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MORPHOLOGY OF OVIDUCTS OF EUROPEAN LOWLAND BISON *BISON BONASUS BONASUS* (L. 1758)

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ABSTRACT

The female genital organs of European bison has not been thoroughly investigated thus far. The aim of the present study was to describe the macroscopic and microscopic structure of oviducts in European bison and their changes throughout postnatal life. As the material served genital organs of 55 European bison females. Animals were divided into two age groups. The first included 36 sexually immature females at the age of 2 months to 1.5 year, whereas the other counting 19 cows included sexually mature females at the age of 2 to 20 years. The morphological analysis of isolated oviducts were performed. The basic morphometric and micrometric parameters were measured. It was stated that oviducts of bison females reach developmental maturity at the age of 1.5–2 years. The morphology and histological features of oviducts resembles that of domestic cattle, however their dimensions differ.

Key words: European bison, morphology, morphometry, oviduct.

INTRODUCTION

Sexual organs are responsible for the reproductive function of a body, assuring species continuity, hence the problem of reproduction is of key importance in animals at risk of extinction. Despite successive restitution, bison may be included amongst these animals [17, 16, 18] owing to a high degree of inbreeding [28, 38]. Thus it seems indispensable to conduct a variety of research addressing the widely understood morphology of this species. Their results will be a cornerstone for further observations in the area of, among others, physiology and reproductive pathology of bison.

The urogenital system of European bison was in part described in two habilitation theses. One of them was devoted to urinary organs [29], and the other to male genitals [36]. In addition, the structure of European bison deferent ducts was the subject of an older study [24] the histological structure of testes was investigated by Bomba [7], Czykier et al. [9], Sysa and Matuszewska [37], and the size and mass of testes in the postnatal development were analyzed by Krasieńska et al. [21].

The morphological structure of female genitals of this species, apart from short mentions, has not been thoroughly investigated thus far. Results of fragmentary observations in this respect were described by Korobko and Kurnosov [20] in the monograph "Bison". Despite data presented, the whole work provides a fairly incomplete picture. In recent years, morphology of the reproductive system of European bison females was the subject of a Ph.D. thesis [27], yet only the first part of this thesis relating to ovaries has been published and thus available to wider audience [26]. The oviducts play a special role in mammalian procreation, what determines their structural and functional traits. In these organs processes essential for reproduction occur including gametes transport, sperm capacitation, fertilization, early embryonic development and embryo transport towards the uterus. In domestic animals the basic construction of the oviduct is well known. The tube reaches a length of 25–30 cm in cattle and horses and 15 cm in small ruminants [3, 30]. The diameter of the bovine oviduct is the smallest in the isthmus (1–2.5 mm), while in the ampulla amounts 3–5 mm [30]. The oviductal mucous membrane forms longitudinal folds and is covered with monolayer columnar, partly ciliated epithelium. The height of the epithelial cells shorten as the uterine tubes progress toward the uterus [34]. Tubal microstructure varies depending on the section and ovarian activity [11]. Progesterone may via specific

receptors modulate oviductal functions to promote early embryo development [33]. The main aim of this study was to describe the macroscopic and microscopic structure of European bison oviducts in the postnatal development based on morphometric analyses. The second basic objective was to trace changes in the structure of those organs as affected by age and sexual maturity of animals.

MATERIALS AND METHODS

The female genitals were isolated from 55 females of European bison *Bison bonasus bonasus* L. 1758, originating from reserve breeding and a wild population of bison from the Białowieża Forest. Information on the age of bison cows (from two months to twenty years), was collected from Pedigree Book or was on-spot determined during the field study based on dentition status [40].

Literature data indicate that in the reserve breeding the youngest pregnant bison females were at the age of 24–28 months [15], and that the oldest bison females delivering calves were at the age of 22 years [19]. Based on these findings, the females were divided into two age groups. The first counting 36 cows (group I) included sexually immature females at the age of 2 months to 1.5 year, whereas the other counting 19 cows (group II) included sexually mature females at the age of 2 to 20 years. The animals originated from selective-reduction culling conducted annually within Białowieża Forest National Park under the supervision of the Ministry of Environmental Protection. The culling was performed since November till February, i.e. in the period of sexual inactivity.

