

# Effect of sex on histological and histochemical structures of interdigital sinus in adult Bakhtiari sheep of Iran

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## Keywords

histochemical, histology, interdigital Sinus, sex, sheep

## Abstract

The aim of this study was to investigate the effect of gender on the histological and histochemical structures of various anatomical regions of the interdigital sinus in the Iranian native sheep. Tissue samples from different anatomical regions of each sinus were obtained from 15 female and 15 male adult Bakhtiari sheep. The sections stained with H&E, Periodic acid- Schiff, Alcian blue, Verhoeff's, Masson's trichrome, and Gomori's method. The sinus was covered by skin and fibrous capsule. The sinus contained descending part, flexure, ascending part, excretory duct and orifice. The sebaceous glands were simple branched acinar and the sweat glands were simple coiled tubular type. The secretory caps were observed in the secretory

cells protruded into the lumen. The various histological and histochemical structures of the interdigital sinus showed no considerable differences among various anatomical regions of the right and left sinuses. Also, no significant sex-based differences were found. The surface epithelial cells of the apocrine glands and their secretion contained both neutral and acidic glycosaminoglycans. It is concluded that the general histological and histochemical structures of the interdigital sinus in Bakhtiari sheep were similar to those of other native sheep, but there were also some differences.

## Abbreviations

H&E: Hematoxylin eosin  
PAS: Periodic acid- Schiff  
AB: Alcian blue

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## Introduction

Sebaceous glands arise from epidermal buds of the skin. In defined body regions of some domestic species especially in the interdigital sinuses of sheep, well developed accumulations of sebaceous glands are obvious [1]. Interdigital sinuses are found on the forelimbs and hindlimbs of sheep of both sexes [2], and located between the digits just above the hoofs [3]. Numerous apocrine sweat glands are associated with the interdigital pouches of sheep [4]. These pouches possess waxy secretion which is discharged through a single opening above the hoofs and serve as a trail marker [2].

The sheep population in Iran is 50 million, belonging to 20 breeds. More than 96% of Iranian sheep are fat-tailed. One of the main Iranian fat-tailed and native breeds is Bakhtiari, which is encountered in the western and southwestern regions of the country especially in Charmahal va Bakhtiari province [5, 6].

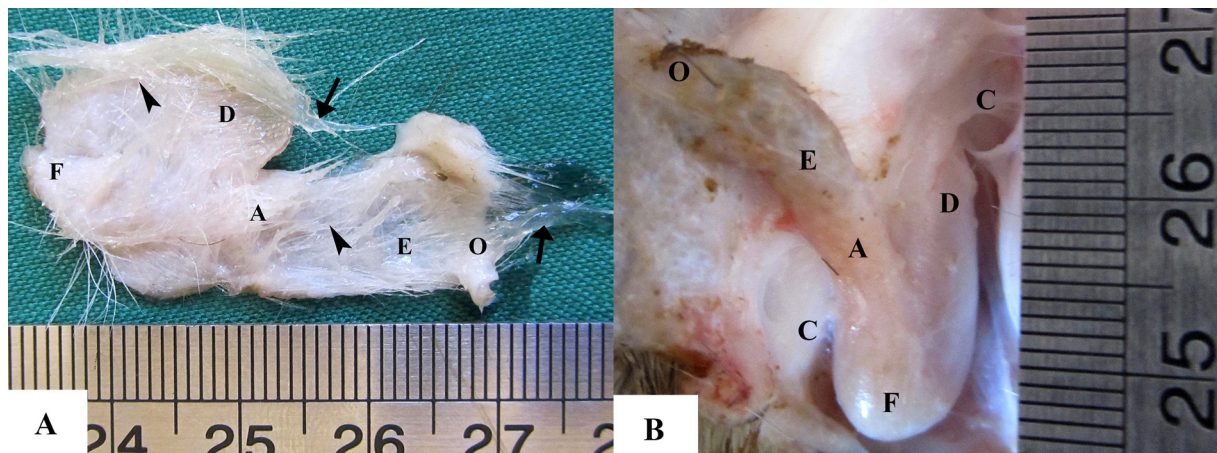
For elucidation of the histology and histochemistry of the interdigital sinus, investigations have been carried out in different native sheep, such as Lori [7], Tuj [8], Dubska pramenka [9], Kivircik [10], and Yankasa [11]. However, there is no information regarding gender effects on the histology and histochemistry of the interdigital sinus in Bakhtiari sheep. The aim of the present study was to describe the histological and histochemical properties of the Bakhtiari interdigital sinus and to reveal if these properties are sex dependent, and to compare the findings with those made in other sheep breeds.

