VETERINARSKI ARHIV 73 (1), 27-37, 2003

Morphohistological characteristics of the interdigital gland in the roebuck (*Capreolus capreolus* L.)

Zdravko Janicki[1]*, Ante Hraste[2], Alen Slavica[1], Dean Konjević[1], Zdravko Marinović[3], and Đurđica Stubičan[4]

[1]*Chair for Game Biology, Pathology and Breeding, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[2]*Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

[3]*'Veterinar d.o.o.' Veterinary Outpatient Department, Slavonski Brod, Croatia*

[4]*Library of the Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

JANICKI, Z., A. HRASTE, A. SLAVICA, D. KONJEVIĆ, Z. MARINOVIĆ, Đ. STUBIČAN: Morphohistological characteristics of the interdigital gland in the roebuck (*Capreolus capreolus* L.). Vet. arhiv 73, 27-37, 2003.

ABSTRACT

This research was conducted on the interdigital gland of the roebuck (*Capreolus capreolus* L.). In the presented study the detailed morphology, type of secretion and anatomical location of this gland are described. The gland is composed of a pouch and an excretory duct. The glandular tissue contains sebaceous acini of the holocrine secretion and tubular-coil glands of the apocrine secretion, and no hair follicles or sweat glands were found. Differential staining established the gland's detailed structure and, using the Sudan Black B method, we have shown the distribution of lipids inside glandular acini.

Key words: roebuck, interdigital gland, histology, histochemistry

* Contact address:
Prof. Dr. Zdravo Janicki, Chair for Game Biology, Pathology and Breeding, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 260; Fax: +385 1 2441 390; E-mail: janicki@vef.hr

ISSN 0372-5480
Printed in Croatia

Introduction

Many species belonging to the Artiodactyla order have well-developed interdigital glands, but they differ morphologically from specie to species. SIVACHELVAN et al. (1992), has reported that it comes as a developed organ. RAESFELD (1978) designates the space between the digits the ''hoof skin organ'', while BAVDEK (1981) calls the same gland in sheep calls ''interdigital sinus''. In sheep, roe deer and Japanese serow the glandular secreted material is inside a pouch with a hair-covered excreting duct, from which secretion trickles (SIVACHELVAN et al., 1992), but in other species this gland is merely a form of skin fold (POCOCK, 1910). This gland produces the apocrine and holocrine types of secretion. The Japanese serow has well-developed interdigital glands on all four extremities (ATOJI et al., 1988) while the roe deer (*Capreolus capreolus* L.*)* has the glands only on the hind feet. Prompted by the dearth of written works on the roebuck's interdigital gland we decided to attempt to correct this omission. Therefore, the primary goal of this study is to demonstrate the primary morphohistochemical characteristics of the roebuck's interdigital gland, or in other words to show its anatomical placement, its appearance and constitution, as well as describing its content and the way in which is produced. Finally, we provide a description of the role the gland plays in the roebuck's life, and also a short comparison between this gland and the glands of other species.

Materials and methods

In order to conduct this research we collected 10 pairs of the roebuck hind feet. All animals were adults and they were shot on the area of Jastrebarsko during a selective hunt during the period May - August 1996. The mating season of the roe deer occurs in July and lasts approximately one month. The roebuck shows a seasonal cycle of testicular and accessory sex gland activity. If young roebuck are in good physical condition they are capable of breeding before the age of one year. The gland preparations were stained using haematoxylin and eosin (HE) and differential staining by the Van Giesen method (ŠVOB, 1974). For proving of the lipids we used the Sudan Black B method (ROMEIS, 1968). Acid glycosanimoglycanes were proven using the method of staining with alcian blue at pH 2.5 (PEARSE,

1968). Glycogen distribution was determined using the PAS staining method (PEARSE, 1968).

Results

The roebuck interdigital gland is located in the space between the digits (regio interdigitalis) on its hind feet. The glands are situated inside the loose connective tissue and covered with skin, so that only the opening duct is visible in the interdigital space (Fig. 1).