Immediately after death, individual animals were weighed with an accuracy of ± 1 kg. The preliminary macroscopic evaluation of oviducts was performed at necropsy. The evaluation included checking abnormalities in anatomical structure, symptoms of inflammation or any other pathological lesions. Once the organs had been isolated, their total length including infundibulum was measured using an electronic slide caliper exact to 0.01 mm. The whole oviducts were collected for histological analyses. Samples were fixed in a buffered 5% aqueous solution of formaldehyde, embedded in paraffin blocks, and cut into 10 μ m thick sections using a rotary microtome. Afterwards, they were stained with a review HE method – hematoxylin and eosin. Thus prepared material was subjected to microscopic analyses using a MultiScanBase v. 8.08TM software. The following parameters were measured: lumen diameter, height of endosalpinx epithelium, thickness of myosalpinx and thickness of serous membrane. The inner diameter was measured at the central part of the ampulla. Additional cross-sections of the isthmus and uterine part were used for the observation of their microstructure.

Results of measurements were subjected to a statistical analysis using Statistica 5.0TM and SPSSSTM software. The Pearson's correlation coefficient was computed to estimate the strength of a linear correlation between the parameters examined. Calculations were made separately in both age groups for both macroscopic and microscopic parameters. A correlation between macroscopic parameters and body weight was analyzed as well. Additional analyses were carried out for the dependence of the length of both oviducts and their diameters on the age of the females and for the dependence of the microscopic parameters: height of epithelium cells, thickness of endosalpinx epithelium and myosalpinx on the age of the animals. In all above-mentioned tests, the significance of differences was adopted at a level of 0.01 – denoted with a symbol **, and at a level of 0.001 – denoted with a symbol †.

RESULTS AND DISCUSSION

An oviduct (uterine tube, Fallopian tube) is a narrow, stranded duct running from the tubal end of the ovary to the apex of the respective uterine horn. It is suspended on a relatively long, thin and vascularized mesosalpinx. Figure 1 presents isolated oviduct of European bison. In our study the infundibulum of sexually mature females was wide and had well expressed fimbriae red-orange in color. Herein, in one of the investigated animals, there were observed small follicles filled with serous fluid (Fig. 2). The abdominal opening of the oviduct was wide and well visible, and its ampulla, which constitutes 2/3 of the whole oviduct length, was wider than the other parts of this organ. The isthmus was short and thin; and straight at its distal section. In the uterine part, the oviduct with the maximum length of 0.4 cm without any distinct boundary, gently extends into the uterine horn. In sexually immature females, the entire oviduct and its infundibulum in particular, was narrower than in mature females, whereas fimbriae were more poorly developed.

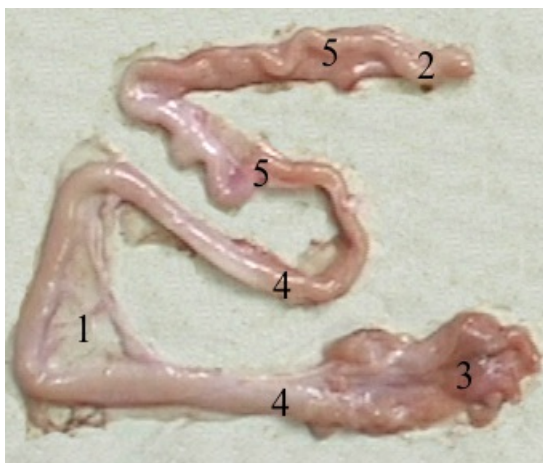


Fig. 1. Oviduct of European bison (1 – mesosalpinx, 2 – uterine part, 3 – infundibulum, 4 – ampulla, 5 – isthmus)

