, excretion duct, bend, and corpus. Our sagittal view of the interdigital gland showed that it was a pipe-shaped structure consisting of three parts: a narrow neck with an orifice at the proximal tip, a wide body, and a bend connecting these two parts.

Szer et al. (7) reported that the weight of the interdigital sinus in Kivircik sheep was 0.84 g, whereas Abbasi et al. (13) reported that the average weight of the gland was 1.16 g in the forelimbs and hind limbs of male and female Iranian domestic sheep in both the breeding and nonbreeding periods. The average weight of the gland in Awassi sheep (1.03 \pm 0.03 g) was higher than that reported in Kivircik sheep and lower than that reported in

Iranian domestic sheep (13). These differences might be due to the different body weights of the breeds examined.

Demiraslan et al. (16) reported a corpus length of 21.9 mm, corpus width of 4.7 mm, duct length of 26.8 mm, and channel diameter of 2 mm for the interdigital gland in Kivircik sheep. Süzer et al. (7) reported a length of 16.74 mm, duct diameter of 4.6 mm, and trunk diameter of the body of 6.95 mm in their study on the forelimb interdigital gland of female Kivircik sheep. Abbasi (13) observed that the length, width, thickness, and duct length of the gland in Iranian domestic sheep were 15.80, 7.60, 7.71, and 26.06 mm, respectively. Misk and Misk (22) reported a duct length of about 30 mm, pouch length of 25 mm, and width of 6 mm in sheep. In our study, we found that mean body length, body width, duct length, diameter of the duct, and diameter of the orifice were 17.42, 8.12, 26.81, 4.65, and 3.53 mm, respectively. The morphometric measurements obtained in this study are consistent with those reported by other researchers.

In our study, all of the morphometric parameters in males had higher values than in females. These findings were in contrast to those reported by Atoji et al. (5) and Awaad et al. (21). This might be attributed to species or breed differences. However, our results were in accordance with those reported by Abbasi et al. (13), who compared the interdigital glands of male and female sheep in both the breeding and nonbreeding periods and reported that the glands of males in the breeding season had higher values in terms of volume and weight than in the nonbreeding season. The higher values observed in

males compared to females might be explained by the live weight differences between the sexes. Meyer (1) reported that average gland size and body weight are correlated. Average body weight of males is higher than that of females in Awassi sheep raised in different regions (26). In accordance with the findings published by Abbasi et al. (13), values in the present study were generally higher in the breeding season than in the nonbreeding season. In accordance with the results reported by several researchers (5,13,21) the morphometric parameters were also significantly higher in the forelimbs ($P < 0.001$). The wall of the body and neck consisted of the epidermis, dermis, and fibrous capsule. The epidermis consisted of stratified squamous keratinized epithelium. Sebaceous glands, apocrine sweat glands, hair follicles, and erector pili muscles were observed in the dermis. The sebaceous glands were closer to the surface, around the hair follicles. Apocrine sweat glands were located in the lower part of the dermis and were composed of a single layer of cubic cells. The fibrous capsule around the gland was composed of connective tissue, blood vessels, nerves, and fat cells. Our histological data are consistent with findings from previous studies (7,12,13,16,25).

We determined that the morphometric and histologic characteristics of the interdigital gland in Awassi sheep were similar to those of other sheep breeds. Higher morphometric values were observed during the breeding season compared to the nonbreeding season, and the lumen of the glands of the forelimbs was larger than that of the hind limbs, supporting the hypothesis that the interdigital gland plays a role in sexual communication.

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