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Comparative morphology of corpus luteum in European bison and European moose

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Abstract: The aim of this study was histological characteristic of *corpus luteum* in lowland European bison (*Bison bonasus bonasus*) and its comparison with histological structure of *corpus luteum* in European moose (*Alces alces*).

The study group consisted of 7 females of European bison 5–20 years old living in Białowieża Forest. Histology of European bison *corpora lutea* were compared with group of 7 females of European moose 4–12 years old living in north-eastern Poland. All animals included in this study were in early pregnancy. The weight and size of each ovary was measured. The surface of each gonad was examined for presence of *corpora lutea*. Then, the ovaries were fixed in 10% neutral buffered formalin, processed by common paraffin technique and stained with haematoxylin and eosin. Microscopic analysis has shown significant interspecies differences in *corpus luteum* histology. The *corpus luteum* in E. bison has more organized structure and more abundant connective tissue compared to that of moose . Large lutein cells and small lutein cells of E. bison *corpus luteum* have lower metabolic activity than in moose. However, detailed analysis of histological structure of E. bison *corpus nucleus* at all stages of pregnancy are necessary to draw more definitive conclusions and further studies are needed.

Key words: European bison, European moose, *corpus luteum*, histology

Introduction

In Poland, European bison live mainly in free ranging herds. Despite that their population in our country remains large, European bison faces a number of health problems characteristic for this species (Anusz *et al.* 2007). Abnormalities of the reproductive tract are relatively frequently diagnosed in sectioned individuals (Olbrych 2002; Katkiewicz *et al*. 2006). For this reasons, E. bison reproduction should be permanently monitored and investigations of particular parts of E. bison reproductive tract should be continued and extended. Especially, analysis of ovary's structure may provide substantial information regarding reproductive parameters, such as number and time of ovulations, fetus presence or number of previous pregnancies (Langvatn 1992). The most informative in this respect is luteal tissue constituting the specific endocrine organ i.e. *corpus luteum*. It develops from ruptured mature follicle following the release of a secondary oocyte during ovulation. Ruptured follicle collapses, folds and temporarily hemorrhages. The granulosa cells expand in their size and number and they become transformed into large lutein cells. As a consequence the luteal tissue develops in ruptured follicle, growing from the periphery to the center of arising *corpus luteum*. It is accompanied by extending of the vasculature from the theca interna into the luteal tissue, where the small blood vessels form a dense network. Simultaneously, yellow pigment lutein accumulates within the cytoplasm of lutein cells. The lutein granules differ in their size. Finally, the ruptured mature follicle transform into large, fully developed *corpus luteum*. This process is called luteinisation. Formation of *corpus luteum* is dependent on luteinizing hormone (LH, gonadotropin II, prolan B) released by pars distalis of pituitary gland. In ruminants, including E. bison and moose, after fertilization, the *corpus luteum* persists through the whole pregnancy. It produces progesterone responsible for embryo implantation in the endometrium as well as maintenance and normal course of pregnancy. Moreover, lutein cells release oxytocin, vasopressin, neurophysin and relaxin. All of those hormones are involved in regulation of the course of pregnancy and parturition.

Histological structure of ovaries varies between different species of wild ruminants (Langvatn 1992). Thus, the aim of this study was comparative analysis of corpora lutea histology in European bison and European moose. Such comparison was possible because of similarities in physiology of reproduction in those two species as well as availability of some data regarding moose ovary anatomy. E. bison is considered as a polyestral seasonal animal. It means that in certain seasons estrous cycles are repeated several times. The peak of its breading season falls in August--September. Females reach full physical maturity at about 4–5 years, but they can be successfully matted being 24–28 months old. The pregnancy lasts 260–270 days and births take place in May-June. If the cow is not fertilized she will enter the estrus again after 20–30 days (Gill 1999).

Moose are also polyestral seasonal animals. They reach sexual maturity at 16–28 months (Rausch *et al.* 2008). Estrus cycle lasts 24–25 days and the heat lasts 15–26 hours (Franzmann 1981). Pregnancy in moose lasts 231–240 days. The peak of reproductive activity falls in August-October.

