

# Evaluation of vaginal swabs in European bison (*Bison bonasus* L.) – preliminary results.

Katarzyna Olbrych<sup>1</sup>, Kaja Urbańska<sup>1</sup>, Michał Krzysiak<sup>2</sup>, Tomasz Szara<sup>1</sup>,  
Bartłomiej J. Bartyzel<sup>1</sup>

<sup>1</sup> Department of Morphological Sciences, Warsaw University of Life Sciences,

<sup>2</sup> Białowieża National Park, Białowieża

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**Abstract:** Recent breeding and conservation of European bison are hindered by numerous health problems. These animals have a low resistance and poor tolerance to adverse environmental conditions. In E. bison frequently are diagnosed abnormalities and dysfunctions of reproductive organs. This is undoubtedly associated with a high rate of inbreeding in this species. In males more often so called preputial disease, referred by some authors as *posthithis* or *balanoposthithis* was diagnosed. The etiology of the latter is unknown until now. Females are probably also affected because similar necrotic alterations enclosing the vulvar area were observed in cows. These and other anomalies in the obvious way, impair the ability of individuals to reproduce. Therefore regular monitoring of the health condition of the bison is necessary. One of the methods of health assessments in females is to perform Pap tests, based on vaginal swabs, and their microscopic evaluation. From a technical point of view, these tests are easy to perform, even in free living females, and unlike any other methods of assessing the morphology of genital organs and reproductive capacity of females they do not require the acquisition of an animal after killing. Preparation and attempt to interpret cytological swabs in female bison seem to have a great educational and practical value.

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## Introduction

Present farming and the protection of European bison faces a number of health problems relating to this species (Anusz 2007; Anusz *et al.* 2010). It has long been known that these animals have a low immunity and poor tolerance to adverse environmental conditions (Gill 1999; Olech 2006; Kita, Anusz 2006). This is mainly due to high of homozygosity of herds (Olech 2009), which is the causing the dysfunction of genital organs which obviously affects the ability to reproduce. European bison in the Red Book of Species has been classified as an endangered species. Therefore, a proper assessment of condition of genital organs is so important especially in females. It is extremely difficult in a situation where the animals live in the wild in an inaccessible terrain. Therefore initiated was the search for a test method that is fast and easy to perform in difficult conditions and allows for intravital examination of animals. Until now observations of genital organs were carried out only *post mortem* (Świeżyński 1968). For these reasons, attempted was a collection vaginal swab samples. Cytological test results can be used not only for

assessing reproductive capacity of females, but also the overall health of the herd. In males more often so called preputial disease (Gill 1999), referred by some authors as *posthithis* or *balanoposthithis* was diagnosed (Kita, Anusz 2006). The etiology of the latter is unknown until now. It is commonly believed that necrotic changes resulting from this disease, occur exclusively in males, but recently an increasing number of females are diagnosed with similar changes in the vulva. Probably the disease affects both sexes. This should be an incentive for further study of the issue not only in males, but also in females. A disease leading to infertility in a rare species may lead to its extinction.

## Material and methods

All studied animals came from a free-living herd in the Bialowieska Forest. The cows were divided into three experimental groups. Swab samples were collected from seven females captured in May 2012, held in quarantine, which preceded the export of these individuals into another breeding center. It was the first experimental group. Age of quarantined animals ranged from 3 to 6 years. Next two groups accounted females killed during a planned annual elimination in the period from December 2011 to March 2012. The second group includes females, aged 6 months, 4 and 20 years old, free of lesions at genitals. The third group were also three individuals at the age of 7, 10 and 20 years who have had changes within these organs.

For females living in enclosure, the material was acquired using the combined anesthesia with Immobilon and 10% Xylazine, by the pneumatic injection with rifle Daninject. The animals were euthanized because of routine official examination and to determine the current health condition as well as to perform standard diagnostic and preventive procedures required before shipment. Blood samples were collected from all individuals. Tuberculin test and worm treatment were also performed. The vaginal swabs were collected at same time. After the above actions animals were awoken by using Revivon combined with Antisedan. These animals were healthy and fully reproductively valuable.

