

RESEARCH ARTICLE

Morphological, Morphometrical and Histological Structure of the Interdigital Gland in Norduz Sheep

Semine DALGA ^{1,a (*)} Serap İLHAN AKSU ^{2,b} Kadir ASLAN ^{1,c}
Turgay DEPREM ^{2,d} Reşit UĞRAN ^{2,e} Rezzan BAYRAM ^{1,f}

¹ Kafkas University, Faculty of Veterinary Medicine, Department of Anatomy, TR-36100 Kars - TURKEY

² Kafkas University, Faculty of Veterinary Medicine, Department of Histology and Embryology, TR-36100 Kars - TURKEY
ORCID: ^a 0000-0001-7227-2513; ^b 0000-0003-3100-498X; ^c 0000-0002-7617-0175; ^d 0000-0002-5523-8150; ^e 0000-0003-3431-8351

^f 0000-0002-0464-7333

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Abstract

In this study, it was aimed to determine the morphological, morphometric, and histological features of the interdigital gland in Norduz sheep. In our study, the interdigital gland in the fore and hind feet of 12 male and 12 female Norduz sheep were examined by first dissecting and removing the interdigital glands from the feet. The study material was used for morphometric and histological examinations after fixation in 10% formaldehyde solution. It was determined that the interdigital gland, located between two digits, has a large corpus located on the corpus of the phalanx media and consists of a long excretory duct opening to the anterior end of the joint between the phalanx proximalis and phalanx media extending from the corpus. The gland had pipe-like appearance. The length and diameter of excretory duct and diameter of the orifice of excretory duct were measured using a digital caliper through the excretory duct of the interdigital gland. The proximal, middle, and distal diameter of the corpus and the length of the corpus were measured using the same method through the corpus of the glands. The mean corpus length of the interdigital gland was 15.05±4.80 mm on the right side, 15.90±4.39 mm on the left side in females, and 15.58±4.00 mm on the right side and 16.14±3.02 mm on the left side in males. The length of the excretory duct was determined as 20.98±4.50 mm, 21.39±5.51 mm in females, and 21.94±2.61 mm and 21.94±2.91 mm in males. L2 and L7 parameters of the fore-feet and hind feet in male Norduz sheep were statistically significant. Tissue sections were stained with Crossmann's modified triple staining technique (Triple staining), hematoxylin and eosin (H&E), and Periodic acid-Schiff (PAS) staining methods. It was observed that the interdigital gland consisted of epidermis, dermis, and a capsule. Apocrine glands were found to be located close to the capsule in the dermis. It was determined that PAS+ areas in male sheep were more intense than in female sheep. There was no histological difference in the interdigital glands of female and male Norduz sheep.

Keywords: Interdigital gland, Norduz sheep, Morphometry, Morphology, Histology

Norduz Koyunlarında Interdigital Bezın Morfolojik, Morfometrik ve Histolojik Yapısı

