Morphology of external female reproductive organs in European bison (Bison bonasus L.)

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Abstract: This study was aimed at providing a description of the morphology and metric evaluation of reproductive organs of a female bison. Its additional objective was to track changes proceeding in the structure of these organs depending on age and sexual maturity of the animals. An attempt was also undertaken for a comparative analysis of those organs with these of domestic cattle.

The experimental material were reproductive organs of 55 females of Lowland European bison inhabiting the Białowieża Forest. The animals were divided into two age classes, including females aged 2 months to 1.5 year (group I) and the sexually matured ones aged 2 to 20 years (group II). Observations were made and description was provided of the vagina, its vestibule and external reproductive organs. In order to analyze microscopic structures, samples of particular tissues were collected for histological examinations. The preparations were stained with a reference HE method. Results obtained were subjected to metric analysis and compared with respective values reported for domestic cattle.

The structure of the reproductive organs of female bison is usually similar to that of domestic cattle, however their dimensions are smaller. The organs display structural characteristics that may be linked with specific seasonality of the reproductive cycle of the species.

Key words: European bison, reproduction, female reproductive organs

Introduction

Apart from short reports, the morphology of female reproductive organs in European bison has not been extensively studied so far. Results of fragmentary observations in this respect have been published by Korobko and Kurnosow (1979). In a monograph entitled "European Bison" they have devoted a seven-page chapter to these organs. It begins with a description of ovaries, which is the most extensive fragment of the chapter, then the authors present a more superficial description of oviducts and uterus, and finally provide very scant information on remaining reproductive organs. Apart from the description of morphology, these authors were also investigating and discussing selected morphometric traits. The monographs includes also a comparative assessment of European bison with domestic cattle, yet referring only to selected structures of the female reproductive organs. Quite interesting is a fragment of this chapter presenting a short description of reproductive

organs originating from archeological excavations, from the so-called postglacial permafrost. Nevertheless, despite the provided information, the entire work constitutes a very incomplete picture.

The recognition of morphological details of the vagina, its vestibule, as well as of the external reproductive organs may be of great significance to the prophylactic and therapeutic measures. In the case of cattle these organs are subject to routine examination by a veterinarian during fertility assessment.

This work is aimed at undertaking both descriptive and morphometric analysis of those organs as well as their comparative analysis with respective organs of domestic cattle, an all-Polish species being the most close in terms of systematic classification to European bison. The similarity of these species as well as ample works addressing the anatomy and histology of female reproductive organs of domestic cattle enable the above-mentioned comparative analysis. Hence, for better understanding of the subject matter, it would be advisable to provide a short, synthetic revision of the anatomy of female reproductive organs in domestic cattle.

In the case of a cow, the vagina is from 20 to 28 cm in length (the length of the entire copulating organ with the vestibule reaches 30 - 40 cm). In the anterior part, there occurs the already mentioned vagina vault, ascending ca. 3 cm on average over the external orifice of the uterus. The wall of the vagina is constituted by mucosa, muscular coat (muscularis) and adventitia or - in the cranial section - the perimetrium. The mucosa, devoid of glands, is coated with stratified squamous epithelium. Its lamina propria in connective tissue stroma contains lymphatic follicles. In turn, the myometrium is constituted by the inner thicker circular layer and the outer thinner longitudinal layer. The latter, in a short fragment evolves into a wall of uterus. The connective tissue adventitia contains large blood vessels, nerves and ganglia. Cranially it is substituted by serosa which, likewise in the uterus, has its own muscular coat with longitudinal fibres. This membrane is in part separated from muscles of the wall with a vascular layer and partly merges with it. On the border of the vagina and vaginal vestibule, there is no hymen. A fragmentary transverse fold of the mucosa may sometimes be observed. At its height, embedded in the ventral wall of the vagina, is the external urethral opening (ostium urethrae externum). Posterior-inferior to this opening there occurs the suburethral diverticulum (diverticulum suburethrale).