Vaginal swabs from shot-killed cows were obtained while keeping identical methodology as for the intravital collection. Genital organs were then dissected and post mortem examination was performed, to determine any abnormalities. These individuals were eliminated in order to keep the structure of the herd and the removal with the animals deviate from the standards of its habit of the species or in poor health. There were females with various pathological changes in the liver, lung, locomotor apparatus, the digestive system, as well as on the genital organs. These changes were clearly revealed in the form of clinical symptoms. In all animals of the second group and a third, even in six months heifer foci in the liver, liver fluke, emphysema with pneumonia and pulmonary nematodes were found. In addition, the oldest 20-year-old female,

from the second group suffered from gastrocnemius bursitis, which caused considerable lameness of the right pelvic limb. In the same group, four-years old female has been eliminated because of a broken left horn and a local abscess in left mandibular region. The third group included females having changes relating to genital organs. In 20-year-old female were found, in addition to severe cachexia, endometritis and in other, 10 years old one, the cysts on both ovaries. Where, in a 7-year-old cow were found four-month female fetus in the left horn of the uterus and vaginitis with erosions of the labial area.

The material was collected with a sterile swab from the mucosa of vaginal vault. Then the obtained samples were transferred by roller movement to microscope slide. The fixation buffer Cytifix was then applied before transportation. Immediately after delivery to the laboratory preparations were stained with Giemsa. The assessment of smears and photographic documentation was drawn using an optical microscope Nikon Eclipse with magnification 40× and 100×.

## **Results**

Swabs from the females of the first group which were obtained in May were identified as atrophic. In the cytological image a clear dominance of the contiguous cells of intermediate and parabasal layers was noted. These cells formed teams of 3–4, sometimes overlapping each other. Isolated oval cells with regular contours were also present in the preparation. Cell membrane borders were usually indistinct. The cytoplasm of most cells was basophilic. Cells showing acidic affinity were sparse. In the central part of the cell was observed large, vesicular nucleus, clearly demarcated from the cytoplasm by the nuclear membrane, having fine-grained, evenly distributed chromatin. The ratio of the nucleus to the cytoplasm was shifted in favor of the nucleus. The result of reduced estrogen activity in females at the time of swab samples acquisition, was cytolysis of most of scattered cells, evidenced their presence only by naked isolated nuclei. The background of the slide was impure. Single glanyocytes have been seen in microscopic image as well as a small amount of mucus that has accumulated in the bands or lumps. The cells of superficial layers, nor erythrocytes were not present. Swabs from eliminated females of the second group at age 6 months and 4 years, were taken in February while from female aged 20 years, in March. The cytological examination revealed the presence of parabasal and intermediate cells, mostly basophilic, arranged in groups or dispersed. Has been observed also quite numerous naked nuclei. In some cells, relative size of the nucleus to the cytoplasm ratio has been moved in favor of the nucleus. There were also individual cases of vacuolization of cytoplasm. Neutrophils were sparse. The remaining third group was represented by females with lesions of genital organs. The first, 20-years-old

cow suffered from endometritis and the swab was obtained in February. Its basal and intermediate epithelial cells characterized by basophilic cytoplasm, although acidophilic cells has been also found. A microscopic examination appeared a large number of neutrophils.

The second case was lactating 10-years-old female, in which cysts at tubal extemities of both ovaries has been found during the autopsy. Cytological swab was also acquired in February. Naked, isolated nuclei were dominated at microscopic slide.

The last case was a seven-year-old pregnant female with macroscopically visible inflammatory lesions of the vaginal mucosa. In the swab, derived from end of February aggregated or single epithelial cells has been observed. The cytoplasm of most cells was basophylic. A microscopic examination appeared a large number of neutrophils and erythrocytes.