Material and methods

The study group consisted of 7 sexually mature females of lowland European bison 5–20 years old. All samples were obtained through annual culling performed during November-February in Białowieża Forest. Because all animals included in this study were in early pregnancy (the first half of pregnancy), only *corpora lutea graviditatis* were present in examined ovaries.

Histology of European bison *corpora lutea* was compared with group of 7 females of European moose 4–12 years old. All individuals sampled for this study were obtained through culling performed in north-eastern Poland under the framework of the project "The strategy for moose protection and management in Poland". The material was collected in November, and similarly to E. bison, all moose females were in early pregnancy.

Immediately after culling the ovaries were isolated and their total length, width and height were measured using an electronic slide caliper exact to 0.01 cm. Moreover all gonads were weighted with an accuracy of 0.01 gram with the electronic scale. The surface of each gonad was examined macroscopically for presence and quantity of ovarian follicles and *corpora lutea*. Then, the ovaries were cut longitudinally along their long axis, placed in the containers with 10% neutral buffered formalin and transported to the laboratory. After fixation, specimens were processed by common paraffin technique, cut into 3 μm sections and stained with haematoxylin and eosin.

Moreover, in all cases the uterine horns were also measured using an electronic slide caliper exact to 0.01 cm. Then, they were sectioned along their greater curvature and their content was analyzed. Estimation of pregnancy advance was performed based on size of uterine horns, level of fetus and fetal membranes development.

The *corpus luteum* histology is influenced on stage of ovulation, presence and advancement of pregnancy. At the time of samples collection all individuals belonging to both species were in the first 3–4 months of pregnancy. This enables for comparative analysis of *corpus luteus* histology in E. bison and moose.

Results and Discussion

Analysis of histological structure of corpora lutea was performed based on following criteria:

1. Appearance and size of large lutein cells and small lutein cells: cellular shape, nuclear placement, presence of nucleoli and their localization throughout *corpus luteum*
2. The localization of connective tissue – presence of capsule, arrangement of connective tissue within *corpus luteus*
3. Central part of the *corpus luteum* – presence of sheets of connective tissue
4. Vascularisation of *corpus luteum* – type and localization of blood vessels within *corpus luteum*

The large lutein cells constitute a major population of *corpus luteum* parenchyma in both compared species. They are originated from granulosa cells of ovary follicle. In lowland European bison as well as in European moose they are large, oval to polygonal cells with foamy cytoplasm. This characteristic appearance of cytoplasm results from production of progesterone belonging to steroid hormones (Krzymowski and Przała 2005). The nuclei of large lutein cells in both species are large and oval in shape. In E. bison the nuclei of large lutein cells are centrally placed, however in moose they are located eccentrically. In E. bison, the large lutein cell have single clearly visible nucleoli localized next to nucleus membrane. Moose large lutein cells have several nucleoli, mainly 2 or 3, also localized marginally. Increased number of nucleoli in moose large lutein cells compared to E. bison may indicate higher metabolic activity of those cells in moose *corpus luteum* (Kawiak and Zabel 2002).

Small lutein cells are originated from the theca interna of mature ovarian follicle and they are located in both species mainly in the periphery of *corpus luteum* just behind its capsule. Such localization results from their origin. Precursors of small lutein cells constitute the most outer cellular layer of mature ovarian follicle i.e. theca interna. However, those cells are also placed along bands of the connective tissue which penetrate from capsule into the centre of *corpus luteum*. Because of this, in both species single small lutein cells are widespread between large lutein cells. Similarly to large lutein cells, small lutein cells also produce progesterone, however in smaller quantity. Their cytoplasm have also foam appearance resulting from steroid metabolism. In both species, the small lutein cells are smaller than large lutein cells and of irregular shape. The nuclei are oval or cup-shaped. In both species, small luteal cells have several marginally located nucleoli which indicates their high metabolic activity.

