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Original Scientific Article

THE PRESENCE OF SMOOTH MUSCLE CELLS AND ELASTIC FIBERS IN THE BULL VESICULAR GLAND

Elena Marettová[1]*, Jaroslav Legáth[2]

[1]Department of Anatomy, Histology and Physiology, [2]Department of Toxicology and Pharmacology, The University of Veterinary medicine and Pharmacy in Košice, Slovak Republic

*Corresponding author, E-mail: marettova@uvm.sk

Summary: Vesicular gland is a compact, lobulated organ surrounded by a capsule of dense irregular connective tissue with a few smooth muscle cells. Smooth muscle cells and elastic fibers as supporting and contractile structures were examined in the vesicular glands (VG) of the bull by the immunohistochemical method. The elastin and -smooth muscle actin (α-SMA) antibodies were used for their detection. Bundles of smooth muscle cells (SMC) positive for smooth muscle actin form thick muscular layer of the gland. Elastic fibers as loose network are inserted among the muscle cells and the bundles of the muscular layer. Thick connective tissue trabeculae rich in smooth muscle cells were seen to arrise from the muscle layer and enter the mucosa. In the stroma of the mucosa layer, smooth muscle cells form bands of different dimensions and arangement. Individual smooth muscle cells were seen just beneath the secretory epithelium. An accumulation of elastic fibers was seen in the connective tissue that separates the secretory alveoles. Dense concentration of elastic fibers have been seen to be located close to secretory epithelium. High accumulation of elastic fibers of the vesicular gland together with smooth muscle cells are supposed to participate in a rapid releasing of the secretory product and rearrangement of the mucosa during and after the ejection.

Key words: vesicular glands; smooth muscle cells; elastic fibers; bull; immunohistochemistry

Introduction

The vesicular gland (*glandula vesicularis*), seminal vesicle are androgen-dependent secretory glands of the male genital tract which together with prostate produce a bulk of the seminal secretions. The vesicular gland was studied in relation to stromal maturation during the ontogeny and in the adult stromal composition after hormonal influences or in tumours. Stromal development of the vesicular gland of the rat was examined by immunocytochemical methods during the pre- and postnatal developmental periods (1). Histological quantitative analysis of collagen and smooth muscle in seminal vesicle stroma after estradiol-17β administration to the immature castrated rat were performed (2).

Only a few studies are there relating the presence of contractile smooth muscle cells (SMC) in the vesicular gland. The distribution of myofibroblasts (MFb) in the stroma of a normal vesicular gland was studied (3). An electron microscope examination revealed the presence of spindle or stellate cells classified as myofibroblasts, distributed in the stroma of the lamina propria. These cells were found to be major stromal components in renal pelvic and ureteral, and in cancers of different organs (4-8). An immunoelectron microscopic examination showed that cells beneath the seminal vesicle epithelium were positive for smooth muscle actin (3).

The reports relating the presence of elastic fibers in the male accessory glands in animals are scarce and were documented mostly in organs of the urinary tract. Distribution of elastic fibers in the upper urinary tract of the human fetus have been published (10). An abundance of elastic fibers among the smooth muscle bundles the human male urethra was (9). In the animals, investigation of the elastic fibre system of the female canine urethra was per-

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formed (11). Morphometric studies on elastic fibers in the urethra have been made in the dog (12), bitch (13), and cat (14). Both male and female guinea pigs showed great amounts of circularly disposed elastic fibers in the vesicourethral junction (15). This particular disposition of fibers may be responsible for imparting resiliency and plasticity to the vesicourethral junction. Elastic fibers in this place have been assumed to contribute to the resting urethral closure pressure. In the bladder base, vesicourethral junction and urethra, the elastic fibers may be partly responsible for the passive occlusive force in this region. The presence of elastic fibers in the vesicular glands, to our knowledge, was not documented. The aim of this work was to study immunohistochemically the distribution of elastic fibers and contractile smooth muscle cells in the bull vesicular glands.

Material and methods

Vesicular gland from five adult bulls was used in this study. The samples of vesicular gland were taken out at the local slaughterhouse immediately upon death. Samples of the tissue were fixed in 10% formaldehyde in 0.2 mol phosphate - buffered saline (PBS) for 24h and routinely embedded in paraffin. The sections 5-μm-thick were cut and stained with haematoxylin-eosin as a general stain. Consecutive sections were used for histological, control and immunohistochemical procedures. For immunohistochemistry, the sections were mounted on slides coated with 3-aminopropyltriethoxysilane.