## Results

In this study, the histology and histochemistry of the interdigital sinus showed no considerable differences among various anatomical regions of the right and left interdigital sinuses. Also, no significant differences were found between the male and female Bakhtiari sheep. The interdigital sinus of Bakhtiari sheep was found on the forelimb and hindlimb of both sexes.

The shape of the interdigital sinus in this native sheep was curved tube-tobacco pipe-shaped and contained descending part or fundus, flexure, ascending part, excretory duct or neck and orifice (Figure 1A). The interdigital sinus of the forelimb ( $3.5 \times 1.77$  cm) was somewhat wider and longer than those of the hindlimb ( $2.88 \times 1.55$  cm). The sinus was located in the interdigital space and covered with a capsule of dense connective tissue and skin (Figure 1B).

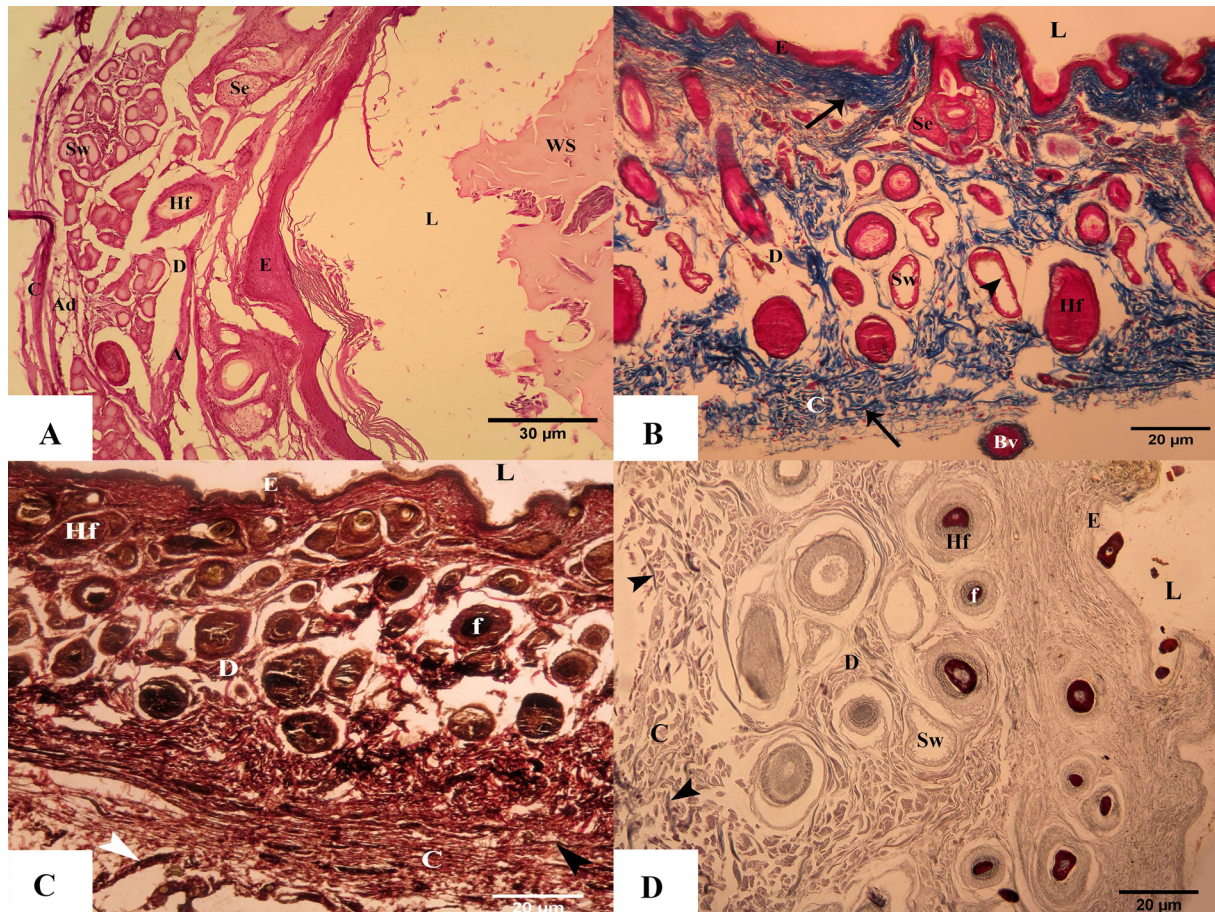
Histologic examination revealed that the interdigital sinus capsule was composed of moderate thick dense connective tissue and contained nerve bundles, adipose tissue (Figure 2A), blood vessels, collagen (Figure 2B), elastic (Figure 2C), and reticular fibers (Figure 2D). The skin of the interdigital sinus was consisted of the epidermis and dermis. The epidermis was composed of keratinized stratified squamous contained stratum basale, spinosum and granulosum. The stratum granulosum was not always well delineated. The melanin granules were observed in stratum basale (Figure 3A).



