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IMMUNOHISTOCHEMICAL STUDY OF THE GOAT *DUCTUS DEFERENS*

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ABSTRACT

***Ductus deferens* plays an important role in sperm transport and participates in the preservation of structure, maturation, and viability of sperm. In this study, we have immunohistochemically examined the *ductus deferens* in the goat. For immunohistochemical study the following monoclonal antibodies were used: cytokeratin 18, α-smooth muscle actin (α-SMA), vimentin and elastin. Morphologically, three distinct layers were identified in the goat *ductus deferens* — tunica mucosa, tunica muscularis and tunica adventitia. The epithelium of the mucosa was intensely stained with cytokeratin 18 (CK 18). The fibroblasts in the *lamina propria* and blood capillaries in the muscle layer showed positive reaction for vimentin. A positive reaction for α-SMA was observed in the smooth muscle cells of the tunica muscularis in the internal, middle and outer sublayers. An intense positive reaction for α-SMA was observed in the wall of the blood vessels. Elastic fibers in the form of a loose meshwork were present in all three layers. The high density of elastic fibers were found in the tunica adventitia.**

Key words: *ductus deferens*; goat; immunohistochemical study

INTRODUCTION

The *ductus deferens* is a channel serving to transport sperm from the epididymis to the ejaculatory duct. Two main structures participate in this transport: epithelium and smooth muscle. Epithelial cells lining the luminal surface provides the environment for the transported sperm, whereas the thick smooth muscle coat, by its peristaltic action, is responsible for the pumping action during ejaculation [20, 22].

Studies relating the the strucure of the *ductus deferens* have been made mainly in man [12, 15, 18]. In animals, histological studies have been made in the rat [8] and in the rabbit [6]. Murakami et al. [13] used scanning and transmission electron microscopy to study the ampullary region of the dog vas deferens with special reference to epithelial phagocytosis of spermatozoa. Various components of the wall of the *ductus deferens* were observed to be spe-

cificaly arranged, compared with other organs of comparable organization [4, 9, 10]. Immunohistochemically, some studies were made on the *ductus deferens* in the donkey and water buffalo bull [3, 4]. Specific types of cytokeratins in the epithelial cells, and desmin in the muscular layers during the various phases of the development, growth, and involution of the human *ductus deferens* has been defined [18]. Francavilla et al. [7] reported on the distribution of actin, myosin and fibronectin during postnatal development of the epididymis and *ductus deferens* in the rat. In our work we have immunohistochemically examined the components of the wall of the goat *ductus deferens*.

MATERIALS AND METHODS

Five adult goats, 35—43 kg b. w., age 2—4 years, clinicaly health were used in this study. The samples of the *ductus deferens* were dissected out in the slaughterhouse. Then, they were fixed in 10 % buffered formalin and were processed for paraffin sectioning. Sections of 5 µm thick were stained with Harris haematoxylin and eosin for histological study. For immunohistochemical study the sections were pretreated with 3 % H_2O_2 in methanol to block endogenous peroxidase activity and preincubated with 2 % goat serum to mask unspecific binding sites. The sections were incubated with the primary antibodies [Tab. 1] and washed in phosphate-balanced salt solution PBS). Afterwards, the sections were incubated with biotinylated secondary antibody for 45 min, washed in PBS, and finely incubated with avidin-biotin-peroxidase complex (ABC kits, Vector Laboratories, USA). The reaction product formation was achieved by incubating for 10 minutes at room temperature, using a mixture of an equal volume of 0.02 % hydrogen peroxide and 0.1 % 3,3´-diaminobenzidine tetrahydrochloride made in Tris buffer. For negative controls, the first antibody was substituted by PBS or by normal rabbit serum.

RESULTS

Light microscopic observations

In the goat *ductus deferens* three distinct layers were identified: tunica mucosa, tunica muscularis and tunica adventitia. The mucosa was made up of columnar pseudostratified epithelium and the *lamina propria*. The epithelium together with the subjacent *lamina propria* formed flat longitudinal mucosal folds. The tunica muscularis was made up of three sublayers — circular, longitudinal, and oblique layers. Fine inner layer consisted of smooth muscle

Tab. 1. Antibodies used in the study

Antibodies	Donor	Code	Isotype	Dilution	Source
Cytokeratin 18	Mouse	C 04	IgG1	1:20	Exbio
Vimentin	Mouse	1074	IgG1	1:50	Imunotech
α-Smooth muscle actin	Mouse	M 851	IgG2a	1:200	Dako
Elastin	Mouse	E 4013	IgG1	1:5000	Sigma

