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## **MORPHOMETRIC STUDY OF THE UTERUS OF LOWLAND EUROPEAN BISON *BISON BONASUS* (LINNAEUS, 1758)**

## **BADANIA MORFOMETRYCZNE MACICY ŻUBRA NIZINNEGO *BISON BONASUS* (LINNAEUS, 1758)**

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**Streszczenie.** Żubr nizinny, pomimo udanej restytucji w Polsce, należy w dalszym ciągu do gatunków zagrożonych. Celem badań było opisanie morfologii macicy żubra i przeprowadzenie analizy morfometrycznej narządu, a także porównanie ze znanymi odpowiednimi parametrami bydła domowego. Materiał do badań pochodził od 55 samic żyjących w Puszczy Białowieskiej. Zwierzęta podzielono na dwie grupy. Grupa I składała się z 36 osobników niedojrzałych płciowo – w wieku od 2 miesięcy do 1,5 roku włącznie. Do grupy II zaliczono 19 dojrzałych samic w wieku 2–20 lat. Stwierdzono istotne różnice pomiędzy badanymi grupami. U młodych samic macice, z wyjątkiem doczaszkowych części rogów, leżały w jamie miedniczej. U starszych zwierząt, które w większości już rodziły, macice były większe, w związku z czym tylko połowa szyjki była umiejscowiona w miednicy, pozostała część narządu znajdowała się w jamie brzusznej. Ogólnie macica żubra jest podobna do tego narządu u bydła domowego, jednak wykazano kilka szczegółowych różnic, być może związanych z odmiennym trybem życia.

**Key words:** European bison, uterus, morphology, morphometry.

**Słowa kluczowe:** żubr, macica, morfologia, morfometria.

## **INTRODUCTION**

Although restitution of European bison on Polish territory was effective, this animal still belongs to endangered species. Acquiring detailed knowledge of the biology and anatomy of these animals expands the possibilities of rational conservation of the species using cutting-edge methods of reproduction control. It may also be important in the diagnosis of congenital defects and diseases (Olbrych 2002). Morphology of the female genital organs, except for brief mentions, has not been thoroughly investigated yet. Results of fragmentary observations in this area were published by Korobko and Kurnosov (Korobko and Kurnosov 1979). However, there are no extensive studies describing the morphology and microstructure of the genital

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organs of female of European bison. Without precise knowledge of the structure of these organs, it is difficult to imagine further work on the physiology and pathology of reproduction, as well as conservation and protection of this interesting species.

The aim of this study was to describe the macroscopic structure and morphometric analysis of the uterus of European bison in the postnatal development. In addition, changes were investigated in the structure of this organ in relation to age and sexual maturity, and results achieved were compared with available data on domestic cattle

## MATERIAL AND METHODS

The material consisted of isolated female genital organs, *organa genitalia feminina*, obtained from 55 females of lowland European bison *Bison bonasus* (Linnaeus 1758), living in the Białowieża Forest. Information about the age of females was acquired from the European Bison Pedigree Book or was determined on-site during field research, based on the state of dentition (Węgrzyn and Serwatka 1984). Animals came from selective culling. Only sick and wounded individuals were sacrificed and only organs showing no lesions were tested. Research material has been divided into two age groups, based on data from studies by Jaczewski and Koch (Koch 1956, Jaczewski 1958). Group I consisted of 36 sexually immature females, aged from 2 months to 1.5 years inclusive, while Group II comprised 19 sexually mature animals at the age from 2 to 20 years. Immediately after death, individuals tested were weighed with an accuracy of 2 kg; skeletotomy, holotomy and syntopy were determined. Then genital organs were removed by cutting the broad ligament of the uterus at its attachment to the pelvic wall. Uterine horns, body and cervix were cut longitudinally, in order to determine the shape, size, arrangement, course, and the color of the tissues including the degree of congestion. Next, the measurements were analyzed using MultiScanBase 8.08™ software. Photographic documentation was prepared at each stage. Anatomical terms were established on the basis of Nomina Anatomica Veterinaria (Nomina anatomia veterinaria). The results were statistically analyzed using the Statistica 5.0™ software package.

