

## Mast Cells Distribution in the Domestic Swine Urinary Bladder's Wall

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The localization and density of toluidine blue (TB) stained mast cells (MCs) in *lamina propria mucosae* and muscular tunic of pig's urinary bladder in order to elucidate their role in organ function, were studied. It was established that in propria, the MCs were localized predominantly close to vessels of microcirculatory bed. Their number was highest in *trigonum* –  $4.00 \pm 0.84$  and *corpus* –  $3.44 \pm 0.51$  ( $P < 0.001$ ), followed by *apex* –  $2.56 \pm 0.51$  and *collum vesicae* –  $1.17 \pm 0.38$  ( $P < 0.001$ ). In the muscular tunic, the MCs were localized near the capillaries' basal lamina, in the adventitia of arteries and veins and between the muscle bundles. The highest MCs density was detected in the muscular tunic of *collum* ( $16.50 \pm 0.51$ ) – and *trigonum* –  $16.61 \pm 0.50$  ( $P < 0.001$ ), followed by *apex* –  $15.89 \pm 0.83$ , and *corpus* –  $12.33 \pm 0.48$  ( $P < 0.001$ ) *vesicae*. In conclusion the higher number of MCs in muscular tunic than propria ( $P < 0.001$ ) allowed to suggest their significant role in the regulation of smooth muscle contractility.

*Key words:* Mast cells, urinary bladder, domestic swine

### Introduction

Urine storage and urination depend on the coordinated activity of the two organs – the bladder and urethra [1]. The bladder receives and stores incoming urine, and the urethra ensures its retention of the urinary reflex [7].

In the last 20 years and more, the domestic pig has been an extremely suitable animal model for human research [2, 3]. In this regard, studies related to immunohistochemical detection of biologically active substances in the sensory neurons of the lumbar and sacral spinal ganglia into the bladder wall in pigs are of interest [5, 8]. Having in mind these finding with clinical importance, the presence of mast cells in the bladder wall in domestic pigs is of particular interest. Enzyme – and immunohistochemical studies

on mast cells in the ureter, the male urethra, and renal blood vessels in pig have shown expression of several their substances [4, 10, 11]. The lack of data on the distribution of mast cells in the bladder wall in domestic pigs, related to motility of organ's smooth muscle cells gave motivation for the present study.

## Materials and Methods

The material was taken from four areas of the bladder – triangle, tip, body and neck, immediately after slaughter of twelve, 6 month pigs – 6 males and 6 females (Landrace × Bulgarian White) at 90 – 100 kg/b.w. The animals were slaughtered for meat consumption in full compliance with national legislation.

The sections of about 1 cm<sup>2</sup> from the wall of four areas were placed for immersion fixation in Carnoy fluid for 4 hours, dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin. Serial 5 µm sections were prepared, and were stained with 0.1% toluidine blue in Mc Ilvane buffer, pH 3.

### Statistical analysis

The number of mast cells with clearly visible nuclei was recorded on serial sections. The density of the observed cells was determined by Leica DM 1000 light microscope, digital camera Leica DFC 290 and software LAS V4.10.0 2016, as the number of microscopic fields (×200). The analysis was performed using GraphPadPrism 6 for Windows, one-way ANOVA and Tukey-Kramer test. Significance of the difference in mast cell count was reported at P<0.05 using one-way ANOVA.

## Results

Light microscopic observations showed the presence of mast cells in all four sections of the bladder wall examined. In the mucosal layer, the mast cells were unevenly distributed, and they were located mainly near to arterioles, capillaries and venules. The number of mast cells in the propia varied from region to region. The highest density of mast cells was found in the *trigonum vesicae* and in the *corpus vesicae* – (P<0.001), followed by their density in the *apex vesicae* and that in the *collum vesicae* (P< 0.001), (Table 1).

**Table 1.** Density of mast cells in microscopic fields (×200) of the propia and musculature in the different areas of urinary bladder

Area \ Layer	<i>Lamina propria</i>	<i>Tunica muscularis</i>
<i>Apex vesicae</i>	2.56±0.51	15.89±0.83
<i>Corpus vesicae</i>	3.44±0.51	12.33±0.48
<i>Trigonum vesicae</i>	4.00±0.84	16.61±0.50
<i>Collum vesicae</i>	1.17±0.38	16.50±0.51

Between and next to the smooth muscle bundles in the middle shell of the bladder wall, mast cells were located mainly near the basement membrane of capillary endothelial cells, in the adventitia of arteries and veins, and in the connective tissue between bundles formed by smooth muscle cells. The highest density of mast cells was observed in the muscular coat of the *collum vesicae* – and the *trigonum vesicae* – ( $P < 0.001$ ), followed by that at the *apex vesicae* – and in *corpus vesicae* – ( $P < 0.001$ ), of the bladder (**Table 1**).

The percentage of mast cells localized in the propria was as follows: top – 22.91%; body – 30.8%, triangle – 35.81% and neck – 10.47%, and for the muscular sheet, respectively: top – 25.9%, body – 20.1%, triangle – 27.1% and neck – 26.9%.

## Discussion

Of particular note of this original data is the percentage of toluidine-positive mast cells in the propria and the muscular layer of the bladder. The highest percentages found in the „boundary zone“ between the ureter and the bladder, namely the triangle, could be explained by the important role that mast cells play through the release of biologically active substances, both in maintaining the local microenvironment (homeostasis) and in the motility of smooth muscle cells.

It is quite logical to assume that the role of smooth muscle cells in this narrowed compared to the broad lumen of the body of the bladder, in the passage of urine to the beginning of the urethra after dilatation of *m. urethralis*, is indisputable. Of course, the involvement of mast cells in influencing the motility of smooth muscle cells in the apex and body also deserves attention.

The hypothesis proposed more than 15 years ago [10] for the participation of mast cells not only in maintaining local homeostasis (microenvironment) of the porcine ureter, but also in the motility of smooth muscle cells on its wall was recently confirmed by [6].

## Conclusion

The original data obtained supplement the lack of knowledge about mast cells in the bladder wall. Along with what is known about them in the ureter, ureterovesical junction [9] and urethra, a clearer and more complete picture of the presence and role of mast cells in the extrarenal part of the urinary tract in domestic pigs is obtained.

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