Öz

Bu çalışma Norduz koyunlarında interdigital bezin morfolojik, morfometrik ve histolojik özelliklerini belirlemek amacıyla yapıldı. Bu amaçla 12 adet erkek ve 12 adet dişi Norduz koyununun ön ve arka ayaklarındaki interdigital bezler kullanıldı. Öncelikle ayaklardan interdigital bezler diseke edilerek alındı. %10 luk formaldehit solüsyonunda tespit edildikten sonra morfometrik ve histolojik incelemeler için kullanıldı. İki parmak arasında bulunan interdigital bezin phalanx media'nın corpus'u üzerinde yerleşmiş geniş bir gövdeye (corpus) sahip olduğu ve gövdeden uzanan phalanx proximalis ile phalanx media arasındaki eklemün ön kısmına açılan uzun bir boşaltım kanalından oluştuğu tespit edildi. Bez şekilsel olarak pipoya benzetildi. İnterdigital bezlerin akıtıcı kanal kısmının üzerinden digital kumpas yardımı ile akıtıcı kanalın uzunluğu, çapı ve kanal açıklığının çapı ölçüldü. Bezlerin corpus'u üzerinden ise corpus'un proximal, orta ve distal çapı ile corpus'un uzunluğu aynı yöntem ile ölçüldü. Dişilerde interdigital bezin ortalama gövde (corpus) uzunluğu sağ tarafta 15.05±4.80 mm, sol tarafta 15.90±4.39 mm, erkeklerde ise sağ tarafta 15.58±4.00 mm, sol tarafta 16.14±3.02 mm olarak tespit edildi. Akıtıcı kanal uzunluğu ise dişilerde 20.98±4.50 mm, 21.39±5.51 mm, erkeklerde 21.94±2.61 mm, 21.94±2.91 mm olarak tespit edildi. Erkek Norduz koyunlarının ön ve arka ayaklarından ölçülen L2 ve L7 parametreleri istatistiksel olarak anlamlıydı. Doku kesitlerine Triple, Hematoksilin&Eozin (H&E) ve Perodik Asit Schiff (PAS) boyamaları uygulandı. İnterdigital bezin epidermis, dermis ve kapsül bölümlerinden oluştuğu görüldü. Dermis'in kapsüle yakın olan bölümünde apokrin bezler ayırt edildi. Erkek koyunlarda PAS+ alanların dişi koyunlarına göre daha yoğun olduğu belirlendi. Dişi ve erkek Norduz koyunlarının interdigital bezlerinde histolojik olarak herhangi bir farklılık saptanmadı.

Anahtar sözcükler: Interdigital bez, Norduz koyunu, Morfometri, Morfoloji, Histoloji

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(*) Corresponding Author

Tel: +90 474 242 6839-5308 Cellular phone: +90 541 582 0218

E-mail: seminedalga@kafkas.edu.tr (S. Dalga)



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INTRODUCTION

Norduz sheep, a domestic sheep breed, are reared in rural areas in Norduz region in Gürpınar county of Van province, Turkey. The whole neck of the sheep is covered with fleece. White, followed by ashen color is frequently seen, a small amount of gray-white and brown-white colors are also observed [1]. Mammals have skin glands that differ in size, shape, and location. In ungulates, reproductive activity is thought to be mediated by odors associated with cutaneous glands, localized in specific parts of the body including infraorbital, tarsal, and interdigital areas [2,3]. The interdigital gland within the artiodactyla is a hoof-skin organ which involved in apocrine and holocrine secretion. The interdigital gland, which contributes to the elasticity of the skin in the surrounding tissues, provides protection against ultraviolet rays, and also has fungicidal and bactericidal effects [4,5]. In addition, these glands act as olfactory glands that produce odorous signals and pheromones that play important biological roles in chemical communication, such as active demarcation of territory and expressing social behavior [5,6]. Although these glands are found on all four limbs in sheep, in some species they are only observed in the hind feet [7]. Due to their location on the body, infections such as interdigital sinusitis occur, particularly in spring owing to poor hygienic conditions and high humidity levels, as well as mechanical injuries caused by foreign bodies entering the gland [8]. Pourlis [9] reported that although number of studies has increased recently, detailed morphological data on the functionality of sheep interdigital glands are not available.

Studies on the anatomical location, morphological, morphometric, and histological structure of interdigital glands in small ruminants are available in the literature [6,10-16]. The present study aimed to conduct morphological, morphometric, and histological examination of the interdigital glands in the fore and hind feet of male and female Norduz sheep (*Ovis aries*), which is of great importance and widely reared in the region, as well as to identify the location and structure of the interdigital glands, thereby providing data related to practice patterns for operative intervention.

MATERIAL AND METHODS

Ethical Approval

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee [KAÜ-HADYЕК] (Approval no: 2021/087).