## RESULTS AND DISCUSSION

Female genital organs of the European bison lie in the abdomen and pelvic cavity, at half-height of cranial pelvic aperture. They are attached to the lateral walls of the pelvis by relatively long broad ligament of the uterus (Fig. 1). This ligament has a complex structure and may extend cranially to the fourth lumbar vertebra. Its proximal section constitutes the mesovarium, which extends caudally into the mesosalpinx, which in turn continues into the mesometrium. Mesovarium and oviduct delimits large ovarian bursa. The broad ligament of the uterus in older animals has a strong and compact structure, while in young females it is much weaker, of flabby consistency. Therefore, obliterated umbilical arteries could easily be seen through them as well as round ligaments of the bladder, 0.5 cm in diameter, lying on the cranial borders of the lateral ligaments of the bladder. At the free cranial edge of the broad ligament of the uterus, from the proximal part of the respective uterine horn to the inguinal canal, the round ligament of the uterus is situated. It is much less pronounced compared to the aforementioned round ligament of the bladder. Clearly visible ureter runs in the lateral part of the broad ligament of the uterus, and aims toward the bladder.

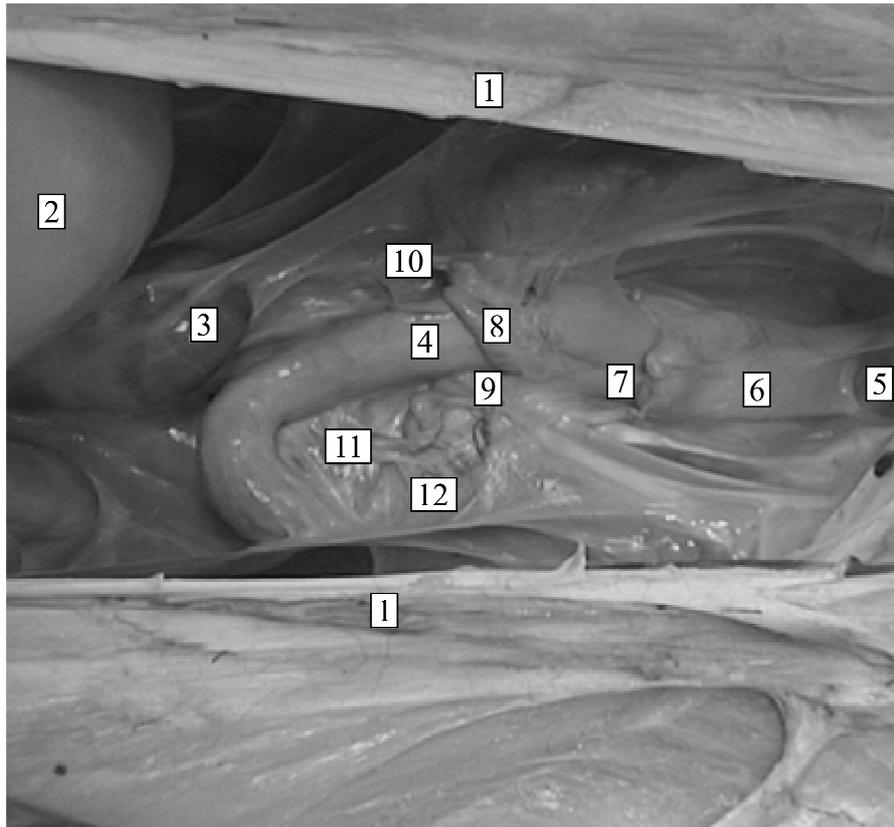


Fig. 1. Location of female genital organs in abdominal cavity and pelvic cavity. 1 – abdominal wall, 2 – rumen, 3 – left kidney, 4 – colon, 5 – vagina, 6 – urinary bladder, 7 – uterine cervix, 8 – right uterine horn, 9 – left uterine horn, 11 – left ovarian bursa, 12 – right ovarian bursa, 12 – broad ligament of the uterus