The vestibule of vagina (vestibulum vaginae) is from 10 to 14 cm in length. Its mucosa is coated with stratified squamous epithelium with many leukocytes. Its lamina propria forms papillas, as well as contains a significantly higher number of elastic fibres than the vagina, and always has numerous lymphatic follicles. Its interior contains a dense, cavitary network of venous vessels. The outer layer of the vaginal vestibule wall is constituted by circularly-and longitudinally-running cross-striated muscle fibres forming a constrictor muscle of vestibule (musculus constrictor vestibule), which ventrally evolves into

an urethral muscle (musculus urethralis). The muscle coat adhering to the constrictor muscle from the inside and built of myocytes constitutes an extension of the vaginal mucosa. At half the height of the lateral side of the vestibule there are urethrally-debranched, paired major vestibular glands (glanulae vestibulares majores), whose efferent ducts escape with large orifices. The distal ends of these ducts are coated with simple squamous epithelium, whereas the outlet ducts - with stratified squamous epithelium. Those glands are composed of lobules and are lying in the submucosa or in the constrictor muscle of the vestibule. Their orifices are located in the lateral wall of the vaginal vestibule, caudally behind the external opening of the urethra. The minor vestibular glands (glandulae vestibulares minores) are dispersed, poorly--developed and located right in front of the clitoris. The clitoris, constituted mainly by elastic tissues, is composed of the corpus (corpus clitoridis), glans (glans clitoridis) and the coating prepuce (praeputium clitoridis) being an extension of the vaginal vestibule. The corpora cavernosa of the clitoris (corpora cavernosa clitoridis) begin from attachments of ischiocavernous muscles, uniting into a round, slightly twisted corpus surrounded by tunica albuginea composed of numerous vessels and nerves. In the terminal section, the corpora cavernosa turn into corpora fibrosa. The free end of the clitoris has a shape of a flat cone ca. 1 cm in height, while its top referred to as glans with the width of 0.5 cm on average, protrudes over fossa of the clitoris (fossa clitoridis) formed by the prepuce fused with the clitoris from the dorsal side, and thus forming the so-called "prepuce lamina" (glans lamina). In cattle, at the end of the clitoris there occurs only a connective tissue theca abundant in elastic fibres, whereas the prepuce of the clitoris is a gland-free mucosa of vaginal vestibule containing numerous neural corpuscles and lymphatic follicles.

A vulvar fissure ($rima\ vulvae$) having $7-10\ cm$ in length is formed by pudendal lips ($labia\ valvae$), i.e. $1-2\ cm$ thick folds covered with short hair. The coat of pudendal lips displays a cutaneous structure, and contains numerous sebaceous and sweat glands. The skin of pudendal lips successively evolves into mucosa of the vulva and vagina's vestibule. It is composed mainly of elastic tissues separated by adipose lining. In the vulvar mucosa, the smooth and cross-striated muscle fibres run circularly around the vulvar fissure and form the constrictor muscle of the vulva ($musculus\ constrictor\ vulvae$). The dorsal commissure of lips ($commisura\ labiorum\ dorsalis$) is rounded, whereas in the ventral commissure ($commisura\ labiorum\ ventralis$) the lips converge at an acute angle. Herein, the lips are covered with longer hair forming some kind of a $3-4\ cm$ long brush (Bielański 1977).

The objective of this study was to provide a description of the macroscopic and microscopic structure, supported by morphometric measurementsy, of the vagina, its vestibule and the internal female reproductive organs of European bison in the extra-fetal development, as well as to conduct a comparative analysis of the anatomy of female reproductive organs in European bison and domestic cattle.

Material and methods

The experimental material were female reproductive organs isolated from 55 females of Lowland European bison (*Bison bonasus*) inhabiting the Białowieża Forest. Information on the age of bison females, which ranged from two months to twenty years, was collected from reserve registers or determined in the course of the field study based on their dental status (Węgrzyn, Serwatka 1984).

Specimens for analysis were obtained from culling conducted since November until February in the years 2000-2005 by the staff of the Białowieża National Park. The criterion of selection was varying, culled were mature sick animals, very old or wounded ones as well as young animals that were born too late in the season with too little chances for surviving the winter, and for the proper development and maturation in the spring season. From the experimental material there were excluded reproductive organs of cachectic animals with visible disorders in the development of the urinary-reproductive system or with detected diseases of that system (Olbrych 2002, Katkiewicz et al. 2006). On the basis of literature data indicating that in the reserve the youngest pregnant bison females were at the age of 24-28 months (Jaczewski 1958), and that the oldest bison females delivering calves were at the age of 22 years (Koch 1956), the analyzed females were divided into two age categories. The first (group I) included sexually immature animals at the age from 2 months to 1.5 year inclusive, whereas the second one (group II) covered sexually mature animals aged from 2 to 20 years. Group I included 36 and group II – 19 bison females.