Fibroblasts are widespread throughout of the *corpus luteum*. They are located between collagenous fibers of *corpus luteum* capsule and in bands of connective tissue penetrating its parenchyma. They were also observed in the sheets of connective tissue in the central part of *corpus luteum* and between large lutein cells. Fibroblasts, are relatively small cells with elongated shape and oval nuclei. They produce the constituents of connective tissue matrix i.e. collagen and proteoglycans (Sawicki 2009). The *corpus luteum* is surrounded by fibrous capsule. It originates from collagenous fibres and fibroblasts of theca externa of mature follicle. The capsule of E. bison *corpus luteum* is well developed and clearly separated from its parenchyma. However, in moose, the capsule is thinner and less pronounced. Thus, the border between fibrous capsule and parenchyma of *corpus luteum* is indistinct.

In E. bison, the bundles of connective tissue penetrating *corpus luteum* parenchyma are broad and clearly visible. They run thorough the *corpus luteum* into its center where they form one or more sheets of connective tissue. However in moose, similarly to the appearance of capsule of *corpus luteum*, the bundles of connective tissue are also poorly visible, much thinner and less numerous.

In E. bison the bundles of connective tissue creates large, clearly visible sheets in the centre of *corpus luteum*. They develop during ovulation. At this time the centre of ruptured follicle is filled with blood. Simultaneously, the migration of fibroblasts from ovary stroma to the cavity of ruptured follicle occurs. The clot resorption and organization take place gradually. Finally, the sheet of connective tissue is formed in the centre of former cavity. Such sheets of connective tissue do not influence on functional activity of *corpus luteum*. They are only remnant of ruptured ovarian follicle. Apart from collagenous fibers and fibroblasts, the blood vessels (mainly veins)

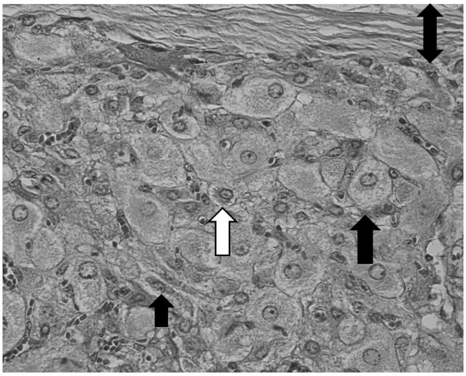

Figure. 1. Moose large luteal cell (black arrow) and small lutein cell (white arrow) morphology and their localization against fibrous capsule (double arrow). Fibroblast is marked with small arrow, for the comparison.

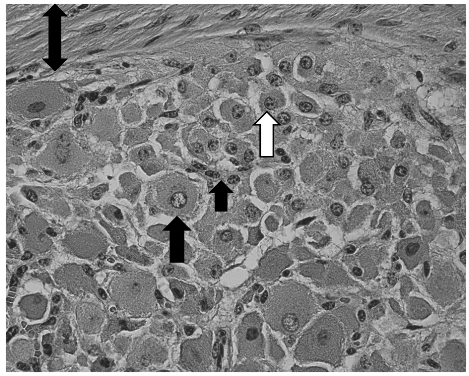

Figure. 2. E. bison large luteal cell (black arrow) and small lutein cell (white arrow) morphology and their localization against fibrous capsule (double arrow). Fibroblast is marked with small arrow, for the comparison.

as well as the lymphatic vessels are also presented in those structures. Interestingly, in E. bison *corpus luteum* the large lutein cells are arranged concentrically around those sheets of connective tissue. In moose, the sheets of connective tissue in the centre of *corpus luteus* are poorly visible and frequently absent. As a result, the moose lutein cells are localized randomly.