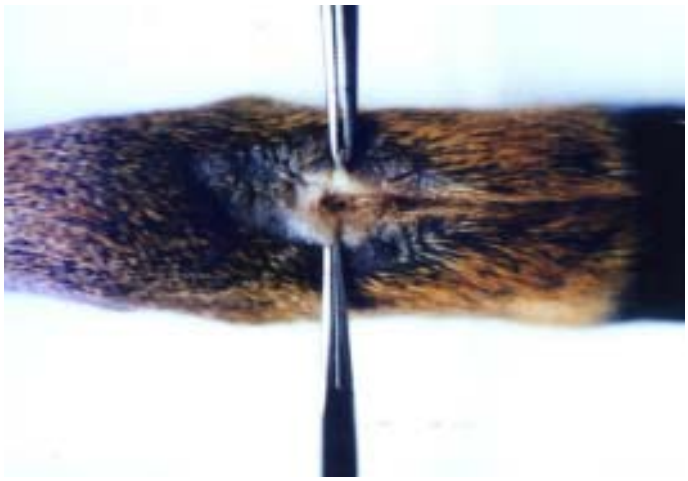

Fig. 1. Opening of an excretory duct on the interdigital skin surface

The glandular tissue forms the pouch, the size of an average hazelnut, and can be divided at the bottom, in the middle part and at the excreting duct. Inside the interdigital gland we could observe the pouch secretion, which at room temperature is thick, with the desquamate epithelium and a thin, delicate, de-pigmented hair within it. The pouch bottom of the interdigital gland is placed at the level of half of the first phalanx and extends to the third phalanx (Fig. 2). The excretory duct is placed almost horizontal to the proximal part of the free interdigital space and ends with an opening to the dorsal surface of the interdigital fold. The lumen of the interdigital pouch is covered with a thin layer of the epidermis. On the sections stained with

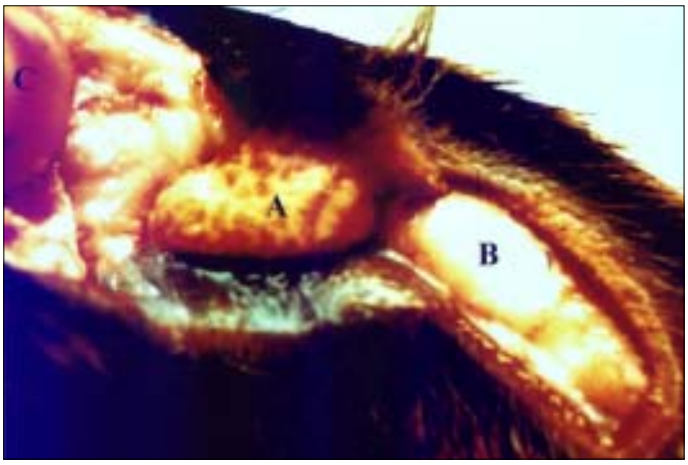

Fig. 2. Gland location in the interdigital space: A) Interdigital gland B) Second phalanx; C) Articular surface of the metacarpal bone

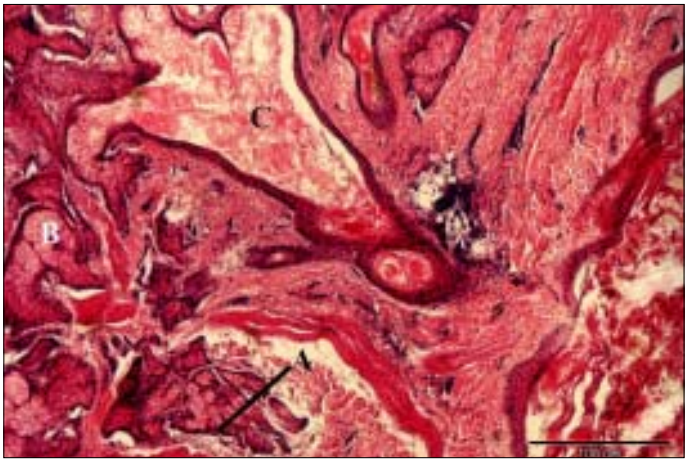

Fig. 3. Interdigital gland in the roebuck, H&E, ×20, scale bar = 100 μm. A) Immature sebaceous cells; B) Glandular parenchyma; C) Excretory duct