Ryc. 1. Narządy płciowe żeńskie w jamie brzusznej i jamie miednicy. 1 – ściana brzucha, 2 – żwacz, 3 – lewa nerka, 4 – okrężnica, 5 – pochwa, 6 – pęcherz moczowy, 7 – szyjka macicy, 8 – prawy róg macicy, 9 – lewy róg macicy, 10 – lewa kieszonka jajnika, 11 – prawa kieszonka jajnika, 12 – więzadło szerokie macicy

Position of the uterus is dependent on the age of the animal. In young females, apart from the proximal parts of the uterine horns and ovaries, the remaining genital organs are located within the pelvic cavity. In older females, most of which have already calved, the uterus is larger, thus only caudal half of the cervix, vagina and the vestibule are located in the pelvic cavity. The uterine horns are bent ventrolaterally. The right horn is next to the sigmoid colon and the left horn is placed between the left kidney (cranially) and colon (ventromedially), and the lateral wall of the abdominal cavity. The cervix, vagina and its vestibule lie between the colon (dorsally), and urinary bladder, urethra and the pubic symphysis (ventrally). Detailed parameters are presented in Table 1. In addition, a Kolmogorov-Smirnov test was performed, characterized by a normal distribution, allowing to use parametric Student's t-test in further analysis. The latter test enabled comparison of the groups and showed that the groups studied significantly differed in almost all analyzed traits.

In anatomico-comparative terms, uterus of the European bison belongs to the bicornis subseptus type. Horns narrow down towards the ovaries (Fig. 2 and Fig. 3).

Table 1. Dimensions of uterus [cm] and their basic statistics  
Tabela 1. Wymiary macicy [cm] i ich ocean statystyczna

	Group I + II Grupa I + II			Group I Grupa I			Group II Grupa II					
	n	min – max	SD	n	min – max	SD	n	min – max	SD			
1	55	13.42	5.90 – 40.83	6.73	36	10.02	5.90 – 16.05	7.73	19	19.84	9.25 – 40.83	7.73
2	55	13.38	5.43 – 35.40	6.53	36	9.93	5.43 – 14.40	7.10	19	19.92	9.46 – 35.40	7.10
3	55	1.36	0.67 – 3.79	0.74	36	0.96	0.67 – 1.69	0.75	19	2.13	0.89 – 3.79	0.75
4	55	1.36	0.57 – 3.19	0.68	36	0.97	0.57 – 1.59	0.64	19	2.09	1.10 – 3.19	0.64
5	55	3.22	1.19 – 6.56	1.41	36	2.56	1.19 – 4.12	0.67	19	4.46	1.97 – 6.56	1.62
6	55	3.75	0.99 – 8.20	1.82	36	3.08	0.99 – 4.94	0.96	19	5.02	1.32 – 8.20	2.35
7	55	2.00	0.90 – 4.42	1.07	36	1.44	0.90 – 2.36	0.35	19	3.07	1.20 – 4.42	1.00
8	55	1.30	0.18 – 6.60	1.20	36	0.72	0.18 – 1.82	0.34	19	2.41	0.74 – 6.60	1.46
9	55	4.02	2.01 – 10.30	2.07	36	2.84	2.01 – 4.50	0.57	19	6.25	3.08 – 10.30	2.05
10	55	1.93	0.62 – 4.40	1.10	36	1.26	0.62 – 1.90	0.25	19	3.20	1.64 – 4.40	0.97

1 – the length of right and 2 – left uterine horn, 3 – the width of the right and 4 – left uterine horn, 5 – the length of the velum uteri, 6 – the length of the uterine body, 7 – the width of the body, 8 – the length of intercornual ligament, 9 – the length of the cervix, and 10 – the width of the cervix.

1 – długość rogu prawego macicy, 2 – długość lewego rogu macicy, 3 – szerokość rogu prawego, 4 – szerokość rogu lewego, 5 – długość żagla macicy, 6 – długość trzonu macicy, 7 – szerokość trzonu macicy, 8 – długość więzadła międzyrożnego macicy, 9 – długość szyjki macicy, 10 – szerokość szyjki macicy.