## Discussion

The smear test is a routine method supporting the qualification of effective mating term, but recently it has become very helpful in diagnosing pathological alterations of female genital organs. These lesions are caused by various factors, such as neoplastic diseases, endocrine disorders or bacterial infections (Goodman 2002). The morphology of the cells lining the vagina and vaginal portion of the cervix is subject to cyclical changes that are dependent on estrogen, and age of the animal (Chretien *et al.* 1998). However, the variable number and type of cells in cytological examination, can also result from vaginal biocenosis disorders or pathological changes at female genital organs (Malarewicz, Szymkiewicz 2004). Therefore, the vaginal swabs can be very useful in diagnosing pathology in European bison. In addition, these findings become a valuable source of information on estrus cycle while monitoring reproductive capacity females of this species. It allows to observe the different phases of the estrus cycle, which gives more complete information on hormonal changes during the year, especially since intravital acquisition of samples. In this regard, the smear test, become attractive diagnostic technique for female genital organs in European bison.

Smears derived from females of the first group in May was defined as atrophic, with a distinct dominance of parabasal cells. As in the material collected from the animals of the second group, in February and March, there was no superficial layers of cells. Frequently occurred parabasal and intermediate cells, as well as a few neutrophils. Preliminary diagnosis revealed that during the winter and spring bison cows can be found in anestrus phase of the cycle. Furthermore no features of genital organs disease have been found. The remaining, third group was represented by females diagnosed *post mortem* with endometritis, vaginitis and cysts on the ovaries. The swabs were taken in February. On the microscopic slide cytoplasm of intermediate and parabasal

cells showed an affinity for basic dyes, although acidophilic staining cells were also found. A microscopic examination revealed a large number of neutrophils and erythrocytes, and prevailed naked, isolated nuclei, which proves the existence of inflammation or other abnormalities of the organs examined. Epithelial cells forming aggregations or singly existing were also observed. This clearly shows that these animals during sample collection were in the silent hormonal phase and sexual inactivity. A common feature of swab samples obtained from animals in phases proestrus, diestrus and anestrus is the presence of intermediate layers cells (Dubiel *et al.* 2004), generally in similar proportions, whereas in the estrus phase, the number of these cells is small (Reddy *et al.* 2011). On cytodiagnostic examination in diestrus and anestrus phase the dominance of parabasal cells is also marked by, which in mears of proestrus and estrus phase states occasionally (Reddy *et al.* 2011).

A significant increase in the number of cells of superficial layers recorded in the proestrus phase ( $65.30 \pm 1.40$ ) and the highest value of the maturation index, defined as the percentage of specific cell types, in order from the superficial parabasal, is observed in the estrus ( $89.94 \pm 0.63$ ). Number of keratinized superficial cells decreases ( $18.52 \pm 0.94$ ) with the onset of diestrus phase, reaching the lowest value in anestrus ( $12 \pm 0.70$ ) (Reddy *et al.* 2011). It is believed that the presence of neutrophil in cytological examination is specific for the quiet period of sexual activity (Kim *et al.* 2007). Theses of (Groppetti *et al.* 2012) have shown however, that the designation of diestrus exclusively on the basis of the presence of neutrophils in smears is not appropriate, since raised estrogen levels leads to congestion of the endometrium increasing the permeability of blood vessels. As a consequence neutrophils passes through the walls of these vessels into vagina also in proestrus phase. An important criterion for differentiating cytodiagnostic samples from female animals at various stages of the cycle is the presence of erythrocytes, found in the proestrus phase (Nizański 2008). It should be noted that some researchers question the relevance of erythrocytes in the recognition of phases of the sexual cycle (Nizański 2003).

In the case of impaired biocenosis of female genital organs, the smear test shows inflamatory and degenerative changes of cells which are often grouped to form aggregations varying sizes. The result of the progressive process of cytolysis is presence in the the whole preparation naked, separate cell nuclei (Malarewicz, Szymkiewicz 2004). Increase in the number of naked cellular nuclei may be associated with abnormalities of genital organs. Prolapsed nuclei clearly characterized a swab collected in February, from the cow with diagnosed cysts on the ovaries. In human females it may suggest the occurrence of ectopic pregnancy, such as the tubal. The probability diagnosing pregnancy of with incorrectly implanted embryo increases when the Arias-Stella cells are also visible on the microscopic slide (Kobayashi *et al.* 1983). Also, in women with vaginitis caused the accumulation of a large number of lactobacilli