Table 2. Immunolocalization of different proteins in layers of the goat *ductus deferens*

	CK 18	Vim	SMA	El
Epithelium	+++	–	–	–
Lamina propria	–	++	–	+
Tunica muscularis	–	+	+++	+
Adventitia	–	++	–	++

Reactivity: – —negative; +—weak; ++—moderate reactivity; +++—strong reactivity

cells arranged circularly. The middle layer contained bundles of smooth muscle cells arranged mainly circularly and helically. The outer longitudinal layer consisted of coarse bundles of smooth muscle cells arranged obliquely. The loose connective tissue of the adventitia diffusely merged with the surrounding supportive tissue of the spermatic cord.

Immunohistochemical observations

Immunostaining revealed positive reactions for CK 18 in the lining epithelial cells of the mucosa in which differences in the reactivity were found. The apical cell membrane gave strong reaction the resting epithelial body displayed moderate reactivity [Fig. 1], whereas the basal cells were stained less intensely. The loose connective tissue of the mucosa was rich in fibroblasts positive for vimentin. The blood capillaries stained with vimentin, and were perforating the muscular coat and adventitia [Fig. 2]. The muscular coat with smooth muscle cells was stained by α-SMA. More intense reaction was observed in the inner layer [Fig. 3]. Additionally, α-SMA staining was seen in the smooth muscle cells of the blood vessels [Fig. 4]. The elastic fibres positively stained with elastin were distributed in all three layers: in the mucosa they formed fine loose networks, in the muscular layer elastic fibers surrounded the bundles of smooth muscle cells. A dense layer of elastic fibres was seen on the periphery of the muscular coat and in the tunica adventitia where the elastic fibers formed dense mesh works [Fig. 5]. A higher concentration of elastic fibers was found around the blood vessels.

DISCUSSION

Comparative histological studies showed that the mucosa of *ductus deferens* from different animal species reveal some species-specific characteristics. The mucosa of the goat *ductus deferens* constitutes folds different from other animal species. In the rat, the mucosal folds are variable while in the rabbit the *ductus deferens* are characterized by a plethora of complex mucosal folds [9]. In the goat the mucosal folds were flat and the number of folds ranged from 2 to 3. Also, differences in the lining epithelium are found in various animal species. In the rabbit, the epithelium is simple cuboidal and columnar, in the donkey, it is high cuboidal, in the buffalo, it is low columnar pseudostratified, in the human it is tall columnar pseudostratified and pseudostratified columnar with stereocilia in the rat [9]. In the

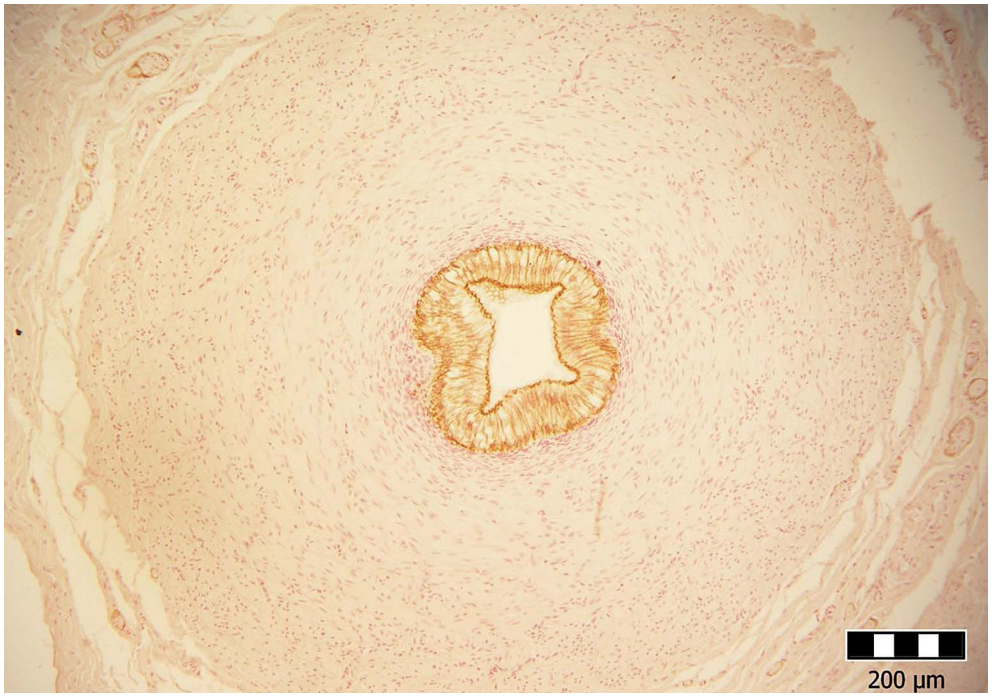

Fig. 1. Localisation of CK 18.
The CK reaction was only confined to the the lining epithelium (EP) of the *tunica mucosa* and to basal cells. Intense immunoreaction was in the apical cell membrane