For immunostaining procedure, histological sections were deparaffinised and rehydrated, pretreated with 3% H_2O_2 in methanol to block endogenous peroxidase activity and preincubated with 2% goat serum to mask unspecific binding sites. Washed sections were incubated overnight with primary antibody – monoclonal mouse anti-α-SMA (Dako), dilution 1:200, and monoclonal mouse anti-elastin (Sigma), dilution 1:5000. The sections were washed in phosphate-balanced salt solution (PBS) and incubated with biotinylated secondary antibody for 30 min. Washed sections in PBS were incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector, Burlingame, USA). After washing with PBS, peroxidase activity was visualized with diaminobenzidine (DAB) and H_2O_2 in TRIS buffer within 5 min at room temperature. Sections were counterstained with Mayer's hematoxylin. For negative controls, the primary antibody was substituted by PBS.

Results

Anatomically, vesicular gland in bull is a compact, lobulated organ, and histologically it is a paired, compound tubuloalveolar gland. A muscular coat consists of an inner layer of circularly arranged smooth muscle cells and an outer layer in which the smooth muscle cells have a longitudinal orientation. External to the muscle coat is adventitia, consisting of loose connective tissue. The highly vascularised loose connective tissue of the lamina propria – submucosa is continuous with the dense connective tissue trabeculae. The interlobular septa are predominantly muscular, derived from the thick tunica muscularis. The mucosa of the vesicular gland consists of secretory alveolae separated by loose connective tissue trabeculae of different thickness. Intralobular secretory ducts drain the slightly coiled tubular portions of the tubuloalveolar gland. The secretory columnar cells and the basal cells have lipid droplets, often in an infranuclear position. An immunohistologic examination showed that the cells positive for α-smooth muscle actin (α-SMA) were observed to form thick tunica muscularis (Fig. 1). The capsule contains a few smooth muscle cells. Groups of smooth muscle cells were observed to arise from muscle layer and enter the mucosa layer. The trabeculae of the lamina propria of different thickness contain bands of SMC which were seen to be localized centrally. Single smooth muscle cells forming incomplete layer were observed beneath the secretory epithelium (Fig. 2). Numerous small blood vessels rich in SMC were present in mucosa layer.

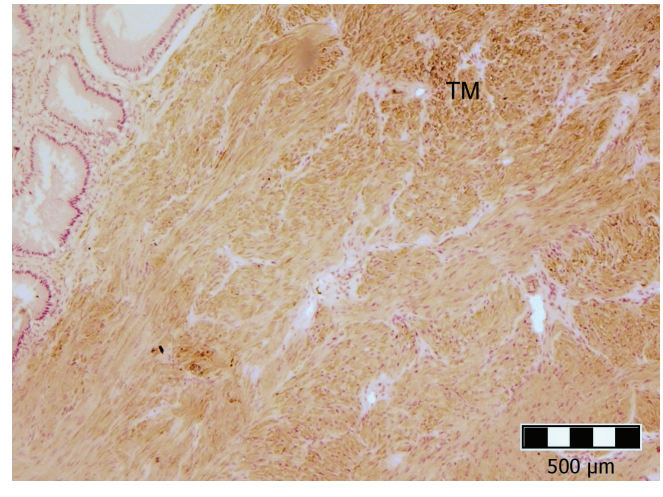

Figure 1: Positive cells for α-smooth actin (brown) forming thick tunica muscularis (TM). Nuclei were counterstained with Meyer's hematoxylin

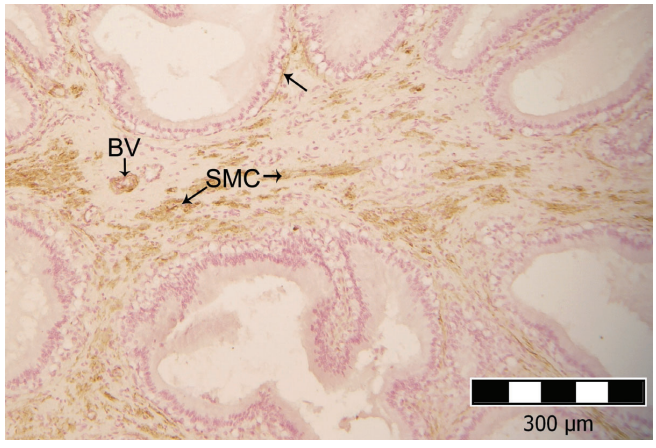

Figure 2: Positive cells for α-smooth actin (brown) localized in trabeculae of lamina propria. Smooth muscle cells are also beneath the secretory epithelium (arrows). Blood vessels (BV) containing smooth muscle cells (SMC). Nuclei were counterstained with Meyer's hematoxylin

Elastic fibers in the vesicular gland were present within all layers – capsule, the muscular layer, submucosa and mucosa. Many elastic fibers occur in the muscular layer and were distributed among bands of the SMC (Fig.3). In the submucosa and in the mucosa layer, elastic fibers were present throughout the area of the lamina propria, forming thick and thin trabeculae. In the connective tissue of mucosa, elastic fibers were seen to be distributed regularly among the muscle cells and collagen fibers. An accumulation of elastic fibers occurred next to secretory alveoles to form thick elastic membranes just beneath the secretory epithelium (Fig.4).

