

Morphological and Immunohistochemical Features of Interdigital Sinus in Kivircik Sheep

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Article Code: KVFD-2015-13902 Received: 22.06.2015 Accepted: 24.08.2015 Published Online: 24.08.2015

Abstract

Interdigital sinus secretions play a role in sexual behavior and reproductive process of sheep. In the present study, it was aimed to characterize the topographical, anatomical and immunohistological features of interdigital sinus in Kivircik sheep. The interdigital sinuses were dissected from 50 forefeet of female Kivircik sheep which were slaughtered in the slaughterhouse. The shape of sinus resembled a pipe and was located between the proximal and distal interphalangeal joints of two digits. The wall of interdigital sinus had three layers as epidermis, dermis and fibrous capsule. Epidermis consisted of a stratified squamous keratinized epithelium. Sebaceous glands, hair follicles, arrector pili muscles, and apocrine sweat glands were in the dermis. Furthermore, there were vessels and adipose tissue in the fibrous capsule. There was immunohistochemical reaction for estrogen receptor expressed in epidermis, apocrin sweat glands and sebaceous glands of interdigital sinus. In conclusion, our results about the topographical, anatomical and immunohistological features of interdigital sinus are very important to understand sexual behavior and reproductive process of sheep, and to make a new approach for its surgery. Furthermore, this study is the first to report the interdigital sinus of Kivircik sheep immunohistochemically.

Keywords: *Interdigital sinus, Kivircik sheep, Morphology, Immunohistochemistry*

Kıvırcık Koyunlarda *Sinus Interdigitalis*'in Morfolojik ve Immunohistokimyasal Özellikleri

Özet

Sinus interdigitalis salgıları koyunlarda seksüel davranış ve üreme sürecinde rol oynamaktadır. Bu çalışma ile Kıvırcık koyunlarda *sinus interdigitalis*'in, topografik, anatomik ve immünohistolojik özelliklerinin tanımlanması amaçlanmıştır. Çalışmada kullanılan *sinus interdigitalis* 'ler, mezbahada kesilen 50 adet Kıvırcık koyunun ön ayaklarından disseke edildi. Şekli bir pipoyu andıran *sinus interdigitalis*'in, iki parmak arasında articulatıo interphalangea proksimalis ve distalis manus hizasında yerleştiği gözlemlendi. *Sinus interdigitalis* duvarının epidermis, dermis ve fibröz kapsül olarak üç katmandan oluştuğu gözlemlendi. Epidermis'in, çok katlı yassı keratinize epitelden oluştuğu saptanırken, dermis katmanında yağ bezleri, kıl folikülleri, mm. arrectores pilorum ve apokrin ter bezlerinin varlığı görüldü. Ayrıca, fibröz kapsül içerisinde kapillar damarlar ve yağ doku bulunmaktaydı. Sinus 'un epidermis, apokrin ter bezleri ve yağ bezlerinde östrojen reseptörleri için immünohistokimyasal tepki olduğu gözlemlendi. Sonuç olarak, *sinus interdigitalis*'in topografik, anatomik ve immünohistolojik özellikleri hakkında elde ettiğimiz sonuçlar, koyunlardaki seksüel davranış ve üreme sürecini anlama ve *sinus interdigitalis*'in operasyonlarına yeni bir yaklaşım geliştirmesi bakımından önemlidir. Çalışma, Kıvırcık koyunlarda *sinus interdigitalis*'in immunohistokimyasal yapısı üzerine yapılan ilk çalışmadır.

Anahtar sözcükler: *Sinus Interdigitalis, Kıvırcık koyunu, Morfoloji, Immunohistokimya*

INTRODUCTION

In mammals, there are different kinds of skin glands such as circumoral, horn, infraorbital and interdigital glands. Shape, size and location of these glands are unlike between in species and even in race. Interdigital glands are located in space between the digits and calls interdigital

sinus in sheep. While these sinuses are found on 4 limbs in sheep of both sexes, a rudimentary type is also present in goat^[1-7].