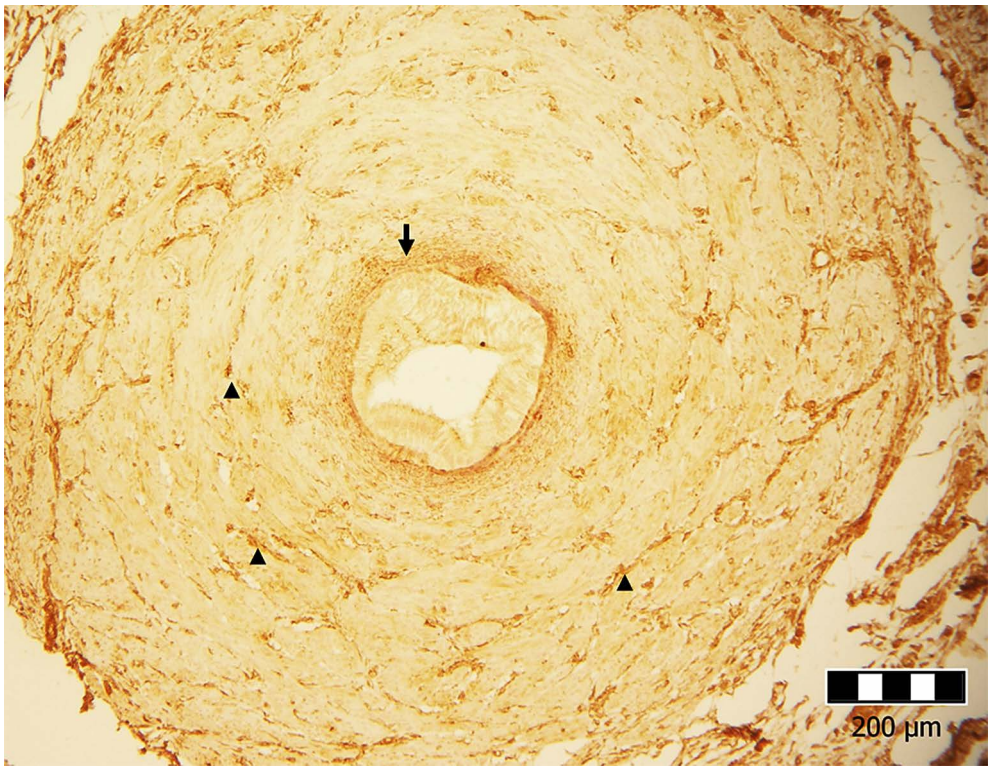

Fig. 2. Localisation of vimentin
Fibroblasts with positive reaction were located in the loose connective tissue of the mucosa (arrow). A positive reaction was confined to blood capillaries among the smooth muscle bundles of the muscular coat (arrowheads).