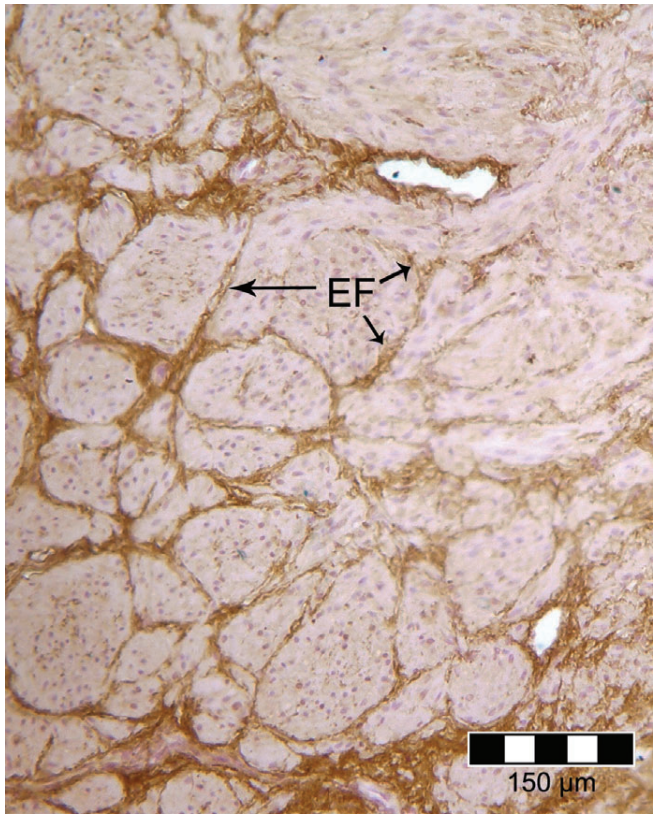

Figure 3: Elastic fibers (EF) are distributed among the bands of the muscular layer. Nuclei were counterstained with Meyer's hematoxylin

Discussion

In the bovine vesicular gland both structures studied - the smooth muscle cells and elastic fibers - were seen to be richly developed. As for cells reacting positively for α-SMA, distributed in all layers, the muscle layer, submucosa and mucosa layer. In the muscle layer, which in bull is very thick, the arrangement of muscle cells is typical for visceral organs. It consists of typical smooth muscle cells which were distributed in bands of different thickness, running mostly circularly. As we have seen, the organization of the SMC in this organ is different from that in other accessory glands. Thick muscle layer and rich saturation of the mucosa layer with smooth muscle cells seem to be specific for these sex accessory organs.

The classification of the muscle cells present in the mucosa, namely those beneath the secretory epithelium, is not clear. Though these cells displayed a strong positive reaction for -SMA and presented

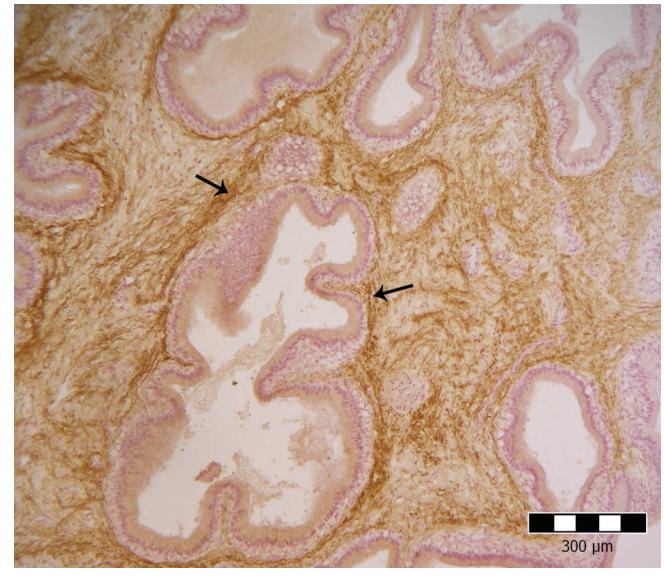

Figure 4: Elastic fibers in the trabeculae of the mucosa form dense notwork. High concentration of elastic fibers are seen beneath the secretory epithelium (arrows). The section was counterstained with Meyer's hematoxylin