Interdigital sinus resembles tobacco pipe shape^[4,8]. This sinus excretes holocrine and apocrine secretion^[1-8]. This chemical secretion plays an important role on bio-



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logical behaviors and territorial demarcation [9,10]. The scent material secreted from interdigital sinus is composed of evaporating elements such as pheromones, ketones, aldehydes and alkanes [11,12]. Pheromones are very important for sexual behavior and reproductive process of sheep and their secretion start especially in the estrus cycles. Estrogen receptors effect on sexual behaviors in mammals, and are very important for secretion of sexual pheromones [13]. Interdigital sinuses are also apt to be contaminated, therefore their location is very important for the surgical intervention.

Kivircik sheep are mainly raised in the northwestern part of Turkey and there is limited report about their interdigital sinus's topographical, anatomical and histological features [14]. Therefore in the present study it was aimed to investigate morphological, topographical and immunohistological features of interdigital sinus of Kivircik sheep to understand more their sexual behavior and reproductive process of sheep, and to make a new approach for its surgery.

MATERIAL and METHODS

In the current study, 50 Kivircik breed, which were slaughtered in the slaughterhouse of Çimet/Bursa in April, were used. Sinuses were dissected (Fig. 1) and collected from forefeet of 3 to 6 months aged female sheeps. The average weights of these sheep were 25 kg. After dissection, interdigital sinus was weighted and length and diameter of the sinus were measured with a digital compass (Mitutuyo Corporation, Kawasaki, Japan). Diameters of the sinus were showed in Fig. 2.

For histological procedures, tissues were fixed in 10% neutral buffered formalin solution for 24 h at room temperature and routine tissue processing procedures were applied. After dehydration in 70% ethanol, 80%

ethanol, 96% ethanol, and absolute ethanol, the interdigital sinus tissues were embedded in paraffin wax and the sections are obtained at 5 µm. intervals. Tissue sections were mounted on polylysine-coated slides and incubated for 1 h at 37°C in an oven. Slides were stained with triple Mallory staining modified by Crossman [15] for investigating the histological structure of interdigital sinus or kept until immunohistochemistry was performed.

The streptavidin biotin peroxidase complex technique was used for immunohistochemical staining. Briefly, sections were deparaffinized and rehydrated, then the slides were placed in 0.05% Saponin solution (Serva, Cat. No: 8047-15-2, Germany) for 20 min at room temperature. Sections were washed three times for 5 min each in phosphate buffered saline (PBS) and blocked with blocking serum-Reagent A (Histostain®-Plus Bulk Kit, 85-6743, Zymed Laboratories, USA). Then, sections were incubated with ready to use monoclonal rabbit anti-estrogen receptor primary antibody (Neomarkers, RM9101) overnight at 4°C in a humidified chamber. After being rinsed three times each in PBS for 5 min, sections were incubated for 10 minutes with biotinylated antibody-Reagent B (Histostain®-Plus Bulk Kit, 85-6743, Zymed Laboratories, USA). Subsequently, slides were washed with PBS and incubated with streptavidin complex containing horseradish peroxidase-Reagent C (Histostain®-Plus Bulk Kit, Zymed Laboratories, USA) for 10 min at room temperature. After washing with PBS, 3, 3' dimethylaminoazobenzene (DAB, Zymed Laboratories, USA) was used as the chromogen which was prepared according to manufacturer's instructions and applied for 5 min. All sections were counterstained with Harris' heamatoxylin, in order to distinguish negative reactions from positive reactions. The slides were then rinsed in tapped water, dehydrated, and mounted with Entellan (Merck, Germany). Negative controls were performed using the same protocol but substituting the primary antibodies with PBS. Slides were examined under



Fig 1. Interdigital sinus and it's oriface (arrow) in Kivircik Sheep

Şekil 1. Kivircik koyunda sinus interdigitalis ve açılışı (ok)

the light microscope (Nikon eclipse 80i Microscope, Tokyo, Japan). Photography was taken with Nikon Ds Camera Control Unit DS-L1 (Tokyo, Japan).

Immunohistochemical staining was scored in a semi-quantitative manner to determine differences between the slides in the distribution patterns of intensity of immunolabelling of the epithelium, sebaceous glands, apocrine glands, and fibrous capsule. All slides were scored as no staining (-), weak (+), moderate (++), and strong (+++) by two researchers in a blind manner. This analysis was performed according to Adams et al.^[16].

RESULTS

Topographically, the interdigital sinus was located between the proximal and distal interphalangeal joints of two digits (Fig. 1).