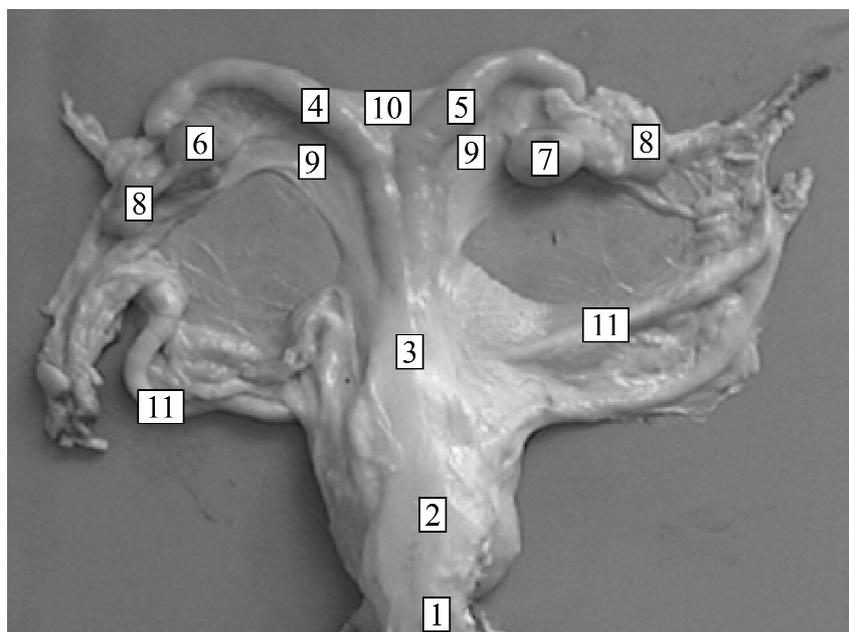


Fig. 2. Genital organs of young female bison. 1 – vaginal vestibule, 2 – vagina, 3 – uterine cervix, 4 – left uterine horn, 5 – right uterine horn, 6 – left ovary, 7 – right ovary, 8 – ovarian bursa, 9 – broad ligament of the uterus, 10 – intercornual ligament, 11 – round ligament of the uterus

Ryc. 2. Narządy płciowe żeńskie młodej samicy żubra. 1 – przedsionek pochwy, 2 – pochwa, 3 – szyjka macicy, 4 – lewy róg macicy, 5 – prawy róg macicy, 6 – lewy jajnik, 7 – prawy jajnik, 8 – kieszonka jajnika, 9 – więzadło szerokie macicy, 10 – więzadło międzyrożne, 11 – więzadło obłe pęcherza moczowego