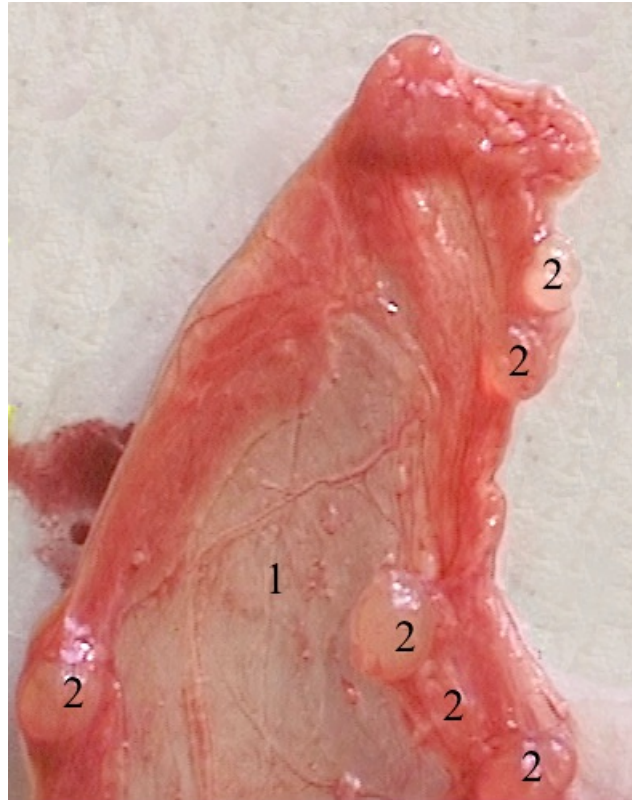


Fig. 2. Fragment of infundibulum of a 17-year-old bison female (1 – mesosalpinx, 2 – lesions in the form of small follicles filled with serous fluid)

The course of the uterine tube displayed high individual diversity both in the young and mature females. Sometimes at the whole length, and sometimes only in the distal section, it was very sinuous (Fig. 3). Often, its straight course was also observed but only in the central part of the oviducts, meaning at the distal section of its ampulla.



Fig. 3. Sinusoidal fragment of the oviduct

In young bison females, the average length of the right oviduct accounted for 14.69 cm, and that of the left oviduct for 15.32 cm, and these values were statistically significantly different (Student's t-test, $p > 0.05$). In domestic cattle, a closely related species, the length of right and left oviduct in slaughter heifers was 16.45 and 16.72 cm on average respectively [10], while in 2-months calves it amounted 15 cm [25]. The mean length of the right oviduct in sexually-mature bison females was significantly greater than in the young females and reached 24.67 cm, whereas that of the left oviduct reached 25.86 cm (Tab. 1). The differences between oviduct lengths in both groups were statistically significant (Student's t-test, $p < 0.001$). The mean diameter of the internal cross-section of the oviduct in group I accounted for 1352.17 μm , whereas in group II for as much as 2180.73 μm , with the differences noted between groups being very large and statistically significant (Student's t-test, $p < 0.001$). The age-related development of the oviduct indicated that its morphological maturation is completed within 20 months (Fig. 4). It corresponded with the general body development.

Table 1. Length of oviducts of European bison [cm]

Groups	Right/left	n	–	min–max	SD	Student's	p
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	oviduct		x			t-test	
I	R	36	14.69	8.94–20.64	2.96	-6.69	0.000†
	L	36	15.32	8.53–23.91	3.11	-6.67	0.000†
II	R	19	24.67	14.01–33.43	6.13	-6.69	0.000†
	L	19	25.86	14.66–33.01	6.50	-6.67	0.000†