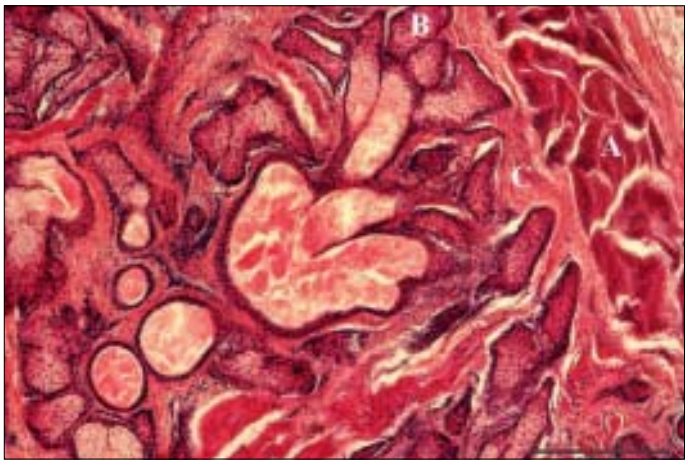

Fig. 4. Interdigital gland in the roebuck, H&E, ×20, scale bar = 100 µm. A) Coil glands; B) Sebaceous glands; C) Connective tissue

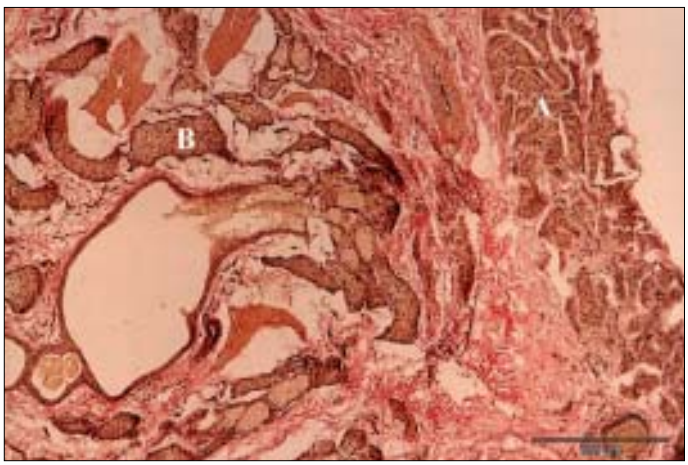

Fig. 5. Interdigital gland in the roebuck, Van Giesen, ×20, scale bar = 100 µm. A) Coil glands; B) Sebaceous glands

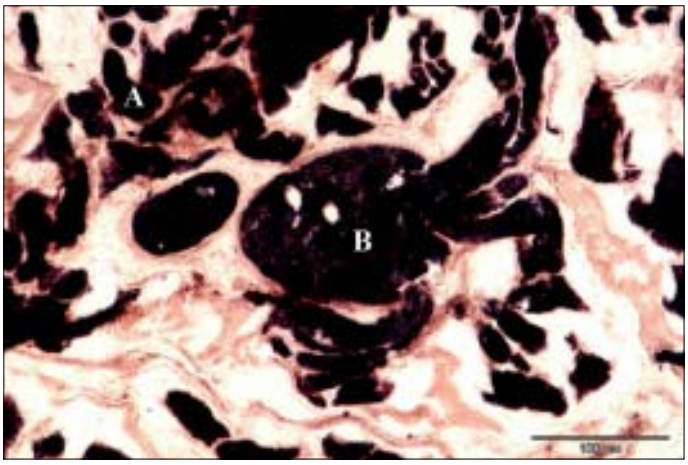

Fig. 6. Interdigital gland in the roebuck, Sudan Black B ×20, scale bar = 100 μm.
A) Sebaceous gland lumen; B) Excretory duct