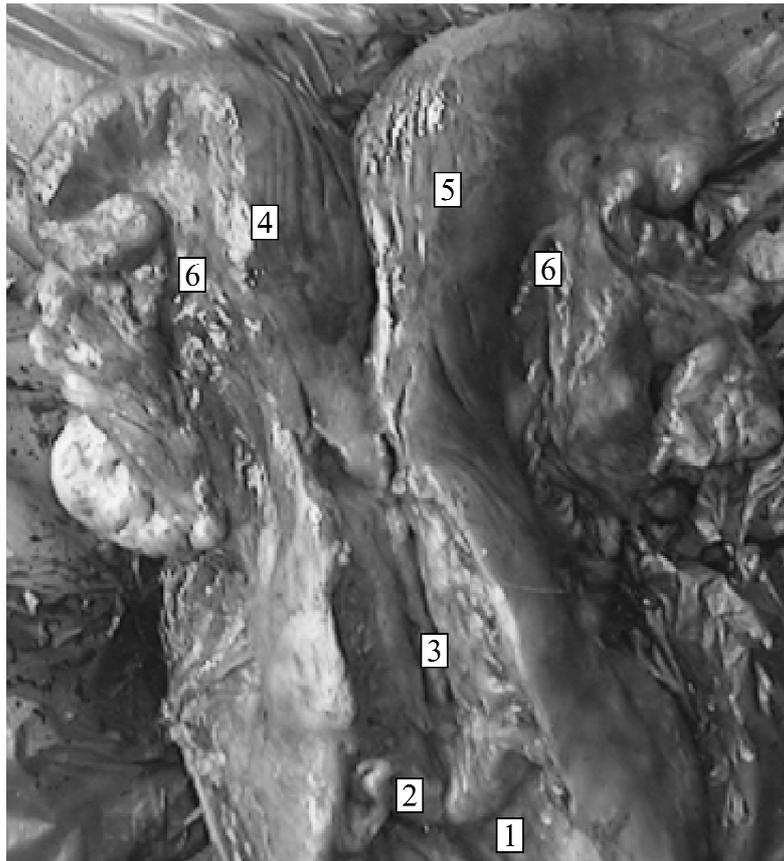


Fig. 3. Genital organs of adult female bison. 1 – vagina, 2 – vaginal portion of the cervix, 3 – cervical canal, 4 – left uterine horn, 5 – right uterine horn, 6 – broad ligament of the uterus

Ryc. 3. Narządy płciowe żeńskie dojrzałej żubrzycy. 1 – pochwa, 2 – część pochwy szyjki macicy, 3 – szyjka macicy, 4 – lewy róg macicy, 5 – prawy róg macicy, 6 – więzadło szerokie macicy

In very young females (several months old), uterine horns are convoluted ventrally in the middle of their length, and their proximal segments are strongly involuted, whereas in adult females (several years of age), they are less involuted and only in the proximal parts. Dimensions of the uterine horns are also dependent on the age and number of gestations.

Uterine caruncles are present on the endometrium and are arranged in 4, rarely 5 rows. These rows lie lengthwise the uterine horns and sometimes they can merge or cross with each other (Fig. 4). Each row contains from 10 to 18 uterine caruncles, with dimensions (length, width) of 0.3 to 0.8 cm in the non-gestating organ. At point of divergence of uterine horns is a double fold of serous membrane called intercornual ligament (Fig. 2). The dimensions of this structure are dependent on the age of the female, and the difference between the groups was statistically significant.

Between this double fold, a triangular cavity is formed about 1.5 cm in width, at the bottom of which bifurcation of uterine body is visible. The body of the uterus is divided in its proximal part by the velum of the uterus. This parameter has significant differences between the groups. Moreover, statistically significant is the average length and width of the uterus. The results show that only 1/8 of the uterine body is not divided by velum uteri. In this descriptive part, the uterine caruncles do not occur. The width of the body is on average less than half of its length. Internal orifice of the uterus begins from the first and smallest circular fold, free

edge of which is directed towards the cervical canal. Number of circular folds in this part of the uterus varies from 3 to 5, sometimes 6, and this is an individual trait. Each of the circular folds has numerous longitudinal folds of different sizes on the surface. Cervical canal contains viscous, gray and pink mucus, which together with the folds constitutes a natural, mechanical and biological barrier, protecting the environment of the uterus from infection. The length and width of the cervix are the values that significantly differ between the groups studied. The last fold is the largest and protrudes a distance from 0.1 cm in the youngest females, to 4.8 cm in the oldest females, towards the vaginal canal, forming the vaginal portion of the cervix (Fig. 3). This part viewed from the vaginal cavity forms the shape of a circle with a diameter of 0.44 cm to 3.9 cm.