Animals

The interdigital gland samples used in the study were collected from Norduz sheep (age, 1-2 years) slaughtered in the commercial abattoirs of Van province in November-December. The interdigital glands in the fore and hind feet

of 12 female and 12 male Norduz sheep were analyzed anatomically and histologically.

Anatomical Method

In total, samples from nine of the sheep were used for morphometric measurements and from three for histological examinations. The interdigital gland between the two digits was first dissected and then fixed in 10% formaldehyde solution (Fig. 1). After the fixation procedures, 7 parameters were measured using the digital caliper on the photographed interdigital glands (Fig. 2).

Parameters

L1: Diameter of excretory duct, **L2:** Length of excretory duct, **L3:** Diameter of corpus proximal end, **L4:** Medium diameter of corpus, **L5:** Diameter of corpus distal end, **L6:** Body length, **L7:** Duct opening diameter.

Histological Method

Histological examinations were performed by obtaining the interdigital gland tissues from the fore and hind feet of female and male Norduz sheep, and fixing the samples in 10% formaldehyde solution for 24 h. Afterwards, the tissues were embedded in paraffin blocks through routine histological tissue processing solutions. 6 µm thick sections of the paraffin-embedded blocks were prepared. To evaluate the tissues histologically, tissue sections were stained with Crossmann's modified triple staining technique (Triple staining), hematoxylin and eosin (H&E), and Periodic acid-Schiff (PAS). Blocks were examined under a light microscope (Olympus BX51, Japan).

Radiological Method

The glandular secretions were emptied by massaging the feet. Then, contrast agent was injected into the gland with a plastic cannula placed at the orifice of the excretory duct using saline solution. Sagittal and coronal images were taken (Fig. 3).

Statistical Analysis

For statistical analysis, the data were subjected to Independent-Samples T Test, using the SPSS 22.0 software package. A P-value of <0.05 was considered statistically significant while evaluating the significance of the differences between the mean values of the sheep interdigital gland.

RESULTS

Macro-anatomical Findings

It was determined that the interdigital gland was located on the corpus of the phalanx media between 2 toes in the fore and hind feet in Norduz sheep. It was observed that the interdigital gland consisted of a corpus, an excretory duct, and an orifice of the excretory duct (Fig. 1, 2, 3). It was

observed that the corpus of the gland was on the phalanx media. The excretory duct had an opening at the anterior edge of the junction of the phalanx proximalis and phalanx media. A large amount of hair was seen in the corpus and excretory duct forming the gland. It was determined that the interdigital gland in male Norduz sheep was larger than the gland in female animals.

Morphometric Analysis

In the statistical analysis between the fore and hind feet in Norduz sheep according to sex, the length of the excretory

duct (L2) and the orifice diameter of the excretory duct (L7) were statistically significant in male Norduz sheep ($P < 0.05$). There was no difference between other parameters in male animals and between all parameters in female animals. According to the statistical analysis of the sides (right/left) between the sexes, the mean corpus length was 15.05 ± 4.80 mm on the right side 15.90 ± 4.39 mm on the left side in females and 15.58 ± 4.00 mm on the right side and 16.14 ± 3.02 mm on the left side in males. It was determined that the mean values of left side measurements of the parameters were larger than that of right side the in both sex (Table 1).