Anatomically, the interdigital sinus resembled a pipe in shape. It was composed of a blind proximal end, a wide body and long-narrow neck (Fig. 2). The body of the sinus was proximodorsal to distopalmar positioned in-between medial and lateral digits. Then the body was curled up at the level of the distal interphalangeal joints and formed the neck of the sinus. The body of gland was distinguishably had a notable flexure from the neck. Its oriface was determined from dorsal aspect at the level of the anterior part of the interdigital cleft (Fig. 1, arrow). The interdigital sinus was connected to neighbor tissues with connective tissue. Morphometric values of the sinus were shown in Table 1.

The weight of interdigital sinus in Kivircik Sheep was 0.84 ± 0.24 g. The ratio of sinus weight to body weight was found to be 0.003. The sinus' proximal and distal end diameters were close to each other although the body diameter is thicker than that value. The excretory duct of interdigital sinus was quite long. Wool fibers in the excretory duct were found.

Histologically, the wall of interdigital sinus had three layers: epidermis, dermis and fibrous capsule. Epidermis consisted of a stratified squamous keratinized epithelium. In the dermis, there were sebaceous glands, hair follicles, arrector pili muscles, and apocrine sweat glands. Sebaceous glands, hair follicles and arrector pili muscles were located in the upper portions of dermis however apocrine sweat glands were located in the deeper portions of dermis as seen in Fig. 3. Sebaceous glands were lobular with cluster of acini. Each acinus was composed of peripherally located small basal cells, which were surrounded by larger round cells that were filled the remainder of the acinus. Each hair follicle were surrounded by connective tissue and there were arrector pili muscles near hair follicles. The epithelium of apocrine sweat glands were simple columnar in the body however the epithelium of apocrine sweat glands were simple cuboidal in the neck. Moreover, myoepithelial cells were surround by the apocrine sweat glands. The outer part of the interdigital sinus was of fibrous capsule and had vessels and adipose tissue (Fig. 3).

The cellular localization of estrogen receptor in the interdigital sinus was ascertained by streptavidin biotin complex method (Fig. 4). In this protocol, a positive reaction is characterized by the deposition of a brown

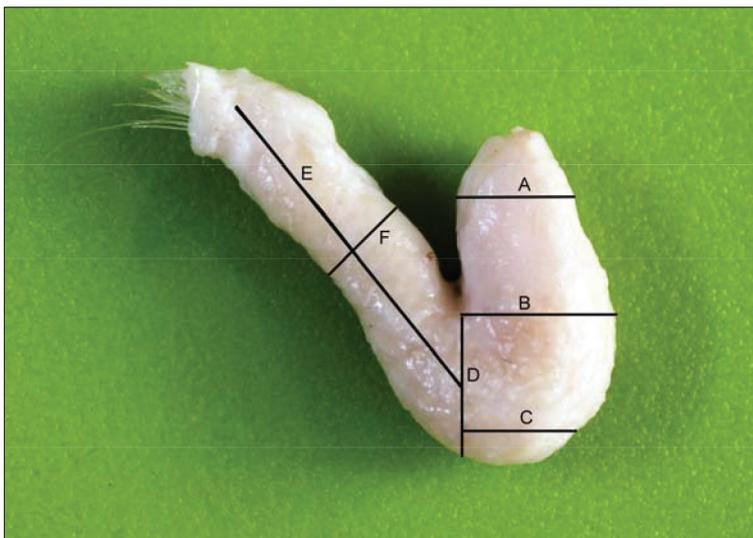


Fig 2. Interdigital sinus and its parts

A: Diameter of proximal end, B: Diameter of body, C: Diameter of distal end, D: Diameter of duct and sinus anastomose, E: Length of duct, F: Diameter of duct

Şekil 2. Sinus interdigitalis ve bölümleri

A: Proximal ucun çapı, B: Gövde çapı, C: Distal ucun çapı, D: Kanal ve sinus birleşme yerinde çapı, E: Kanalın uzunluğu, F: Kanalın çapı

Table 1. Morphometric values of the interdigital sinus

Tablo 1. Sinus interdigitalis'in morfometrik değerleri

Weight (g) $\bar{X} \pm S\bar{X}$	A (mm) $\bar{X} \pm S\bar{X}$	B (mm) $\bar{X} \pm S\bar{X}$	C (mm) $\bar{X} \pm S\bar{X}$	D (mm) $\bar{X} \pm S\bar{X}$	E (mm) $\bar{X} \pm S\bar{X}$	F (mm) $\bar{X} \pm S\bar{X}$
0.84±0.24	6.47±1.07	6.95±0.97	5.77±0.90	8.62±1.31	16.74±2.21	4.60±0.60