haematoxylin and eosin we could see the glandular parenchyma, the immature sebaceous gland and the excretory duct with the secretion product (Fig. 3). Close to the gland we found the muscle fibers and between them the connective tissue fibres. At the borderline between the subcutis and the dermis (corium) there were accumulations of narrow, coil-tubular glands of the apocrine secretion, and due to their darker pigmentation they can be seen macroscopically on the cross-sections of the prepared gland. These glands are surrounded with connective tissue and muscle fibres (Fig. 4). Close to the borderline between the subcutis and the dermis there are apocrine glandular tubules. Among them we found layers of collagen and muscle fibres of differing density. On the borderline between the subcutis and the dermis there is a larger amount of the elastic and only a smaller amount of the collagen fibres. It would appear that the amount of the elastic fibres gradually increases towards the subcutis and the epidermis, and that the fibres lie parallel to the epidermis. On the other hand, at the bottom of the glandular pouch, collagen and elastic fibres are more densely developed around the coil-tubular glands than around the sebaceous glandular acini.

On the material stained using the differential staining method of Van Giesen we were able to observe sebaceous glandular cells and secretion inside the pouch lumen, ranging from greenish to yellowish, while the coil glands of the apocrine secretion were intensely yellowish-green (Fig. 5). Staining with Sudan Black B showed a high concentration of sudanophilic substance in all layers of the sebaceous and the coil glands (Fig. 6). The combined sebaceous alveoli form a kind of excretory duct through which the secretion is discharge into the pouch lumen, unlike in the body's sebaceous glands. Above the basal membrane of the roebuck's interdigital gland there is a stratum germinativum with cells capable of intensive metabolic activity. The glandular cells located peripherally next to the basal membrane are smaller and flatter, as opposed to the more centrally located cells. During cell growth they become more rounded in shape, with clearly visible cell membrane and nucleus. Cells that are closer to the centre of the alveoli are much bigger, their cytoplasm is weakly stained, the nucleus is invisible, and the cell wall is obviously thinner and cracked, which all that points to their gradual fatty infiltration. This is characterized by the accumulation of lipids, whose concentration is highest in the area of the gland excretory duct. Staining with the PAS method reveals that a small amount of neutral glycosaminoglycan is present in a lower concentration level in the cells, while acid glycosaminoglycans stained with alcian blue are present on the higher amount in the secretory product.

Discussion

In this study we have demonstrated that the roebuck interdigital gland can be found only on its hind feet. This is contrary to findings of RAESFELD (1978) in the moufflon (*Ovis musimon* L.) and the antelope, and to those of ATOJI et al. (1988) in the Japanese serow (*Capricornis crispus*), due to the fact that all those species have interdigital gland on all four feet. The shape and size of the interdigital gland in the roebuck are analogous to the description of the same glands in the Japanese serow and sheep (KLIMOV, 1947; ATOJI et al., 1987; SIVACHELVAN et al., 1992). The size of the gland and of the glandular acini is closely connected with the reproductive cycle, as observed by BULLOUGH and LAURENCE (1960), MULLER et al. (1983) and ODEND'HAL et al. (1992). Our findings regarding the structure and location of the interdigital