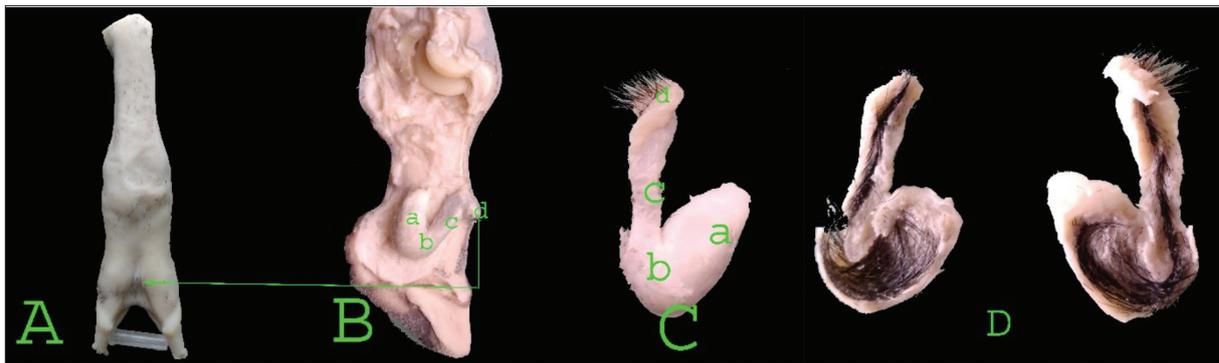


Fig 1. Location, morphological state and excretory duct of interdigital gland in Norduz sheep; (A) Cranial, (B, C) lateral appearance of the gland, (D) Sagittal section of gland in Norduz Sheep, a: body, b: flexure, c: excretory duct, d: orifice

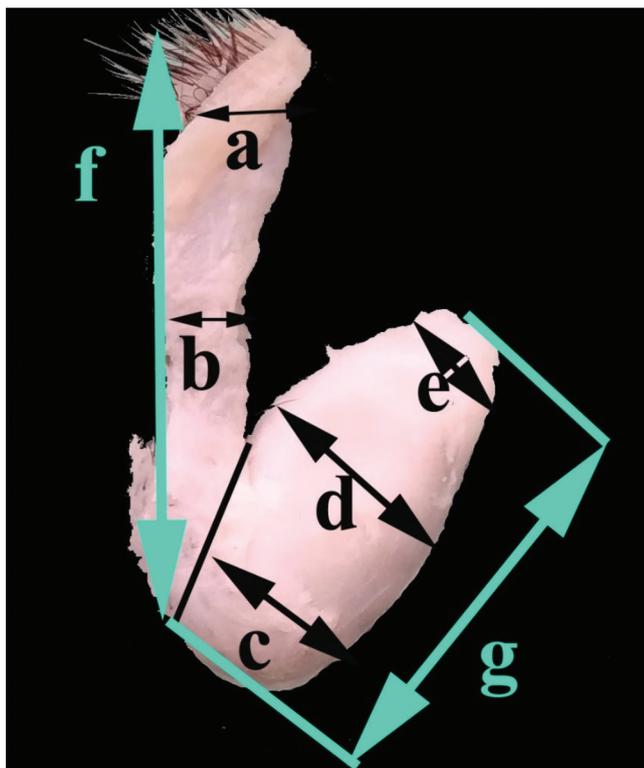


Fig 2. Morphometric measurement points of lateral appearance of the interdigital gland in Norduz sheep, a: duct opening diameter (L7), b: diameter of excretory duct (L1), c: diameter of corpus distal end (L5), d: medium diameter of corpus (L4), e: diameter of corpus proximal end (L3), f: length of excretory duct (L2), g: body length (L6)

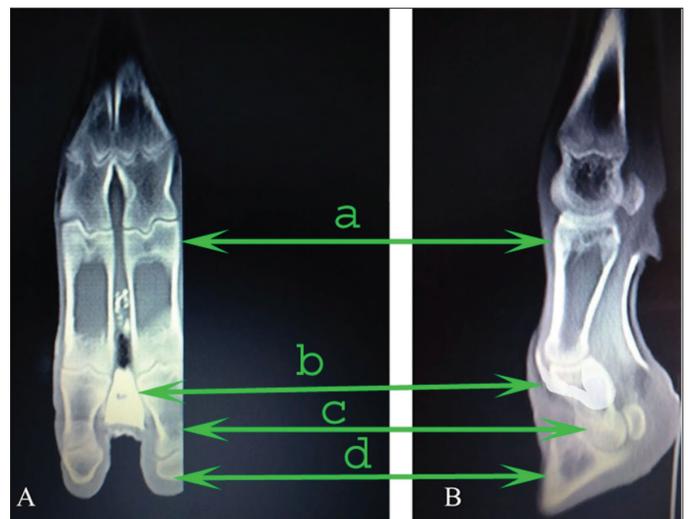


Fig 3. Radiological appearance of interdigital gland in Norduz sheep; (A) Dorsopalmar, (B) mediolateral, a: proximal phalanx, b: interdigital gland, c: medial phalanx, d: distal phalanx