In both species, blood vessel network of *corpus luteum* is well developed. Larger vessels, mainly veins, are localized on the periphery of *corpus luteum*, just behind its capsule. They are also located along bundles of connective tissue, parallel to the fibers orientation. Small blood vessels are numerous and they are lying between large lutein cells. The abundant vascularisation of *corpus luteum* observed in both species is a characteristic feature of this organ, especially in the period of its intensive secretory activity. The study conducted on cattle have shown, that the ovarian blood flow is positively correlated with systemic concentrations of progesterone. It increases with increasing the progesterone level (Ford *et al.* 1981). The main function of *corpus luteum* graviditatis is progesterone production. This hormone is released by both large and small lutein cells. The ovarian blood flow have to increase enough to meet metabolic requirements of the luteal cells which is necessary to effective production of progesterone at the level sufficient to maintainthe pregnancy (Wise *et al.* 1982).

The *corpus luteum* parenchyma consist of large lutein cells and small lutein cells. Their localization throughout *corpus luteum* results from their origin. The large lutein cells are transformed granulosa cells and they are widespread in whole organ. However, the small lutein cells are located mainly behind the capsule of *corpus luteus*,

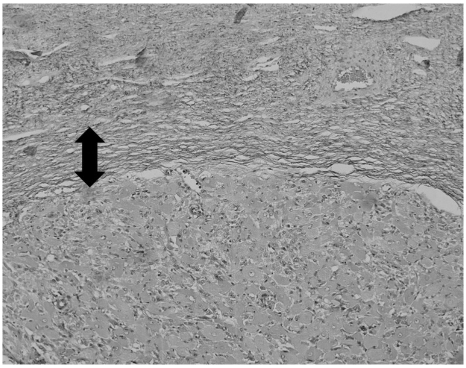

Figure. 3. Thickness of fibrous capsule (double arrow) surrounding *corpus luteum* in moose.

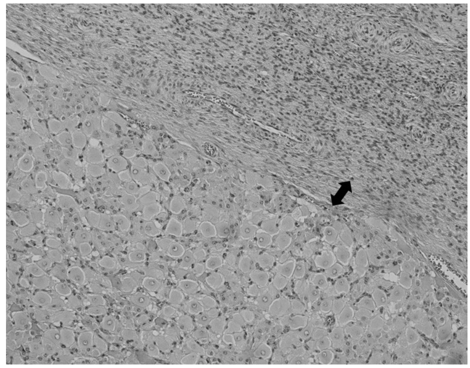

Figure. 4. Thickness of fibrous capsule (double arrow) surrounding *corpus luteum* in E. bison.

as they origin from theca interna cells which surrounds the stratum granulosum of ovarian follicle. Both type of cells have foamy cytoplasm which indicates the steroid metabolism. In European moose, the large lutein cells would be more active as they have several nucleoli. In contrast, E. bison large luteal cells usually have only single nucleoli. In the light of current knowledge it is difficult to explain why luteal cells are more active in moose compared to E. bison. It may result from interspecies difference in levels of hormones and proteins released during pregnancy. Taking into account the appearance of luteal cells, E. bison would have physiologically lower level of progesterone than moose. However, in the available literature there is no data confirming this hypothesis. The E. bison included in this study were in slightly more advanced pregnancy than moose. This can also explain difference in the activity of luteal cells in both compared species, as E. bison produce especially high level of progesterone at the very early stage of pregnancy (Kirkpatrick *et al.* 1991). The studies on white-tailed deer (*Odocoileus virginianus*) have shown that the progesterone level in peripheral blood increases as the volume of *corpus luteum* graviditatis is increasing (Harder and Moorhead 1980). However, in the available literature there is lack of similar data regarding bison and moose species. Some studies focusing on the levels of reproductive hormones in E. bison in various months of the year are available (Gill 1997). Unfortunately those data are incomplete. They do not report the levels of reproductive hormones in full annual reproductive cycle which exclude to draw conclusions.