Denotation of statistical differences between groups:
** p<0.01
† p<0.001

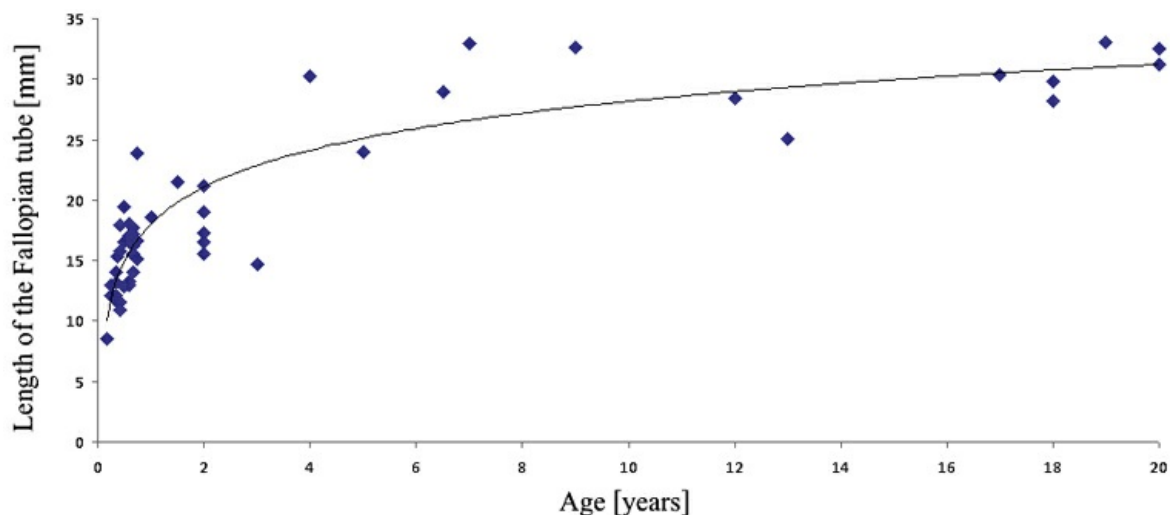


Fig. 4. Age-related development of European bison's oviducts

The folds of the mucous membrane (endosalpinx) protruded to the lumen of the oviduct. Those folds were characterized by a high diversity – from very long and branched with sinuous course, to straight and short, depending on the part of the oviduct they occurred in. In the ampulla, the folds displayed the greatest diversity (Fig. 5), whereas in the other sections, especially in the uterine part, they were short, straight with thick and heavily vascularized lamina propria (Fig. 6). Dimensions of microscopic structures of European bison's oviducts presents Table 2. The folds were lined mainly with simple epithelium resting on the basilemma. Its mean height in group I reached 22.23 μm , and in group II – 23.11 μm . In comparison, in domestic cattle mature heifers the mean height of oviduct epithelial cells amounted from 20.7 to 25.6 μm , depending on the day of oestrous cycle [13] and in young calves this dimension was 23 μm in the isthmus section [25]. In the present study the difference between groups was statistically insignificant. The ciliated and non-ciliated (secretory) cells were observed in the epithelium. The first occurred in majority and were rectangular or triangular in shape, had a dark nuclei and pink-colored cytoplasm. Often, they were arranged tightly, and sometimes in multi-rows, especially at the peripheral parts of the tubal folds. In turn, the latter were lower, with light cytoplasm and occurred individually on the folds. The least numerous were very low and round cells laying next to the basilemma. Their cytoplasm was light, and their nuclei were oval and large. In cattle the counts of ciliary cells varied during the sexual cycle: they reached the maximum (up to 68%) during oestrus. About 13% of cells lost cilia during metoestrus and at the beginning of dioestrus. Reciliation occurred during pro-oestrus [39]. Underneath the epithelium there was the layer of the lamina propria, which was very thin in the folds. It was constituted mainly by fibers of connective tissue, fibroblasts and sparse cells of smooth muscles, and macrophages. It also contained blood vessels. Directly under the endosalpinx there was the muscle coat of the oviduct with the thickness of 262.93 μm on average, whereas separately for groups I and II, its mean thickness accounted for 221.47 and 341.49 μm , respectively. The difference between the groups was statistically significant. This membrane was built of two layers of smooth muscles. In the internal layer, the muscle fibers run circularly, whereas in the outer layer – longitudinally. In the uterine part, the muscular membrane was more developed than in the other parts of the oviduct. Both layers constituting it were indistinctly separated from each other, and blood vessels were located between them. These vessels penetrated into the serous membrane of the uterine tube. It was constituted by a thin layer of connective flaccid tissue that contained numerous collagen and reticular fibers, and smooth muscle cells. It was covered with simple squamous epithelium which extended into the epithelium covering the mesosalpinx. In group I the mean thickness of serous membrane reached 85.26 μm , and in group II – 117.42 μm , and the difference between groups was statistically significant. The statistical analysis demonstrated that the groups examined were differing significantly in almost all analyzed traits. No significant differences was only observed for the height of endosalpinx epithelium. Significantly positive correlations occurred between the length of the right oviduct compared to the left one, in both age groups. In addition, a significantly positive correlation was observed in group I between oviduct diameter and the height of the endosalpinx epithelium. The Pearson's test demonstrated that the macroscopic parameters of the oviducts were significantly positively correlated with body weight of the bison cows in both groups I and II.