glands in the roebuck differ somewhat from the description of this gland in the sheep (BAVDEK, 1981). In sheep the gland is located inside the connective tissue, surrounded by a capsule and also has sweat gland cells (POCOCK, 1910). In our findings we were unable see that the connective tissue forms a capsule that covers the surface of the gland, nor could we find sweat glands cells. The glandular pouch consists of sebaceous and coil glands which are disseminated in the connective tissue, while the skin covers the surface of the gland. Therefore, the opening of excretory duct can be seen macroscopically only from the dorsal side in the interdigital space, while from the plantar side the skin limits the gland. The movement of lifting, gathering, sagging and interspacing of hoofs mechanically discharges the gland. This is why the interdigital space at the level of the second phalange acts as a pump for the gland. The finding of the muscle fibres around the coil glands, according to BAVDEK (1981), KLIMOV (1947) and RAESFELD (1978), confirms that secretion is aided by muscle contractions (ROTHMAN, 1954). According to SOKOLOV et al. (1990) the roebuck marks his territory with the increased secretion of the interdigital gland, while RAESFELD (1978) thinks that it occurs in dangerous situations, e.g. when the roebuck runs from a possible predator. In order to prevent total and immediate discharge of secretion of the interdigital gland a danger situation the gland lumen possesses an intertwined hair (upon which the secretion of the gland gradually seeps). This finding only partially corresponds with the description by SIVACHELVAN et al. (1992) of the foetal and post-natal development of the interdigital gland in the sheep, as we found no hair follicles in the lumen. It seems, therefore, that, as observed by MONTAGNA (1956, 1963), in adults the hair follicles are reduced and gradually completely disappear when the animal matures. The wall of the roebuck's interdigital pouch is thicker and better developed than in other hoofed animals (ATOJI and SUZUKI, 1987). In our findings the amount of phospholipids and neutral fats differs in the interdigital gland. The very high amount of sudanophilic substance is visible in the cells and gland excretory duct. It appears to be somehow less noticeable in the lumen of the glandular acini, while we were unable to observe it in the basal membrane. It is well known that the formation of the sebum inside the glandular cells is the result of the transformation of carbohydrates into lipids. It is therefore easy to understand the unequal

concentration of lipid substances in cells inside the glandular pouch. This is also in accordance with the MÜLLER-SCHWARZE (1978) report on fatty acid distribution inside the sebaceous gland.

This finding confirms the fact that the interdigital gland has a role in lubricating the movable parts of the hoof, while JENKINSON and NAY (1975), MONTAGNA (1963), SOKOLOV and GROMOV (1993), also attribute to it scent function, which is important for intercommunication. In our examination of the coil gland we have found a lipid content which, according to RAESFELD (1978), is a volatile substance, a fragrant component of interdigital secretion. The primary function of the interdigital gland in animals with long feet (elk - red deer - *Cervus elaphus* L.*)* is to lubricate the space between the digits. Therefore, it is not so important for scent communication because it plays a minor role in leaving a scent trail (HOFFMAN and THOME, 1986). The opposite is the case with species with short feet (Japanese serow and roe deer) whose interdigital gland has developed as a specialized skin gland with a cyclic excretory activity, which as a primary function scent marks the trail (ATOJI et al., 1988). According to MONTAGNA (1963) and JENKINSON and NAY (1975) those glands have a fungicidal and a bactericidal activity that helps disinfect the skin, keeps it elastic and protects it from UV radiation. Therefore, we believe that in the roebuck it has the same function, which is why it can be regarded as a specialized organ. The size of the sebaceous glands depends on the part of the body in which they are located, on the range and the thickness of the hair cover, and on hormonal regulation (MONTAGNA, 1962; PRASAD and SINHA, 1979; BULLOUGH and LAURENCE, 1960).

References

ATOJI, Y., Y. SUZUKI (1987): Lecitin histochemistry of the intraorbital gland in Japanese serow. Acta anatomica nipponica 62, 481-488.

ATOJI, Y., M. SUGIMURA, Y. SUZUKI (1988): Lecitin histochemistry of the interdigital gland in the Japanese serow (*Capricornis crispus*) in winter. J. Anatomy 161, 159-170.

BAVDEK, S. (1981): Interdigitalni sinus ovaca. In: Ko•a in njeni derivati. VTOZD. Biotehniške fakultete v Ljubljani.

BULLOUGH, W. S., E. B. LAURENCE (1960): Experimental sebaceous gland secretions in the adult male mouse. J. Invest. Dermatol. 35, 37-42.

HOFFMAN, R. R., H. THOME (1986): Zur functionellen Morphologie und Mikroarchitektur des Zirkumkaudalorgans von *Cervus elaphus* und *Cervus nippon*. Berl. Münch. Tierärztl. Wschr. 99, 418-424.

JENKINSON, D. M., T. NAY (1975): The sweat glands and hair follicles of different species of bovidae. Aust. J. Biol. Sci. 28, 55-68.