**Figure 1**

The interdigital sinus in the Bakhtiari sheep. A) The sinus was curved tube-tobacco pipe-shaped and contained descending part (D), flexure (F), ascending part (A), excretory duct (E) and orifice (O). The lumen of the sinus filled with hairs (arrowheads) and glandular waxy secretion (arrows). B) The sinus located in the interdigital space between the digits is covered with a capsule (C) of dense connective tissue and skin.





**Figure 2**

Structure of the wall of the interdigital sinus of Bakhtiari sheep. A) The interdigital sinus was covered with a capsule (C) and skin, epidermis (E), dermis (D), hair follicles (Hf), sebaceous glands (Se), sweat glands (Sw), arrector pili muscle (A), adipose tissue (Ad), The interdigital lumen (L) filled with waxy secretion (WS), hematoxylin eosin. B) Collagen fibres (arrows) in the capsule (C), and dermis (D) of the interdigital sinus of the Bakhtiari sheep. The secretory caps (arrowhead) observed in the free apical cytoplasm of secretory cells of sweat glands (Sw), Masson's trichrome. C) Cross section of the interdigital sinus stained by Verhoeff's showing abundant amount of elastic fibres (arrowheads) in the capsule (C) of the interdigital sinus of the Bakhtiari sheep. D) Reticular fibres (arrowheads) in the capsule (C) of the interdigital sinus of the Bakhtiari sheep, Gomori's method.

The dermis was contained hair follicles, sebaceous glands, nerve plexus, arrector pili muscles and numerous large apocrine sweat glands (Figure 2A, 2B, 2D).