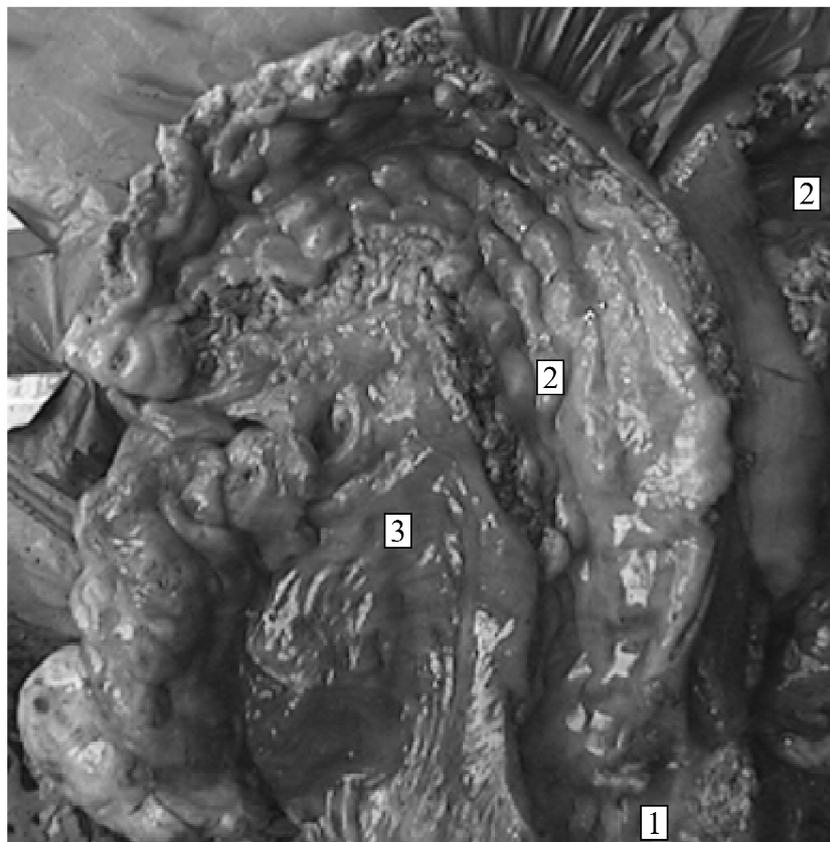


Fig. 4. Uterine horn opened along the greater curvature. 1 – uterine velum, 2 – caruncula, 3 – broad ligament of the uterus

Ryc. 4. Róg macicy po rozcięciu wzdłuż krzywizny większej. 1 – żagiel macicy, 2 – brodawka, 3 – więzadło szerokie macicy

In addition, Pearson's parametric test was performed, which allowed investigating correlations between the variables within each group. This test was performed separately in the group of sexually immature females and the group of mature animals. The analysis showed that significant positive correlations, both in group I and II, occurred for the majority of parameters. There were no significant negative correlations found. Further, a comparison of these correlations was carried out with the use of Fischer's test. As a result of this test,

it was found that the previously demonstrated interrelationships, in all cases of significantly correlated parameters, were significantly higher in the group of mature females compared to young females. Pearson's test showed no significant correlation between body weight and the length of the body of uterus in group I. Other parameters positively correlated with body weight. In group II, all variables significantly positively correlated with body weight. Fischer's test demonstrated significantly higher correlations in group II than I for the following variables: the length and width of the left uterine horn and the length of the body of the uterus and body weight.

This study allows understanding the structure and position of the uterus in females of the European bison. It is also the basis for determining the age-related changes, especially with respect to sexual maturity. Statistical analysis of quantitative traits of individual organs, and a comparison with domestic cattle is the foundation for establishing morphological (anatomical) standards for females of the European bison in prepubertal and reproductive period, which is highly important from the perspective of conservation and reproduction of this species, and its further breeding in reserves (Kobryń 2006).

Position of the uterus in European bison is very similar to the topography of this organ in domestic cattle. Comparison of the two study groups of females of the European bison showed that in young individuals, likewise in domestic cattle heifers, this organ lies almost entirely within the pelvic cavity (Milart 1991). Involution of the uterine horns was observed, starting from their proximal sections, while in cows that had already calved, the uterus did not return precisely to the original position, thus the greater part of the organ was located in the abdominal cavity (Kobryń 1999). In domestic cattle, after birth, uterine horns become symmetrical again, although sometimes one of them (usually the right) may be slightly bigger due to the pregnancies. The overall dimensions of the uterus are somewhat larger after each gestation. Therefore, this organ in multiparous cows is generally larger than in the primiparous cows (Max 2012).