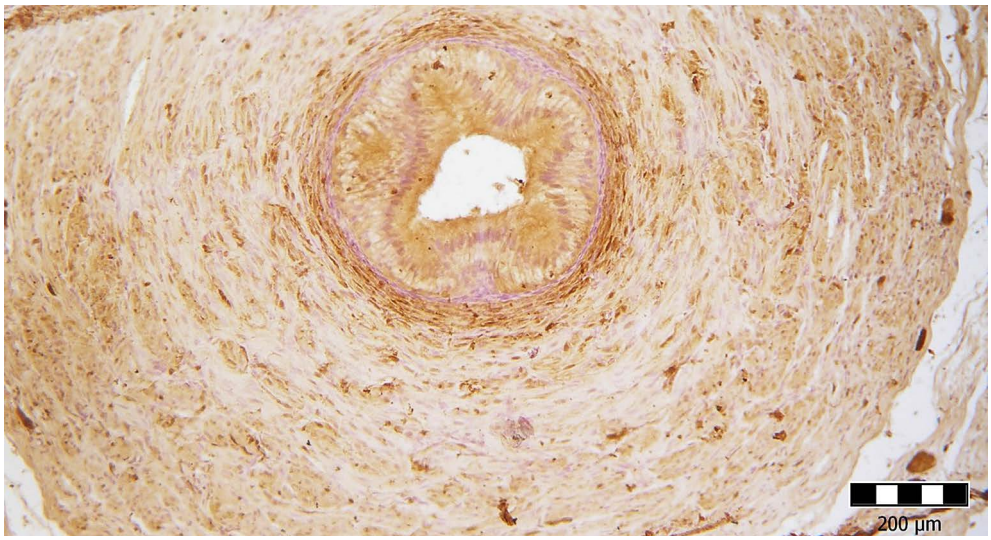

Fig. 3. Localisation of α-smooth muscle actin
Smooth muscle cells of the inner layer of the muscular coat are intensely stained by α-smooth muscle actin (arrow)

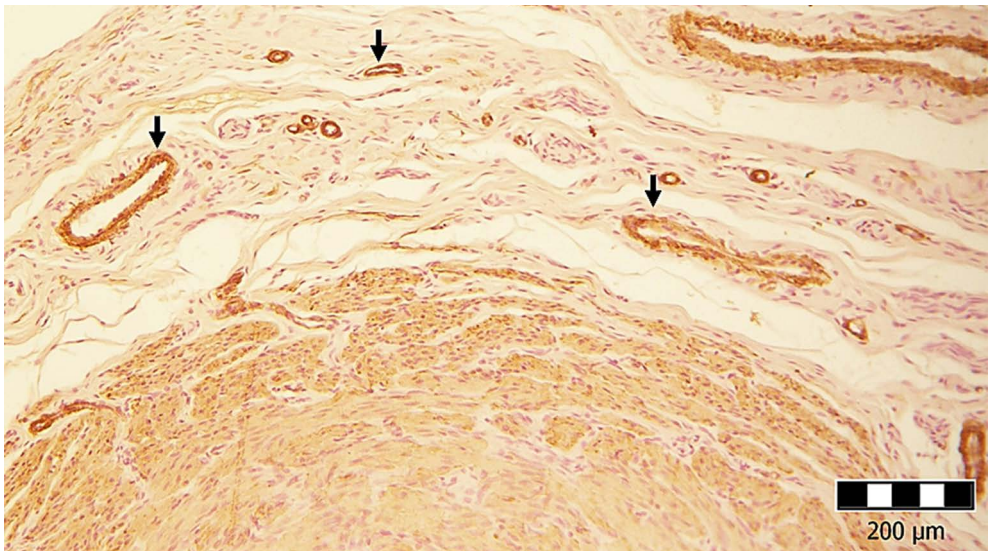

Fig. 4. Localisation of α-smooth muscle actin
Smooth muscle cells of the outer layer of the muscular coat (MC) and of blood vessels in the adventitia are stained by α-smooth muscle actin (arrow)

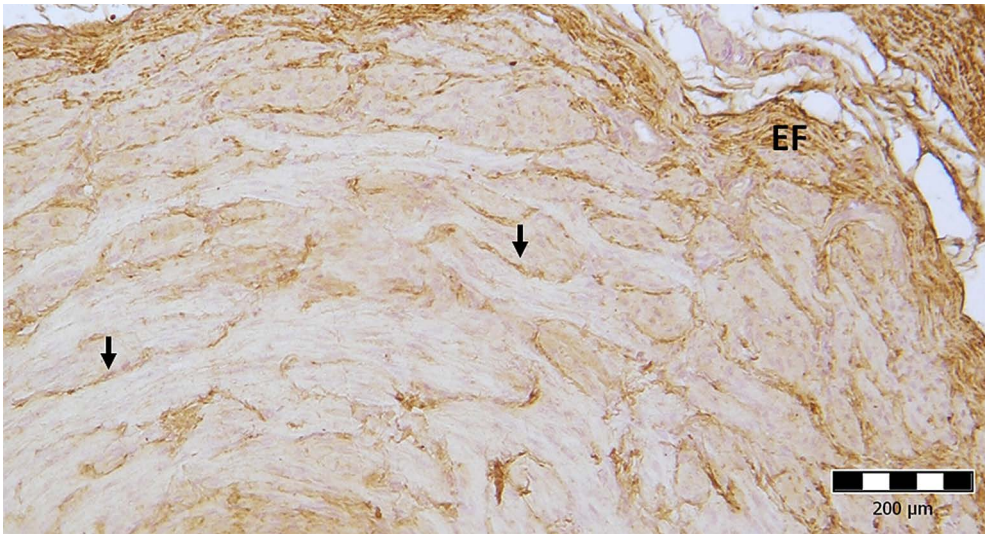

Fig. 5. Localisation of elastin.
Positively stained elastic fibres in the muscular layer are located between bundles of smooth muscle cells (arrow). A higher concentration of elastic fibers were on the periphery of outer muscle sublayer and *tunica adventitia* (EF)

goat the epithelium was found to be high pseudostratified. A three-layered muscularis and a serosa or an adventitia have been found in different mammals [10, 15].