Histological and Histochemical Examination

Interdigital glands sampled from both fore and hind feet of Norduz female and male sheep were examined histologically. The epidermis, dermis, and a capsule of the interdigital gland were distinguished in all samples (Fig. 4-A,B). It was observed that the epidermis surrounding the lumen consisted of stratified squamous keratinized epithelium. In the dermis, intense collagen fibers around

Table 1. Morphometric measurements of interdigital gland in Norduz sheep

Parameters	Female Norduz Sheep (mean±standard deviation)		Male Norduz Sheep (mean±standard deviation)		Female Norduz Sheep (mean±standard deviation)		Male Norduz Sheep (mean±standard deviation)	
	Right	Left	Right	Left	Fore	Hind	Fore	Hind
L1	3.91±0.49	4.43±1.10	4.27±0.46	4.52±0.83	3.85±0.26	4.49±1.14	4.13±0.74	4.66±0.48
L2 ^a	20.98±4.50	21.39±5.51	21.94±2.61	21.94±2.91	23.11±1.98	19.26±6.21	23.28±2.70 ^a	20.60±2.01 ^a
L3	5.27±1.02	5.92±1.36	5.49±0.96	5.52±0.58	5.99±0.74	5.21±1.5	5.48±0.82	5.63±0.77
L4	6.69±1.37	7.43±2.25	6.92±1.54	6.83±1.31	7.32±1.06	6.81±2.44	7.14±1.61	6.61±1.16
L5	5.73±1.72	7.36±2.79	6.79±1.68	6.51±1.25	6.48±1.95	6.61±2.90	6.31±1.43	6.99±1.46
L6	15.05±4.80	15.90±4.39	15.58±4.00	16.14±3.02	16.29±2.49	14.66±3.92	16.03±2.80	15.69±4.18
L7 ^b	3.79±0.66	3.63±0.91	4.59±0.77	4.62±0.81	3.70±0.65	3.72±0.92	4.26±0.65 ^b	4.95±0.74 ^b

^a*b* *P*<0.05; L1: Diameter of excretory duct; L2: Length of excretory duct; L3: Diameter of corpus proximal end; L4: Medium diameter of corpus; L5: Diameter of corpus distal end; L6: Body length; L7: Duct opening diameter