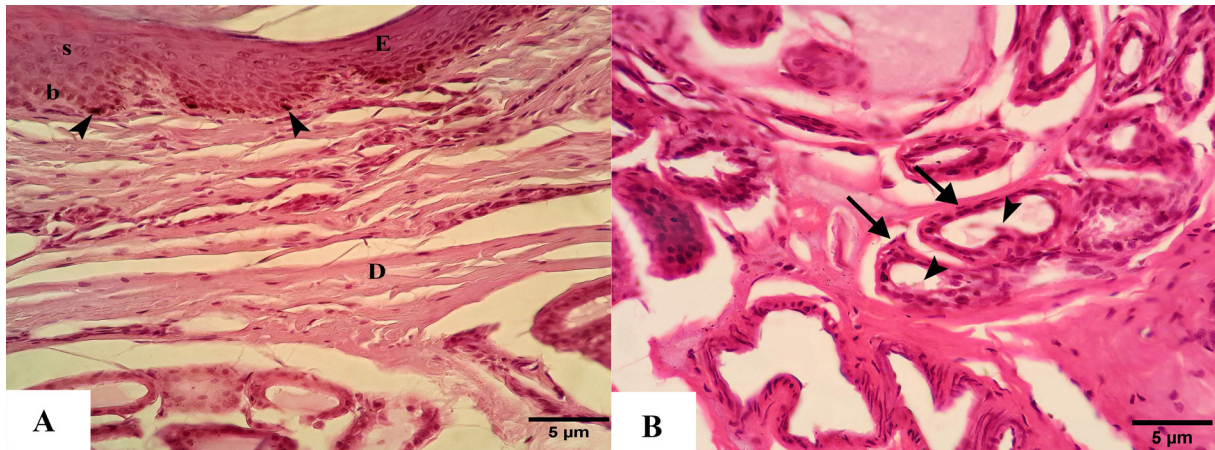
Numerous hairs with different sizes were associated with the interdigital lumen but they were absent in excretory duct. The interdigital lumen was filled with hairs and glandular waxy secretion of the sinus (Figures 1A, 2A). Among sinus regions, the highest and lowest hair follicle densities were found respectively in descending part and orifice. The sebaceous glands in the interdigital sinuses were simple branched acinar types. The sebaceous glands were always associated with hair follicles and located just above the apocrine sweat glands (Figure 2A, 2B). The sebaceous glands were larger and more branched in excretory duct of the

interdigital sinus. The arrector pili muscles were found around the hair follicles. These bundles of smooth muscle cells were inserted on the dermal sheath and attached to the dermis of the interdigital sinus (Figure 2A).

The sweat glands with different shapes and sizes in the interdigital sinuses were simple coiled tubular type. These large apocrine glands were mostly below the sebaceous glands, just between the hair follicles and the boundary of papillary and reticular layer of dermis (Figures 2A, 2B, 2D). The secretory caps that indicated their secretory activity, were observed in the free apical cytoplasm of secretory cells protruded into the lumen (Figures 2B, 3B). The maximum sweat gland frequency was observed in descending part of sinus.

The secretory cells of sweat glands were lined-





**Figure 3**

A) The epidermis (E) of the interdigital sinus of the Bakhtiari sheep was composed of stratum basale (b), stratum spinosum (s), and stratum granulosum (arrow). The melanin granules (arrowheads) observed in stratum basale, D: dermis, hematoxylin eosin. B) The sweat glands of the sinus were simple coiled tubular type. Their secretory cells surrounded by the myoepithelial cells (arrows), the secretory caps observed into the lumen (arrowheads), H&E.

with a simple to stratified cuboidal epithelium. The secretory cells surrounded by one row of myoepithelial cells and a thin layer of connective tissue fibres. Among sinus regions, the myoepithelial cells were more numerous in excretory duct (Figure 3B).

All epithelial cells of the apocrine glands and their secretion reacted positively to periodic acid Schiff (PAS) (Figure 4A), and alcian blue stains (Figure 4B).