Under field conditions, immediately after culling, individual animals were weighed with accuracy to 2 kg. Subsequently, their whole reproductive organs were extracted by cutting off the broad ligament of the uterus right next to its attachment to the wall of pelvis cavity, together with partly preserved rectum, the whole anus perineum and the vulva. Afterwards, photographs were taken in an appropriate scale. Such parameters as: length and width of vagina and its vestibule as well as depth of suburethral diverticulum, were measured on the spot with an electronic slide caliper with accuracy to 0.01 cm. Macroscopic examinations were conducted as well, which enabled eliminating females with observed pathological lesions or developmental disorders of reproductive organs. Particular structures were examined paying special attention to: shape, size, arrangement, course, consistency and tenacity, as well as the color of their tissues considering the extent of their congestion. Observations were made for the shape of the vagina and its vestibule as well as of the pudendal lips with clitoris. Selectively samples were also collected from the vaginal section of the uterine cervix and from half the length of the vagina. Sampled tissues were fixed by placing in buffered 5% aqueous solution of formaldehyde. Once the structural elements had been transported to the laboratory, they were

embedded in paraffin blocks, which were next cut into $10 \mu m$ sections with the use of a rotary microtome. This enabled obtaining histopathological specimens of the cross-section of the above samples. Afterwards, the samples were stained with a standard HE method – hematoxylin and eosine, and the following results of staining were achieved:

- nuclei blue color
- cellular cytoplasm and intercellular substance red color, in different hues. Anatomical and histological nomenclature was adopted following standard nomenclature (Milart 2002), and the last edition of "Animal Anatomy" handbook (Krysiak, Świeżyński 2004)

Results

In the initial section the vagina is considerably expanded and further on is contracting in the caudal direction. The mean dimensions of the vagina determined for all females examined are as follows: 11.01 cm in length, and 4.06 cm in width; with the values of 8.43 cm and 2.81 cm noted for group I, and values of 15.89 cm and 6.44 cm for group II, respectively. Both groups are found to differ significantly regarding values of these variables. The vagina is a membraneous - muscular organ, with pale pink mucosa and very extensible and relatively thin wall. Observations of few isolated, pregnant organs confirmed the great extensibility of both the vagina and its vestibule. Externally, depending on the place of occurrence, the vagina is covered with serosa or adventitia. The latter contains large blood vessels as well as multiple muscle and connective tissue fibres. The border between the vagina and its vestibule is designated by the external urethral opening, being especially discernible in older females. In addition, this opening leads to the suburethral diverticulum, the depth of which may reach 3.93 cm. The mean depth of this diverticulum noted for both groups accounts for 1.40 cm, whereas for individual groups it reaches: 1.02 cm for group I and 2.12 cm for group II. The difference observed between the groups is statistically significant. At both sides of the discussed structure, there are paired efferent ducts of major vestibular glands, that occur in the form of large agglomerations of packets. In addition, a round fold of mucosa – being a vestigial form of hymen, may be observed herein in the case of young bison females. The vestibule of the vagina, being an extension of the vagina, is shorter and narrower but more vascularized than the vagina. Its mean sizes noted for both age groups reach 7.46 cm in length and 2.92 cm in width. In the case of individual groups, they account for, respectively: 6.06 cm and 2.19 cm in group I as well as 10.10 cm and 4.29 cm in group II. The differences determined between groups compared for the values of these sizes of the vaginal vestibule are significant. The mucous membrane of the vestibule is dark brown, and beneath it there is the stroma of muscular coat. Medially from the openings of major vestibular

glands there run clitoris-faced folds that end at the fossa of the clitoris. Therein, there occur small orifices of the minor vestibular glands.

The mucosa of bison vaginal vestibule is coated with simple squamous epithelium. It is composed of a few strata, yet the basal layer is represented mainly by cells oval in shape, with a bright cytoplasm and dark nucleus. Closer to the surface, they are becoming flattened and their cytoplasm is becoming darker. The mucosa forms low folds with flat apexes. Its lamina propria is constituted chiefly by elastic connective tissue, whose thick bands are running longitudinally. In turn, deeper layers include a circular coat of smooth muscles, which without any tangible borderline turns into a longitudinal layer. Both the lamina propria of the mucosa and the muscular coat are heavily vascularized. They are additionally lined with numerous bands of elastic tissue.