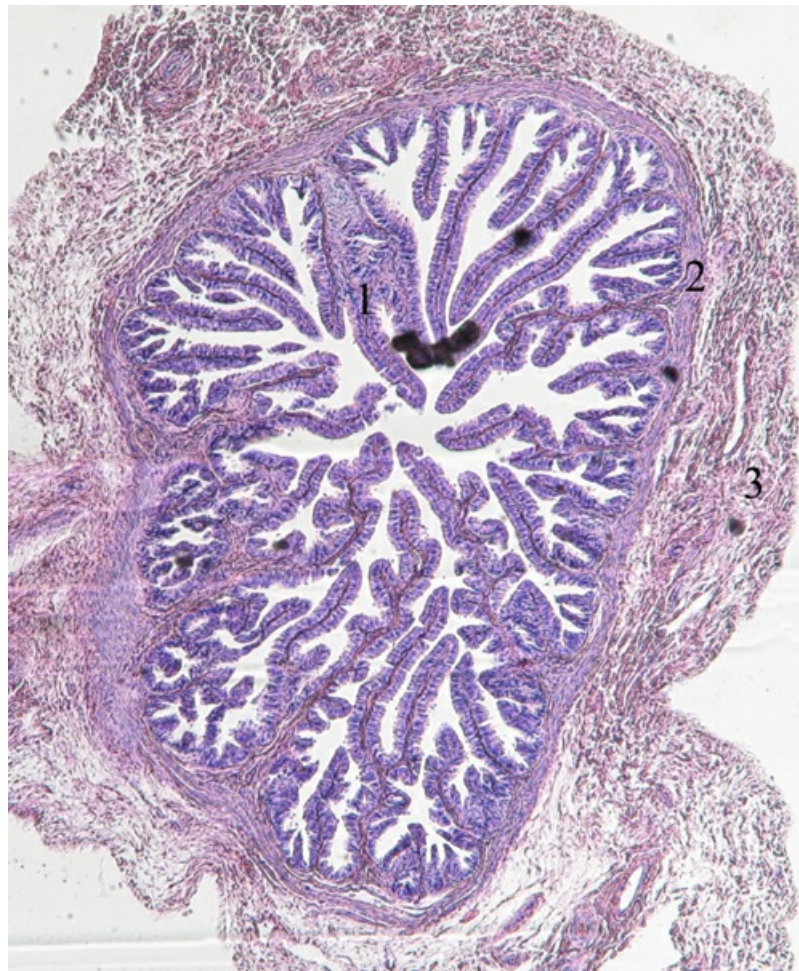


Fig. 5. Oviduct of European bison, ampulla (cross-section), 4 x magnification (1 – mucosal fold, 2 – myosalpinx, 3 – serous membrane)

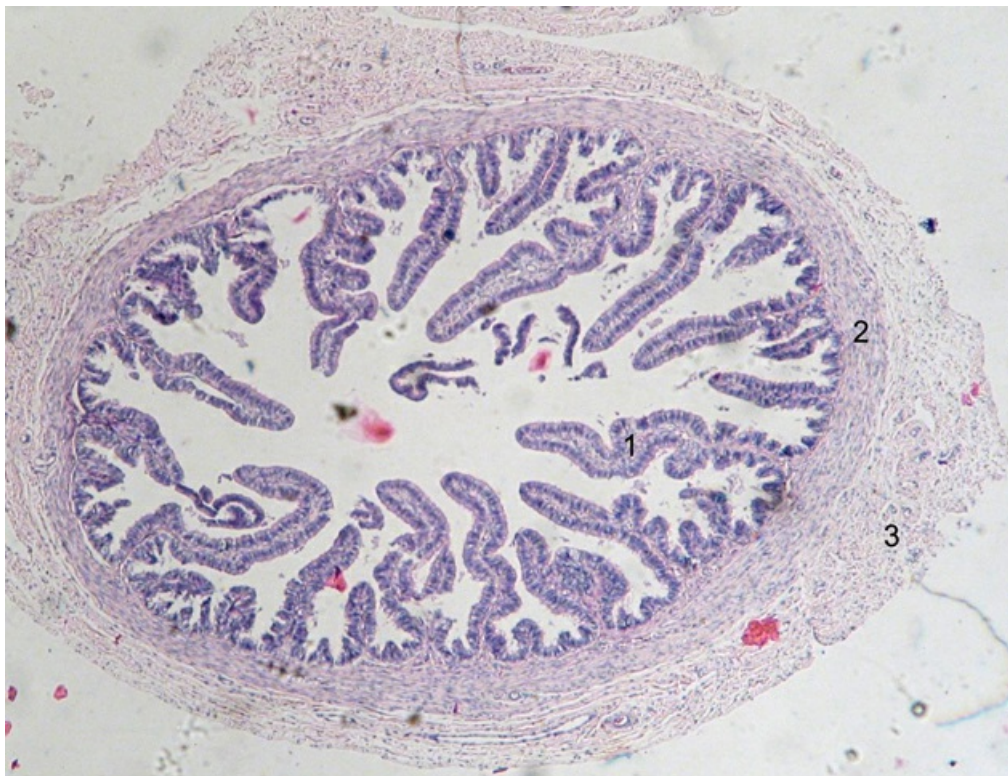


Fig. 6. Oviduct of European bison, uterine portion (cross-section), 4 x magnification (1 – mucosal fold, 2 – myosalpinx, 3 – serous membrane)