Immunohistochemical studies reveal that most epithelia lining the human male reproductive tract, including those in the epididymis, *ductus deferens*, prostate gland, and seminal vesicle, synthesize CK 5, in addition to cytokeratins 7, 8, 18, and 19 [2]. In the ampulla of the *ductus deferens* of camel, the cytokeratin reaction was found in the pseudostratified columnar epithelium and in the secretory columnar epithelium of the submucosal glands, where strong reaction was seen in the apical cytoplasm of columnar and basal cells of the secretory units [1]. The epithelium of the *ductus deferens* in rodents showed absorptive, synthetic and secretory activities [11, 20], thus providing an appropriate luminal environment for sperm before ejaculation. We found a higher concentration of CK 18 in the epithelial cells which may correspond with these activities. Recent studies suggest that the epithelium might modulate the contractility of smooth muscle. Ruan et al. [19]

reported, that ATP inhibition of the vas deferens smooth muscle contraction is epithelium dependent.

The *lamina propria* of the mucosa of the goat *ductus deferens* contained fibroblasts stained with vimentin and myofibroblasts stained with SMA. The myofibroblasts were found subepithelially in many mucosal surfaces throughout almost the whole of the gastrointestinal and genitourinary tracts. The myofibroblasts in other tubular organs have been identified by their expression of various intracellular cytoskeletal proteins — the microfilament α-smooth muscle actin, type 3 intermediate filaments such as vimentin or desmin, and by the absence of epithelial cytokeratins [17]. Within the human bladder lamina propria there was found a layer of cells with the cytological characteristics of both fibroblasts and smooth muscle cells. This combination of features is characteristic of the myofibroblast [21].

In the tunica muscularis, a positive reaction to α-SMA was observed in all three layers. A remarkable intense reaction was found in the inner layer, whereas in the middle layer, the reaction to a-SMA remained only moderately stained. A positive reaction allowed for us to more easily distinguish the different orientation of the smooth muscle cells (SMC) in the midle layer. In the goat, like in other animal species, the SMCs of the middle layer showed an intermingled pattern of orientation. Some outer longitudinal layers penetrate into the circular layer to reenter the outer longitudinal layer and thus make helically arranged muscle loops as described by Williams et al. [22]. The circular profile predominates in the buffalo [4] and also in the goat *ductus deferens*. The outer longitudinal layer was distinct and consisted of coarse bundles of SMCs, particularly in the donkey [3]. In this animal species the muscularis was nearly two folds thicker than that in the buffalo [4]. The strong positive reaction of smooth muscle cells allowed to be seen the high concentration of blood vessels located mainly in the tunica adventitia.

Elastic tissue is a normal component in the genito-urinary region [5, 14]. In the wall of the goat *ductus deferens* we found the elastic fibres in all three layers. Differences were seen in their density and arrangements. While in the *lamina propria*, we found elastic fibres as a loose network in the muscle layer where these were intimately associated with the smooth muscle cells. A similar distribution was observed in the *ductus deferens* of man and monkeys [14]. A high concentration of elastic fibers was seen in the wall of the blood vessels located in adventitia. In these areas, the elastic fibers were observed also in the adult human *ductus deferens* [16]. The function of elastic fibers in the *ductus deferens* has not been fully explained. The role of elastic fibres in the lamina propria of the human *ductus deferens* has been described as providing elastic recoil of the ductus following contraction and dilatation at ejaculation and as participating in peristaltic movements [16]. We suppose that the elastic fibers in the goat *ductus deferens* play a similar role.

CONCLUSIONS

The *ductus deferens* consists of three layers corresponding to other tubular organs. Immunohistochemically, CK 18 stained the lining epithelium, vimentin expression has been observed in fibroblasts and blood capillaries, α-SMA was found in the smooth muscle cells of the muscular layer and blood vessels. Elastic fibers were distributed in all three layers with higher concentrations in the periphery of the organ.

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