spindle shape typical for smooth muscle cells, their cytoplasmic components seemed to be different. The cells lying under the secretory epithelium were seen to be more slender with a small amount of cytoplasm, whereas the cells inside the trabeculae were seen to be rich in cytoplasm. Smooth muscle actin was also proved histochemically in smooth muscle cells of other organs and cells: in pericytes of blood vessels (16), in human normal testicular stroma (17, 18) in normal pancreas and various pancreatic lesions (19), and in highly differentiated fibroblastic cells, the so-called myofibroblasts (20-22). The cells positive for alpha-smooth muscle actin in the stroma of normal seminal vesicles just beneath the epithelium, which the authors characterized as myofibroblasts were observed (3). Though the cells under the epithelium and those in the septa of vesicular gland express α-SMA typical for smooth muscle cells, the specific environment and morphological features of these cells may have also another function than that ascribed for SMC (23).

There are more data about the presence of myofibroblasts in various organs, however, there are still doubts whether these cells are true myofibroblasts (24, 25). Evidences reported (3) that the majority of myoid cells in human testicular seminiferous tubules are myofibroblasts rather than smooth muscle cells, and supposed that these myofibroblasts may play a role in sperm transport. This mechanism has been shown also in the rat seminal vesicle (26). Myofibroblasts have also been identified to play a role in tissue contraction during wound healing and contraction (27-30) and in various pathological conditions and organ fibrosis (31-33). The muscle cells observed in the mucosa layer of vesicular gland may have the functional properties of byofibroblasts.

The connective tissue of the mucosa of the vesicular gland is specific and differs from similar tissue in other glands. The vesicular gland and prostate are androgen-dependent secretory glands of the male genital tract. They produce the bulk of the seminal secretions. In the vesicular gland, androgen receptors were observed in the lamina propria (1). Lamina propria and mainly subepithelial area with muscle cells are very rich in nerve fibers. Around the glandular secretory alveoles and namely below the epithelial lining of the glandular duct, a tightly woven subepithelial plexus was seen which sends short penetrating branches into the basal zone of the epithelium (34).

A dense concentration of the elastic fibers was seen in the bull vesicular glands. Only in a few studies these fibers were observed in animals. Elastic fibers have been demonstrated in the urethra of the dog (12), bitch (13), and cat (14). Both male and female guinea pigs showed great amounts of circularly disposed elastic fibers in the vesicourethral junction (15). It seems that vesicular glands are specific and characteristic with such concentration of elastic fibers.

In conclusion, in the bull vesicular gland smooth muscle cells and elastic fibers form an important structure of the organ. Both structures are localized within the muscle layer, submucosa and mucosa. The organization of muscle cells and elastic fibers is related to the process of the ejection of sperm and seminal plasma. The particular disposition of elastic fibers may be responsible for imparting resiliency and plasticity to the vesicular gland, allowing it to distend and recoil in response to ejaculation.

Acknowledgements

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PRISOTNOST GLADKIH MIŠIČNIH CELIC IN ELASTIČNIH VLAKEN V MEHURNICI BIKOV

E. Marettová, J. Legáth

Mehurnica je čvrst, režnjičast organ, obdan s kapsulo iz gostega, neenotnega vezivnega tkiva, ki vsebuje nekaj gladkih mišičnih celic. S pomočjo imunohistokemičnega barvanja smo proučevali gladke mišične celice in elastična vlakna kot podporne in krčljive strukture v mehurnici bika. Za ugotavljanje so bila uporabljena protitelesa proti elastinu in gladkemu mišičnemu aktinu α (α-SMA). Ugotovljeno je bilo, da snopi gladkih mišičnih celic (SMC), pozitivnih na aktin gladkih mišičnih celic, oblikujejo obilno mišično plast žleze. Elastična vlakna so kot ohlapna mreža vložena med posamezne mišične celice in snope mišičnine. Debelo vezivno tkivo, bogato s trabekulami, v gladkih mišičnih celicah prehaja iz mišične plasti v sluznico. V podpornem tkivu sluznice gladke mišične celice oblikujejo trakove različnih dimenzij, ki so različno razporejeni. Pod epitelijem so bile vidne posamezne mišične celice. Elastična vlaka, ki ločujejo izločevalne mešičke, so bila nakopičena v vezivnem tkivu. V bližini epitelija smo opazili goste snope elastičnih vlaken. Gosti snopi elastičnih vlaken v mehurnici naj bi skupaj z gladkimi mišičnimi celicami sodelovali pri hitrem sproščanju izločkov in prerazporeditvi sluznice pred ejakulacijo in po njej.

Ključne besede: mehurnica; gladke mišične celice; elastična vlakna; bik; imunohistokemija