Table 2. Dimensions of microscopic structures of European bison's oviducts [μm]

Dimension	n	\bar{x}	min-max	Student's t-test	p	SD
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Group I						
Diameter of the oviduct	36	1352.17	574.70–1996.40	-8.81	0.000†	331.52
Height of the epithelium	36	22.23	15.20–30.57	-0.91	0.369	3.88
Thickness of the myosalpinx	36	221.47	112–483.34	-4.00	0.000†	99.84
Thickness of the serous tunic	36	85.26	35.64–163.60	-3.17	0.004**	19.92
Group II						
Dimeter of the oviduct	19	2180.73	1772.10–892.10	-8.81	0.000†	332.33
Height of the epithelium	19	23.11	20.14–27.91	-0.91	0.369	2.43
Thickness of the myosalpinx	19	341.49	210.24–587.85	-4.00	0.000†	116.36
Thickness of the serous tunic	19	117.42	52.06–208.53	-3.17	0.004**	41.86
Denotation of statistical differences between groups:						
** p<0.01						
† p<0.001						

Out of the microscopic parameters, only the thickness of endosalpinx epithelium was poorly correlated with age, whereas the height of cells did not change with age. No correlation was either observed between the thickness of myosalpinx and age.

Based on the morphological analysis of oviducts, it was stated that females of European bison might begin sexual maturation at the age of 1.5 – 2 years [26], which has been confirmed by analyses of oviducts conducted in this study. Most often, however, bison cows reach sexual maturity in the third year of life, and deliver their first calf at the age of four [22, 23]. The length of both oviducts and their diameter was significantly correlated with age of the animals. In one animal fluid-filled follicles were observed within the infundibulum region. They resembled paraovarian cysts reported in other species like horses [5], cattle [1, 32], sheep [35], goats [2] and pigs [14].

The comparison of linear dimensions of the oviducts demonstrated that in bison they were shorter and thicker than in the cattle and the differences appeared to be the greatest between mature individuals. It confirms earlier results of observations made by Korobko and Kurnosov [20]. Unlike in cattle, in examined European bison females the left oviduct was longer than the right one in both groups (Tab. 1). In addition, the oviducts of bisons were characterized by more straight course. The microscopic structure was similar in both species. Korobko and Kurnosov [20] demonstrated that in bison females the quality and type of cells of the endosalpinx and extent of mucus secretion are subject to continuous changes. In their opinion, it is determined by the endocrine secretion of hormones. Similar changes are observed in cattle, which are characterized by a higher number of secretory cells and enhanced production of mucus into the lumen of the oviduct at oestrus [6, 12]. It is confirmed by observations of the analyzed oviducts of bison females that were collected for this study at the time of seasonal sexual inactivity and probably low concentration of gonadal steroids. The observed number of mucus-secreting cells was low. In turn, Czchartiszvil [8] determined a lower number of secretory cells in bison than in other domestic ruminants. Unfortunately, he did not specify the period of the sexual cycle they originated from. In addition, our study showed increased thickness of muscle coat in the uterine section of bison oviduct. Oviductal length, inner diameter and the thickness of tunica serosa appeared to be smaller and the thickness of tunica muscularis bigger than those reported in cattle [4, 11]. Experiments conducted with the muscle coat of oviduct in cattle demonstrated its increased contractility and thickness in the isthmus and in the area of the uterine ostium [Rodríguez-Martínez 31]. It may thus be assumed that in both species bison and cattle, the thickness of the myosalpinx increases in similar parts of the oviduct.

CONCLUSION

The analysis of oviducts morphology of bison females demonstrated that they reach developmental maturity at the age of 1.5–2 years. All dimensions, apart from the height of oviduct epithelium, measured in young bison females, differed statistically significantly from the respective variables in sexually mature females. In majority of cases, stronger correlations of the examined traits were observed in the group of sexually mature females than in the sexually immature ones.

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