The female external reproductive organs (partes genitales feminina externae) include, among other things, the vulva which is the terminal section of the reproductive organs. It is formed by two-sided vulvar lips that are joint together in a dorsal commissure of the lips at the upper side and with the ventral commissure at the lower side. In some females, pachynses could be observed on the skin and mucosa of those lips that were formed by scar tissue resulting probably from damage during parturition or copulation. Between the lips there is a vulvular fissure with the length of 2.00 to 8.76 cm, attaining the shape of a teardrop. Its lower angle restricted by the ventral commissure is sharpened, whereas the upper angle restricted by the dorsal commissure of the lips is rounded. Beneath that commissure, skin is covered with slightly longer hair falling into a shape of a brush. Between the dorsal commissure of the lips and the anus there extends a perineum having the length from 1.51 to 7.90 cm. In the ventral commissure of the lips, there occurs a prepuce fossa restricted by a small fold, with a well visible clitoris. The latter is an organ homologous to a penis in males, hence its structure resembles that of the penis. The clitoris is composed of clitoral legs attached to the pubic arch and corpus ended with a glans whose maximal sizes may account for 0.83 cm in width and 1.91 in length, and the minimal ones - for 0.31 cm in width and 0.42 cm in length.

Discussion of results

The macro-and microscopic observations of the reproductive organs of female bison enabled to describe their anatomy and location. They are additionally fundamental to determine changes proceeding with age and, most of all, in relation to reaching sexual maturity. The metric analysis of the quantitative traits of particular organs of mature animals as well as their comparative analysis with domestic cattle forms a basis for the identification of morphological (anatomical) standards for sexually immature female bison and for

females in the reproductive period, which is of outmost significance from the viewpoint of protection and welfare of this species reproduction and further breeding in natural reserves.

The linear parameters of the vagina and its vestibule are substantially lower in the female bison examined than in domestic cattle. Earlier observations (Korobka, Kurnosowa 1979) prove that the length of those structures in bisons is twice smaller than in cows. In young female bison, the hymen – occurring in the form of a fold – is very clearly visible, in contrast to heifers where it occurs only in the vestigial form. In mature animals of both species, extremely well visible are major vestibular glands with their openings. In cattle the length of these orifices may range from 0.61 in calves to 0.96 cm in mature cows (Reutner, Morgan 1948). Bison females have also well visible openings of the minor vestibular glands, though not as discernible as in the case of the major glands.

In contrast to cows, the vulvar fissure and sizes of clitoris are substantially lesser in bison. The length of the perinenum in cattle fluctuates between 4.5 and 6 cm (Bielański 1977), which is similar to the values reported for bison females.

The comparative analysis conducted between females of bison and domestic cattle enables speculating that the differences presented may be due to different daily activities of these animals. When compared to females of bison, the number of pregnancies and parturitions during a life span of cows is considerably higher. It results from intensive breeding practices and exerts a significant effect on the structure and size of reproductive organs of this species. Free-living bison are affected by variable environmental conditions. Not every season facilitates rearing of calves. Females must adjust to changes ongoing in their habitat. Pregnancy may occur in months that are the most favorable to calves rearing. In addition, the calves may be sucking their mothers for a long period of time. Hence, often a female with a calf does not allow a male for copulation, and has no heat period. Such behaviors contribute to suppressed and less frequent activity of the reproductive organs of the female. And this is the likely cause of smaller sizes of the examined reproductive organs of bison females.

Conclusions

Particular descriptive parts of the reproductive organs of bison as well as the histological picture of the structures examined, correspond to those reported for cattle. However, their linear dimensions are lower, which proves specific seasonality enforced by conditions of the natural habitat of those animals.

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Morfologia zewnętrznych żeńskich narządów płciowych żubra (Bison bonasus L.)

Streszczenie: Badania miały na celu dokonanie opisu budowy morfologicznej oraz ocenę metryczną narządów płciowych samicy żubra. Celem było również prześledzenie zmian w strukturze tych organów w zależności od wieku i dojrzałości płciowej. Podjęto także próbę przeprowadzenia analizy porównawczej tych narządów z organami bydła domowego.

Materiał do badań stanowiły narządy płciowe żeńskie 55 samic żubra nizinnego, zamieszkującego Puszczę Białowieską. Zwierzęta podzielono na dwie grupy wiekowe. Pierwsza to osobniki niedojrzałe płciowo w wieku od 2 miesięcy do 1,5 lat, druga obejmowała samice dojrzałe płciowo liczące od 2 do 20 lat. Dokonano obserwacji i opisu pochwy, jej przedsionka oraz zewnętrznych narządów płciowych. W celu oceny struktur mikroskopowych z materiału pobrano wycinki do badań histologicznych. Preparaty barwiono metodą przeglądową HE. Uzyskane wyniki poddano analizie metrycznej oraz porównano z bydłem domowym.

Narządy płciowe żubrzyc mają na ogół podobną budowę jak u bydła domowego. Jednak ich wartości metryczne są mniejsze. Organy te wykazują cechy budowy, którą można wiązać ze swoistą sezonowością w rozrodzie.