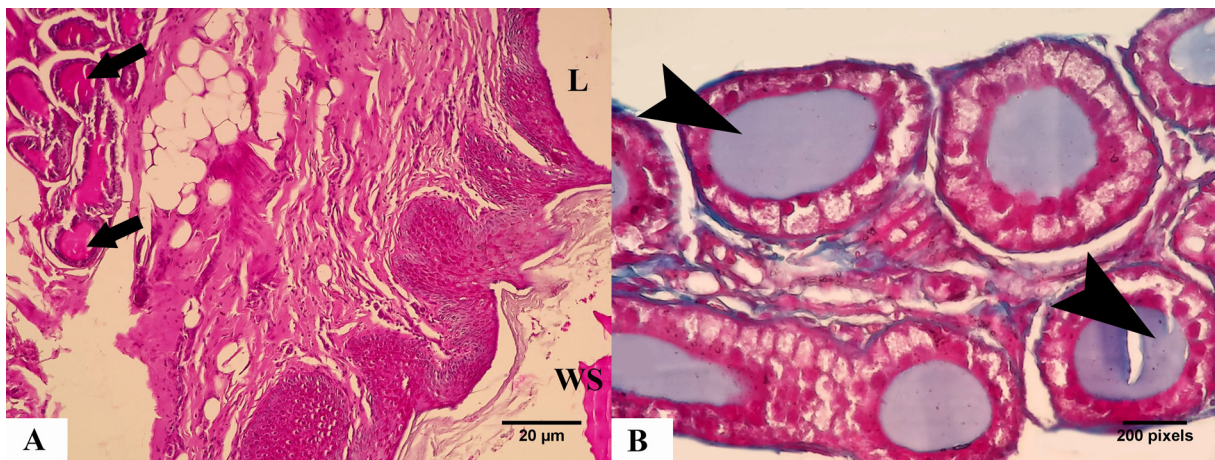
## Discussion

In the present study, the histology of the in-

terdigital sinus showed no significant differences according to sex which is in agreement with the results reported by Aslan et al. [8] in Tuj sheep and Atoji et al. [13] in Japanese sorrow, but Abbasi et al. [7] reported sex histological differences in adult Lori's sheep.

The interdigital sinus of Bakhtiari sheep was found on the forelimbs and hindlimbs of both sexes. This finding is in agreement with what was reported for some native sheep breeds [7-10]. Janicki et al. [14] reported that the interdigital sinus was found only in the hind feet of the roebuck.

The interdigital sinus in Bakhtiari sheep was



**Figure 4**

A) PAS-positive material (arrows) is present in the secretory epithelial cells of the apocrine glands and their secretion (WS), L: interdigital lumen, PAS. B) AB (+) luminal apocrine epithelial cells in the interdigital sinus of Bakhtiari sheep (arrowheads).

curved tube-tobacco pipe-shaped. Similar results were also reported by Abbasi et al. in Lori's sheep [7], Aslan et al. in Tuj sheep [8], Avdic et al. in Dubska pramenka [9], and Demiraslan et al. in Kivircik sheep [10].

Like other native sheep [7, 9], the interdigital sinus of the forelimb of Bakhtiari sheep (3.5×1.77 cm) were also wider and longer than those of the hind feet (2.88×1.55 cm). Aslan et al. [8] reported no sex differences between forelimb and hind feet in Tuj sheep. Although Abbasi et al. [7] divided the interdigital sinus into the secretory and excretory units, Avdic et al. [9] defined the interdigital sinus as the fundus, collum (neck) and corpus. Meanwhile, Karahan et al. [15] divided the interdigital sinus into neck, flexure and body, while Demiraslan et al. [10] described this sinus with the terms corpus, flexure, excretory duct and orifice. In this study, the interdigital sinus was divided into fundus or descending part, flexure, ascending part, neck or excretory duct and orifice.

The interdigital sinus of the Bakhtiari sheep was covered with a capsule of dense connective tissue and skin which accords with the findings of other authors [7-10, 15]. Janicki et al. [14] reported that in the roebuck the interdigital sinus was situated inside the loose connective tissue.

The sinus capsule was consisted of blood vessels, adipose tissue, nerve bundles, reticular, elastic and collagen fibers. Abbasi et al. [7] reported that the capsule was contained of blood vessels, adipose tissue, nerve bundles, and bundles of collagen in Lori's sheep. The skin of the interdigital sinus of the Bakhtiari sheep was consisted of the epidermis and dermis, which corresponds to the findings of other authors [7-10, 14-15].