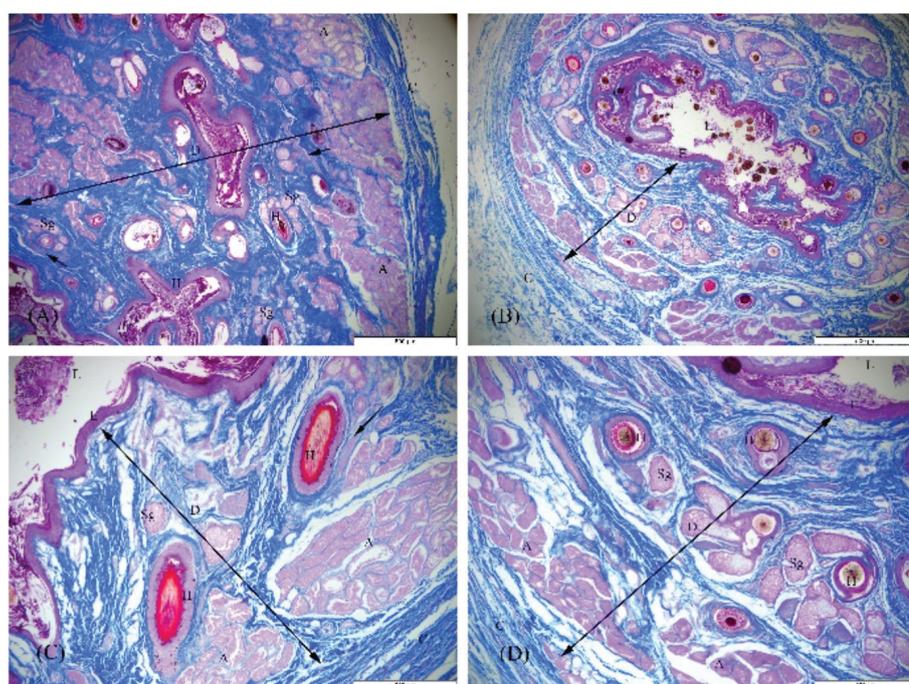


Fig 4. Histological structure of interdigital gland in Norduz sheep. (A-C): Male Norduz sheep, (B-D): Female Norduz sheep. L: Lumen, E: Epidermis, D: Dermis, A: Apocrine gland, C: Capsule, H: Hair follicle, Sg: Sebaceous gland, Arrows: m. erector pili; Triple stain

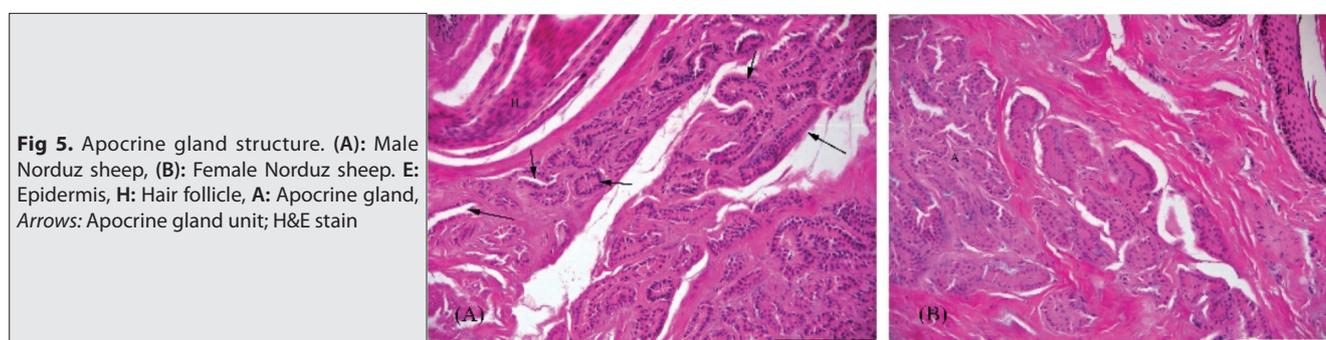


Fig 5. Apocrine gland structure. (A): Male Norduz sheep, (B): Female Norduz sheep. E: Epidermis, H: Hair follicle, A: Apocrine gland, Arrows: Apocrine gland unit; H&E stain

the sebaceous glands, hair follicles, arrector pili muscle, blood vessels, and nerve plexuses were observed (Fig. 4-C,D). The hair follicles surrounded by sebaceous glands were intense in the dermis. Secretory units with narrow lumen and consisting of a single row of cubic cells surrounded by myoepithelial cells were identified in the dermis mostly

close to the capsule (Fig. 5-A,B). It was determined that the outermost layer of the gland, the capsule, had a loose connective tissue structure and contained blood vessels, nerve plexuses, and adipose cells. PAS staining showed that PAS⁺ was observed in the basal membranes of the apocrine glands in the interdigital gland. PAS⁺ areas were