cytological smears are characterized by a significantly high percentage of prolapsed cellular nuclei (Demirezen 2003). In veterinary medicine, vaginal cytological examination performed primarily in companion animals, mainly in bitches (Groppetti *et al.* 2012) and, incomparably less common in cats (Jannifer *et al.* 1979; Mattos *et al.* 2003). In farm animals such as cows and mares, these tests are made occasionally (Ahmadi *et al.* 2006; Santos *et al.* 2008). While, in wild animals in the available literature only single cases of vaginal cytodiagnostic have been described, including dromedary (Ahmadi *et al.* 2006), coyote (Carlson *et al.* 2008) and the maned sloth (Snoeck *et al.* 2011). However, in European bison no smear tests have been performed so far. Over the years, among numerous observations of bison reproduction (Karcov 1903; Wróblewski 1927; Jaczewski 1958; Krasieński 1967; Krasieński 1978; Krasieński and Raczyński 1967), the lack of precise information on the phases of the cycle and the diseases causing disorders in female breeding. On the basis of calving has been established the estrus time and its repeatability. The length of pregnancy was also calculated. However, to date accurate data on this topic are not complete, and the characteristics of the different phases of the estrous cycle has not been carried out. The only information currently available is research on the levels of sex hormones in European bison and their seasonal changes (Gill 1997). This analysis involved both females and males in different age groups, but did not cover all the months of the year. This arose only introduction to diagnosis of female genital organs in European bison. Therefore, it is reasonable to continue the work in the form professional analysis of both morphological and pathological features, and above all cytodiagnostic and hormonal tests that can be performed intravital. Such comprehensive analysis allows to obtain a full diagnostic profile of female genital organs. These are all very important information not only for researchers, but also for people engaged in the breeding and protection of the European bison.

## Conclusions

Cytological observations of vaginal swabs of examined cows indicate that during the winter-spring period those animals are in anestrus phase of the menstrual cycle, on so called hormonal quiet. It was also noted that morphology of cells in animals with lesions of female genitals observed is changing reflecting the above mentioned pathologies. These findings encourage further research, because the simple method can not only diagnose the phase of the cycle but also diseases of the female reproductive organs. Therefore, the work should be continued and expanded to include additional research methods, eg. histological or hormonal examination. In addition, observations should be carried out in different months of the year in order to establish a complete hormonal profile, which is a valuable information concerning the reproduction of these rare animals.

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### Ocena wymazów z pochwy samic żubra (*Bison bonasus L.*) – wyniki wstępne

**Streszczenie:** Współczesna hodowla i ochrona żubra jest utrudniona przez liczne problemy zdrowotne. Zwierzęta te mają niską odporność i słabą tolerancję na zmieniające się warunki środowiska. U żubra regularnie stwierdza się nieprawidłowości i dysfunkcje w układzie rozrodczym. Jest to bez wątpienia związane z wysokim stopniem inbrodu tego gatunku. U samców coraz częściej obserwuje się tak zwaną chorobę napletkową, opisywaną przez niektórych autorów jako *posthithis* lub *balanoposthithis*. Etiologia tej choroby jest do tej pory nieznaną. Samice prawdopodobnie też są dotknięte tą chorobą, ponieważ zmiany martwicze okolic pochwy były u nich stwierdzane. Te i inne nieprawidłowości obniżają zdolności reprodukcyjne osobników. Z tego względu regularny monitoring zdrowia żubrów jest konieczny. Jedną z metod oceny jest zastosowanie u samic testu Pap w stosunku do wymazu z pochwy jak i jego ocenę mikroskopową. Z technicznego punktu widzenia, te testy są łatwe do zastosowania, nawet w stosunku do wolno żyjących samic, w przeciwieństwie do innych metod oceny morfologii narządów rozrodczych i zdolności reprodukcyjnych wymagających unieruchomienia samicy. Przygotowanie, ocena i interpretacja cytologii zmian u samic żubra może mieć duże znaczenie praktyczne i edukacyjne.

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