The connective tissue in *corpus luteum* is more abundant in E. bison than in moose. It has more pronounced fibrous capsule and bundles of connective tissue penetrating *corpus luteum* parenchyma. In E. bison, large lutein cells forms concentric layers around sheets of connective tissue placed in the centre of this organ. It appears that the structure of E. bison *corpus luteum* is well organized. In moose *corpus*

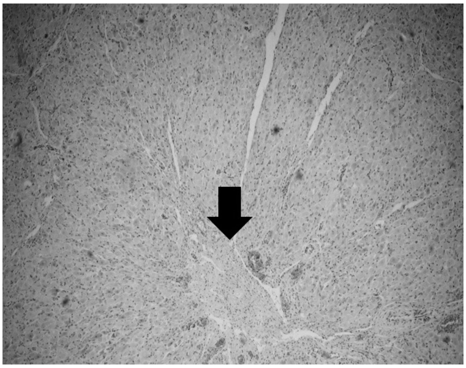

Figure. 6. Central part of E. bison *corpus luteum* with distinct sheet of connective tissue and luteal cells arranged concentrically.

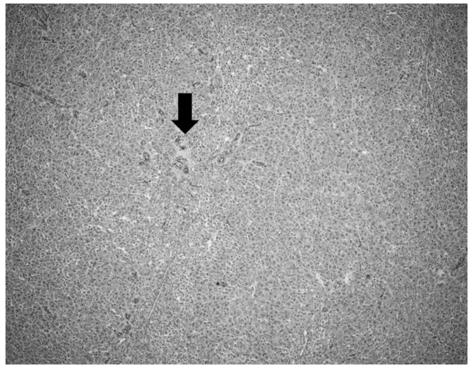

Figure. 5. Central part of moose *corpus luteum* with poorly visible connective tissue.

luteum, the connective tissue is much less obvious than in E. bison and large lutein cells are localized randomly. The explanation of this phenomenon is difficult. The connective tissue composes the stroma of *corpus luteum*, and it does not influence activity of this organ, thus the differences in its pattern and quantity can by considered as interspecies difference with no impact on function of *corpus luteum*. However, some studies conducted on cattle have shown an increase in the connective tissue, fibroblasts and extracellular matrix in *corpus luteum* towards the end of the pregnancy, being more pronounced after 190 days of pregnancy (Xavier *et al.* 2012).

Conclusions

Further studies are necessary to fully describe the histology of the *corpus luteum* in lowland European bison. They should focused on histological analysis of *corpus luteum* in various stages of pregnancy in relation to blood progesterone level. Especially, the morphological appearance of large luteal cells and small luteal cells should by described. Unfortunately, in our study such comparison was not feasible because of lack of relevant data available in the literature. However, such studies should be conducted in future, as they expand our knowledge of wild animal reproduction and allow more successful protection of threatened species.

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Morfologia porównawcza ciałka żółtego żubra i łosia.

Streszczenie: Celem niniejszej pracy było dokonanie opisu budowy mikroskopowej ciałka żółtego żubra nizinnego, oraz przeprowadzenie analizy porównawczej z odpowiednimi strukturami łosia europejskiego.

Grupę badawczą stanowiło 7 samic żubra nizinnego, w wieku od 5 do 20 lat, pochodzących z terenu Puszczy Białowieskiej. Ciałka żółte żubrów porównano z grupą 7 samic łosia europejskiego, w wieku od 4 do 12 lat pochodzących z terenu północno-wschodniej Polski. Wszystkie pozyskane zwierzęta znajdowały się we wczesnym etapie ciąży. Pobrane gonady zostały zważone i zmierzone za pomocą suwmiarki. Oględzinom poddano powierzchnię zewnętrzną jajników, pod kątem występowania ciałek żółtych. Następnie gonady zatopiono w formalinie, pokrojono na mikrotomie rotacyjnym i zabarwiono metodą hematoksylina-eozyna.

Histologiczna analiza struktur ciałka żółtego wykazała znaczne różnice między gatunkami. Komórki luteinowe i paraluteinowe w ciałku żółtym żubra wykazują mniejszą aktywność metaboliczną niż u łosia. Struktura ciałka żółtego żubra jest bardziej uporządkowana i przerośnięta tkanką łączną. W celu wyciągnięcia dokładnych wniosków konieczna jest jednak analiza ciałek żółtych żubrów na wszystkich etapach ciąży co wymaga dalszych badań.