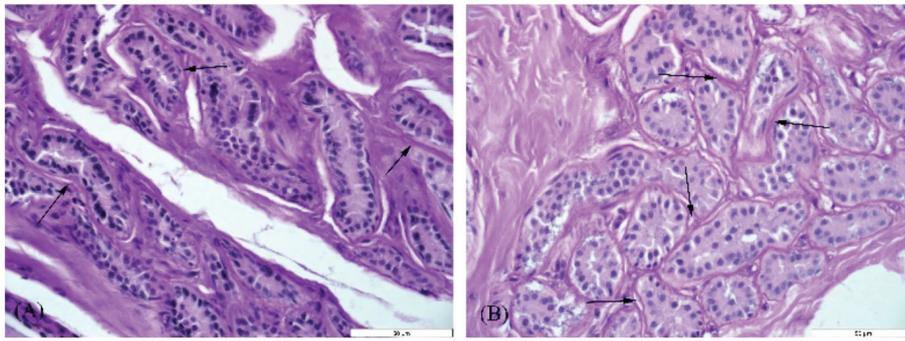


Fig 6. PAS stain for apocrine glands. (A): Male Norduz sheep, (B): Female Norduz sheep. Arrows: PAS+ areas; PAS stain

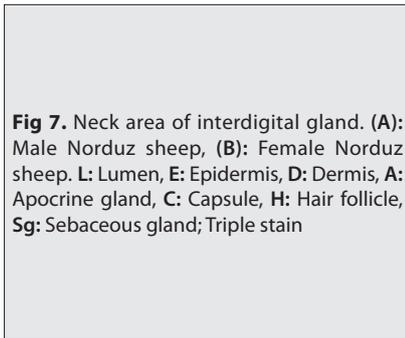
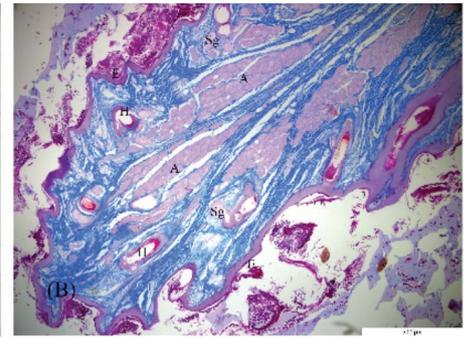
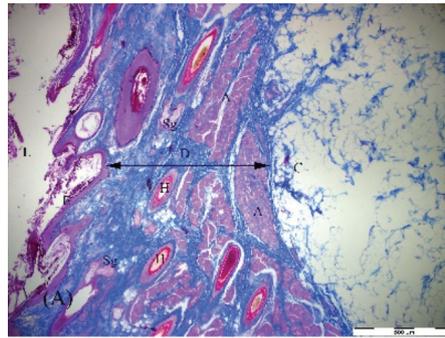


Fig 7. Neck area of interdigital gland. (A): Male Norduz sheep, (B): Female Norduz sheep. L: Lumen, E: Epidermis, D: Dermis, A: Apocrine gland, C: Capsule, H: Hair follicle, Sg: Sebaceous gland; Triple stain



not found in the lumens of the secretion units. It was determined that PAS+ areas were more intense in male sheep than in female sheep (Fig. 6-A,B). Histologically, the neck regions of the interdigital gland composed of similar structures as the corpus region (Fig. 7-A,B). There was no histological difference in the interdigital glands of female and male Norduz sheep. It was observed that the interdigital glands of the fore and hind legs consisted of the same structures.

DISCUSSION

In this study, morphological, morphometric and histological features of interdigital gland were determined in Norduz sheep. Accordingly, Gürbüz et al.^[17] have reported that the length of the gland was 30.18 ± 1.93 mm in the fore-feet and 25.67 ± 1.77 mm in hind feet in male Hemshin sheep. They also have stated that the interdigital gland in fore-feet was significantly larger than that of the hind-feet its collected in December. In our study, it was observed that measurements of hind-feet were greater than that of the fore-feet, and intersex comparisons showed males have larger gland parameters than females. This result is similar to Lori's sheep^[4]. Süzer et al.^[10] have examined the morphological and immunohistochemical features of the sinus interdigitalis in Kivircik sheep and reported that the sinus has a tube-like appearance and is located between the proximal and distal interphalangeal joints between the two toes. In Norduz sheep, it was observed that the gland has a corpus located on the phalanx media between two toes and a long excretory duct opening to the distal edge of the phalanx proximalis in both sexes. Interdigital glands had

a pipe-like appearance in our study, which was consistent with the literature^[4,14].