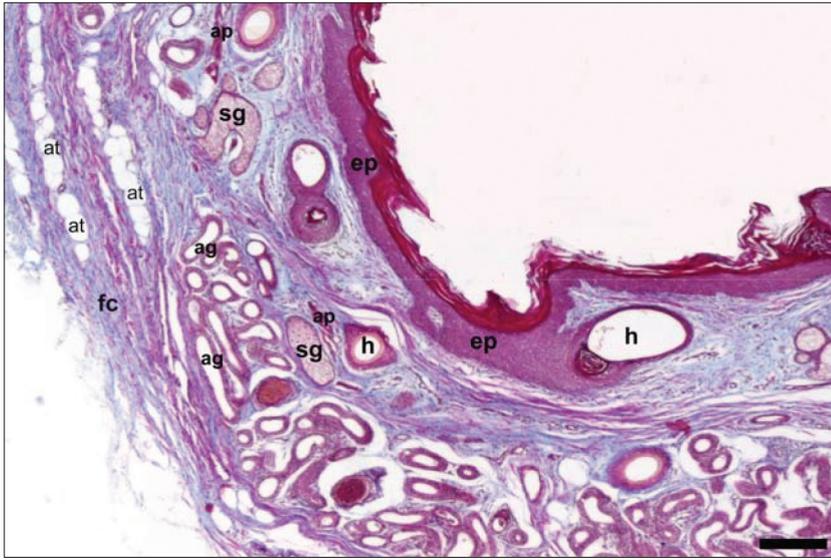


Fig 3. Transverse section of interdigital sinus. Stratified squamous keratinized epithelium (ep), sebaceous gland (sg), apocrine sweat gland (ag), arrector pili muscle (ap), hair follicle (h), adipose tissue (at), fibrous capsule (fc). Crossman's triple staining technique. Bar: 100 μ m

Şekil 3. *Sinus interdigitalis*'in transversal kesiti. Çok katlı yassı keratinize epitel (ep), yağ bezi (sg), apokrin ter bezi (ag), m. arrector pili (ap), kıl folikülü (h), yağ dokusu (at), fibröz kapsül (fc). Crossman'ın üçlü boyama tekniği. Bar: 100 μ m

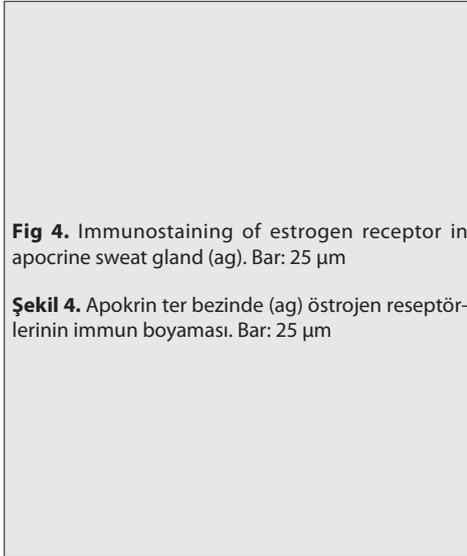
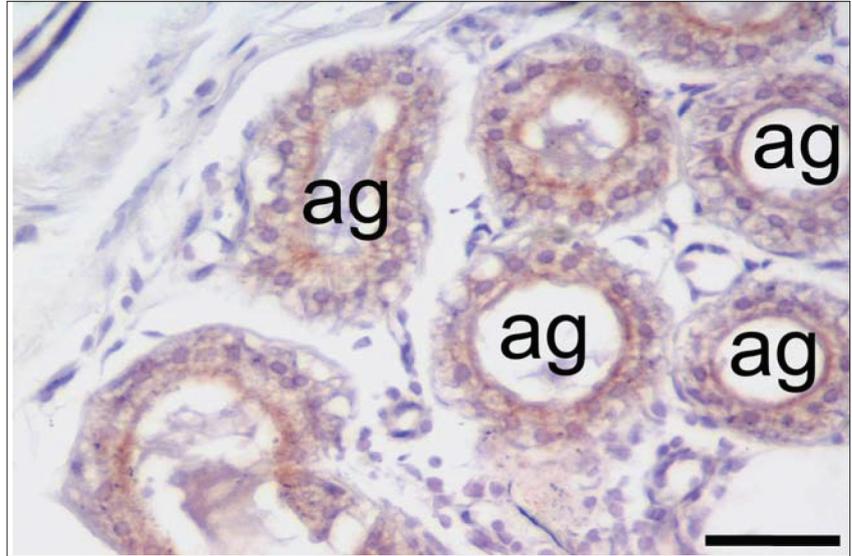