The epidermis was composed of keratinized stratified squamous including stratum basale, spinosum and granulosum. Abbasi et al. [7] reported only stratified squamous epithelium with a prominent keratin layer in Lori's sheep. In this study, the dermis was composed of hair follicles, sebaceous glands, nerve plexus, arrector pili muscles and numerous large apocrine sweat glands which are similar to previous findings [3, 7-10]. Unlike Tuj sheep [8], lymph follicles were not found in the dermis of the interdigital sinus in Bakhtiari sheep.

Some researchers [7, 9-10, 14-15], reported that the lumen of the interdigital sinus was filled with secretory material. This finding is in agreement with our results. These waxy secretions may serve as a trail marker [2] or play a role in the pro-

duction of pheromones [16] or odoriferous signals in the social life of animals [8].

In the Alcian blue staining, positive reaction was observed in surface epithelial cells of the apocrine glands and their secretion. Similar results were also reported by Abbasi et al. in Lori's sheep [7], Demiraslan et al. in Kivircik sheep [10], and Janicki et al. in roebuck [14]. This reaction indicated that the apocrine secretion consisted of neutral glycosaminoglycan.

It was determined that the highest hair follicle densities were found in descending part of the interdigital sinus, while in Dubska pramenka the hair follicles were more numerous around the orifice of the interdigital sinus [9]. In the present study, the wall of the interdigital sinus had well-developed sebaceous glands and numerous large apocrine sweat glands that were in matching with results of the literature [3-4, 7, 9-10, 14-15].

Janicki et al. [14] reported that the sebaceous glands are compound alveolar, but it may also be simple alveolar [7]. The observations made in this study exposed the sebaceous glands of interdigital sinus in Bakhtiari sheep were from branched acinar type.

In the present study, the arrector pili muscles were found around the hair follicles of the interdigital sinus which agreements with the findings of Abbasi et al. in Lori's sheep [7]. The sweat glands of interdigital sinus in Bakhtiari sheep were simple coiled tubular type which is similar to previous findings [7, 14]. The secretory cells of these apocrine glands surrounded by one row of myoepithelial cells which is according to the findings of Abbasi et al. in Lori's sheep [7].

The histological and histochemical features of the interdigital sinus of the Bakhtiari sheep were similar to those in the other native sheep except the special features for the capsule; with the connective tissue fibres, epidermis; which composed of stratum basale, spinosum and granulosum, the absence of lymph follicles in the dermis and the presence of more numerous hair follicle in descending part of the interdigital sinus. There were no significant sex differences on the histology and histochemistry of the interdigital sinus.

## Materials and Methods

A total of 120 interdigital sinuses in the forefeet and hind feet of the 30 adult, healthy Iranian Bakhtiari sheep (15 females and 15 males) aged 1-2 years, were examined. The feet of the Bakhtiari sheep were obtained from the Shahrekord

Municipality Slaughterhouse. The interdigital sinuses were removed from the subjects and the samples were taken from different regions of the sinuses. Tissue samples from different anatomical regions of each interdigital sinus were fixed in 10% neutral buffered formaldehyde for 48 h and processed to embed in paraffin.

The serial sections (5 µm) were stained with haematoxylin eosin for general histological observations and special techniques: Masson's trichrome (for collagen fibres), Verhoeff's (for elastic fibres), and Gomori's methods (for reticular fibres). To investigate the chemical character (pH) of the secretion material in the epithelial cells, Alcian blue reaction (pH, 2.5) was employed to determine acidic mucosubstance and Periodic acid-Schiff (PAS) was used for determining neutral mucosubstances [12]. The histological and histochemical studies on stained sections were carried out by light microscopy (Olympus BX50, Japan).

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## Author Contributions

Conceived, designed and performed the experiments: B. Mobini. Contributed reagents/materials/analysis tools: V. Ranjkesh Adermanabadi.

## Conflict of Interest

The authors have no conflict of interest to declare.

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