Kara et al.^[15] reported that the corpus and excretory duct lengths of the interdigital gland were 17.25 ± 1.09 mm, 26.39 ± 2.22 mm in fore-feet and 13.67 ± 0.85 mm, 22.64 ± 2.30 mm in hind feet in Hasmer sheep and 16.33 ± 0.92 mm, 29.65 ± 0.82 mm in fore-feet, 12.89 ± 0.66 mm, 23.51 ± 1.21 mm in hind feet Hasak sheep, respectively. Similarly, in the study Yılmaz et al.^[14] investigating the effects of sex and breeding season (June-July) on the morphological and histological structures of the interdigital gland in Awassi sheep, the corpus and excretory duct lengths of the gland were 19.87 ± 0.39 mm and 29.47 ± 0.5 mm in fore-feet and 14.96 ± 0.26 mm and 24.14 ± 0.34 mm in hind feet, respectively. In addition, Yılmaz et al.^[14] have stated that all parameters of the gland in Awassi sheep have higher values in males compared to females. Similarly, it was observed in our study that the values of parameters in males were higher among Norduz sheep.

The histological examinations of Norduz sheep revealed that interdigital gland wall structure consists of epidermis, dermis and a capsule as observed in Lori's sheep^[4], Kivircik sheep^[11], Baladi sheep^[18], Hemshin sheep^[17], Awassi sheep^[14], Hasmer and Hasak sheep^[15]. As in other sheep breeds, the interdigital gland of Norduz sheep was composed of stratified keratinized epithelium, and sebaceous glands, hair follicles, arrector pili muscle, blood vessels, and nerve plexuses were observed in the dermis.

Abbasi et al.^[4] have reported the presence of sweat glands located close to the capsule and surrounded by myoepithelial cells, and that sweat glands occupy more

space in the interdigital gland compared to the sebaceous glands located in the superficial area. In Norduz sheep, it was observed that the apocrine glands were located close to the capsule and occupy a large area in the interdigital gland. Unlike other studies, we determined that the apocrine gland units consisted of a single row of cubic cells but had a narrow lumen. This suggests that the interdigital gland, which has been reported to have activity during the reproductive period, may cause periodic or age-dependent changes in the histology of apocrine glands^[5,14].

Janicki et al.^[7] in their study examining the interdigital gland structure of roe deer, reported that the interdigital gland structure is not surrounded by connective tissue (capsule) and has no sweat glands. Similar to other studies^[4,11,18], we determined that the interdigital gland of Norduz sheep was surrounded by a capsule formed by connective tissue, and there were blood vessels, nerve plexuses and fat cells in the capsule. In our study, PAS staining showed no neutral glycoprotein in the lumen of apocrine glands in contrast to Kivircik sheep^[11] and similar to Lori's sheep^[4]. In addition, no histological difference was observed between male and female interdigital glands.

Interdigital gland in Norduz sheep is considered to be similar in shape and structure to other sheep breeds, but different in terms of morphometrics. The similarities and differences of the morphological, morphometric and histological features of the interdigital gland will form the basis of future sexual function studies in Norduz sheep.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that data supporting the study findings are also available to the corresponding author.

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This work was not supported by any institution.

COMPETING INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

SD and SİA designed the study. SD, KA, and RB performed the macro-anatomical and morphometric analysis. SİA, TD, and RU carried out histological examination. SD and SİA carried out the statistical analysis. SD, SİA, and KA performed the imaging all section. The manuscript was written by SD, SİA, and RU. All authors approved the final version.

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