KLIMOV, A. F. (1947): Anatomija domaćih •ivotinja. I dio, Ko•ne •lijezde. Nakladni zavod Hrvatske. Zagreb. p. 392.

MONTAGNA, W. (1956): The Structure and Function of Skin. 1st ed. Academic Press. New York.

MONTAGNA, W. (1962): The structure and function of the skin. 2nd ed. Academic Press. New York.

MONTAGNA, W. (1963): Comparative aspects of sebaceous glands. In: Advances in Biology of Skin and Sebaceous Glands. Vol. 4, (Montagna, W., R. A. Oxford, Eds.). Pergamon press. Oxford. pp. 32-45.

MULLER, G. H., R. W. KIRK, D. W. SCOTT (1983): Small Animal Dermatology. 3rd ed. W. B. Saunders Company. Philadelphia. p. 889.

MÜLLER-SCHWARZE, D. (1978): Responses of reindeer to interdigital secretions of conspecifics. J. Chem. Ecol. 4, 325-335.

ODEND'HAL, S., K. V. MILLER, D. M. HOFFMAN (1992): Preputial glands in the white-tailed deer (*Odocoileus virginianus*). J. Mamm. 73, 299-302.

PEARSE, A. G. E. (1968): Histochemistry Theoretical and Applied. J. A. Churchill Ltd., London.

POCOCK, R. I. (1910): On the specialized cutaneous glands of ruminants. Proceedings of the Zoological Society of London, pp. 840-986.

PRASAD, R., R. D. SINHA (1979): Histological and certain histochemicals studies on sebaceous glands and their modifications in the eyelids of the Indian buffalo (*Bubalus bubalia*). Indian. Vet. J. 56, 672-674.

RAESFELD, V. F. (1978): Das Rehwild. Verlag Paul Parey, Hamburg und Berlin, pp. 10-15.

ROMEIS, B. (1968): Mikroskopische Technik. Oldenbourg Verlag. München, Wien.

ROTHMAN, S. (1954): Physiology and Biochemistry of the Skin. University of Chicago Press. Chicago.

SIVACHELVAN, M. N., A. YAHAYA, A. G. CHIBUZO (1992): Developmental changes in the interdigital pouch of Yankasa sheep. Small Ruminant Res. 9, 303-312.

SOKOLOV, V. E., S. GROMOV, S. V. SIMAK (1990): Role of excretions from specifics cutaneous glands in the chemical communication of Tartarian roes. Dokladi Akademii Nauk SSSR 315, 1507-1510.

SOKOLOV, V. E., S. GROMOV (1993): Chemical communication in roe deer (*Capreolus capreolus*). Zoologicheski Zhurnal 72, 123-136.

ŠVOB, M. (1974): Histološke i histokemijske metode. Izdavačko poduzeće ''Svjetlost''. Sarajevo.

Received: 7 November 2002
Accepted: 14 February 2003

JANICKI, Z., A. HRASTE, A. SLAVICA, D. KONJEVIĆ, Z. MARINOVIĆ, Đ. STUBIČAN: Morfohistološke značajke interdigitalnih žlijezda srnjaka (*Capreolus capreolus* L.). Vet. arhiv 73, 27-37, 2003.

SAŽETAK

Istraživanje je provedeno na uzorcima interdigitalnih žlijezda srnjaka (*Capreolus capreolus* L.). Smještaj, morfološke osobitosti i sekrecija navedene žlijezde su detaljno opisani. žlijezda ima oblik vrećice s izvodnim kanalom. U njenom tkivu razlikuju se lojni acinusi holokrine sekrecije i tubularne klupčaste stanice apokrine sekrecije. Dlačni folikuli i znojne žlijezde nisu utvrđeni. Diferencijalnim bojenjem utvrđena je struktura žlijezde, a pomoću Sudan Black B metode prikazan je raspored lipida u žlijezdanim acinima.

Ključne riječi: srnjak, interdigitalne žlijezde, histologija, histokemija