The uterus of domestic cattle and European bison belongs to the bicornuate septal type of uteri. In cattle, the uterine horns are strongly involuted in the shape of ram horns, and the degree of involution is dependent on the phase of the estrous cycle, which is particularly pronounced in heifers (Pierson and Ginther 1987; Bonafos et al. 1995). In the European bison females belonging only to group I, i.e. those who had not given birth yet, horns had a similar shape and orientation. Older females had uterine horns with straighter course, sometimes only the proximal parts were bent ventrolaterally. This study showed that the linear dimensions of the uterine horns of the European bison are smaller than in cattle. Nevertheless, the number of uterine caruncles in European bison is slightly larger and it may be due to their smaller size compared to cattle. This fact was already pointed out earlier (Korobko and Kurnosov 1979). These authors showed that the caruncles were smaller in young European bisons compared to adults, but they did not indicate the exact number of the caruncles.

Significant differences between the two compared species occur in the linear dimensions of the intercornual ligament, as these values in European bison are lower. In contrast, the dimensions of the velum uteri and the body of the uterus are similar in both species. The dimensions of the cervix also vary, particularly in a group of young females. The parameters of this structure are much higher in cattle heifers than in young females of the European

bison. This difference disappears in mature individuals of both species. Vaginal portion of the cervix has smaller size in European bison than in cattle; it is also less developed than in cows. Based on the above comparative analysis between European bisons and domestic cattle, it can be speculated that the presented differences may be due to the different lifestyles of these animals. Cows, compared to European bisons, more often become pregnant and give birth during their lives, as a consequence of domestication. This is due to the intensive use of cattle by man, which exerts a great impact on the structure and size of the genital organs of this species. European bisons living in the wild have to cope with varying environmental conditions and not every season is conducive to giving birth and rearing calves (Kraśiński and Raczyński 1967). Females of the European bison need to adapt to changes in their environment (Kraśiński 1978). Births occur in the months that are most favorable for raising the young (Kraśiński and Raczyński 1969; Daleszczyk and Kraśiński 2001). And the offspring may suck the mother for a long time. Studies showed ten months old, and even last year's calves that still were sucking their mothers (Daleszczyk 2004). Therefore, female of the European bison with the young may not go into estrus and would not allow males to approach her. This is confirmed by the publications showing that in natural conditions of the Białowieża Forest, females of the European bison born calves every other year (Wróblewski 1927; Kraśiński and Raczyński 1969; Kraśińska and Kraśiński 2004). This behavior leads to reduced activity of the female genital organs. This is probably the cause of the smaller size of the female genital organs, including the uterus of the European bison.

Similarly as in cattle, most of the parameters considered in this work are significantly statistically higher in the group of sexually mature females of the European bison compared to the young ones. This is undoubtedly associated with reaching sexual maturity by females. Stronger correlations between the variables with respect to particular parts of the uterus in sexually mature females confirm this hypothesis. In adult females, a stronger relationship is also observed between body weight and all the parameters of the organ discussed. This shows a much greater development of those structures in this group of individuals caused by the increased function and physical maturity.

## CONCLUSIONS

Topographical position of the uterus in European bison resembles that of domestic cattle. The linear dimensions of this organ are mostly smaller than in domestic cows. However the uterus is somewhat larger after each gestation. The differences between species are more distinct in young females. The specificity of the female genital organs morphology in European bison reflects its adaptation to the environment.

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**Abstract.** Despite successful restitution in Poland, European bison still belongs to endangered species. The aim of study was to describe the morphology of the uterus of European bison and to perform morphometric analysis of the organ as well as to compare findings with available data on domestic cattle. The material consisted of female genital organs obtained from 55 females living in the Białowieża Forest. The animals were divided into two age groups. Group I consisted of 36 sexually immature females, while group II comprised 19 sexually mature animals. Significant differences were observed between the two age groups. In young female uteri, except cranial parts of horns, lay in the pelvic cavity. In older animals, most of which have already given birth, uteri were bigger, therefore only a distal half of the cervix was located in the pelvis, while the rest of the organ stretched to the abdominal cavity. Generally, the uterus of the European bison is similar to that organ in domestic cows, however some differences were specified, may be related to a different lifestyle.