Fig 4. Immunostaining of estrogen receptor in apocrine sweat gland (ag). Bar: 25 μ m

Şekil 4. Apokrin ter bezinde (ag) östrojen reseptörlerinin immun boyaması. Bar: 25 μ m



precipitate at the site of the antigen-antibody interaction. It is notable that, in all positive immunoreactions were found in intracytoplasmic location and nuclear staining was not observed. Estrogen receptor immunoreactivity was only seen in epidermis, apocrin sweat glands and sebaceous glands. The expression of estrogen receptor was weak (+) in sebaceous gland, moderate (++) in epidermis and strong (+++) in apocrine sweat glands. In addition that, there was no immunostaining in fibrous capsule and no immunoreactivity in the negative control was observed.

DISCUSSION

The histomorphological structure of the interdigital sinus has been described in many animal species [1-4,11,12,17,18]. This study presents some characteristics of interdigital sinus in Kivircik sheep, which is usually raised in West Anatolia.

Embryologically, the interdigital sinus's epidermis and associated glands are formed by ectoderm and connective tissue of dermis and hypodermis are formed by mesoderm [5,19,20]. So, it contains sweat and sebaceous glands, hairs. In agreement with previous articles, the luminal surface of interdigital sinus in Kivircik sheep contained wool fibers. The localization of interdigital sinus was similar to the findings reported by the literature [2,4,5,20].

The interdigital sinus, a pipe shaped, contained a blind proximal end, a wide body and long-narrow neck, as documented by the literature [3-5,20]. The body diameter values of sinus had close values in sheep [21,22] and in Iranian Native Breed of sheep [4]. While the average length of duct in Kivircik sheep was 16.74 mm, the same parameter was 18-20 mm in sheep [22] and 10 mm. for Japanese serow [18]. While the diameter of duct in sheep was reported as 2-4 mm [22], we measured as 4.60 mm in Kivircik sheep, as in Iranian Native Breed of sheep [4]. Some minimal value

differences may result from breeding and non-breeding season of sheep.

We showed that the wall of interdigital sinus in the Kivircik sheep had similarities with other small ruminants' skin histological structures due to having three layers such as epidermis, dermis and fibrous capsule. Epidermis consisted of a stratified squamous keratinized epithelium. In the dermis, there were a great number of sebaceous glands, hair follicles, arrector pili muscles, and apocrine sweat glands. Also, there were vessels and adipose tissue in the fibrous capsule. Our findings are agree with the results of previous studies that showed the histological structures of Iranian Native Breed of sheep [4], Akkaraman breed sheep [5] and Tuj sheep [20].

The estrogen receptor belongs to the steroid hormone nuclear receptor superfamily [23], and it plays important roles on either regulation of various functions in skin, mainly hair growth, regulate sebaceous and sweat gland activity [24] or secretion of sexual pheromones [25]. Especially, apocrine sweat glands regarded as pheromone-producing scent glands [24] and their secretions play a role in demarcation of territorial area [6]. The interdigital sinus is a structure which is rich in apocrine glands.

In our study, estrogen receptor was detected only in epidermis, apocrine sweat glands and sebaceous glands. The immunoreaction of estrogen receptor was stronger in apocrine sweat glands. Estrogen receptor was observed in human skin and it was reported that there were immunoreaction of estrogen receptor in epidermis, sweat and sebaceous glands [24,26]. Our findings are consistent with these reports.

As a result, this study is the first to report the histological structures of the interdigital sinus including immunohistochemical localization of estrogen receptor in Kivircik sheep. Our immunohistochemical results showed that interdigital sinus's secretions may play a role on mammalian sexual behavior and reproductive process and also these results provide information for future investigation on the roles of steroid hormone either on skin functions or in sexual behaviors in sheep.

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