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COMPARATIVE DESCRIPTION OF THE MAMMARY GLAND OF DUBSKA PRAMENKA DURING THE DRY PERIOD AND LACTATION

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Abstract: *Glandulla mammae* is a secondary sexual characteristic. The composition and structure of the mammary gland depends on the functional state of the gland and is affected by hormones. The mammary gland reaches its full development during gestation and it becomes functional and secretory upon delivery. Lactocytes of the mammary gland may be squamous in shape, low or high prismatic, depending on the phase of the glandular cycle. Lactocytes are affected by LTH hormones due to which they are called prolactin. The function of the mammary gland is also affected by STH, and indirectly by TTH. Examined material was taken from Central Bosnia Canton, under field conditions of veterinary practice. The assay of histological features of the mammary gland of Dubska pramenka was conducted during the dry period as well as during lactation. The total number of animals was 16, eight (8) during the dry period and eight (8) during lactation period. Following the development of histological preparations, we conducted a descriptive overview - interpretation of the histological structures. The study of histological preparations of the mammary gland of Dubska pramenka during the dry period and lactation shows that the parenchyma is well developed, more or less, with reduced intersticium. Histological appereance of the lactocytes resembles that of the perifollicular tissue, the presence of different cells is higher during the lactation - the onset of glandural cycle. Well-developed lactiferous ducts with cavity, with or without secretion, but with preserved epithelium are the characteristics of both periods. The glandural tissue is well-developed and the secretion was present regardless of the examination period.

Key words: Dubska pramenka, dry period, lactation, mammary gland

Introduction

The mammary gland (*Glandulla mammae*) in sheep develops in pair. It is an external (exocrine) tubulo-alveolal skin gland with apocrine type of secretion. During genesis, it goes through a phase before lactation and lactation, changing both functionally and structurally (*Z. Kozarić, 1997*). Epithelial cells of lobuloalveolar tissue conduct synthesis of proteins, lipids, lactose and other substances during milk secretion. Substances required for the composition of mammary gland secretion – milk are provided through blood circulation. Particular change - involution of the mammary gland occurs during cessation of lactation i.e. while breastfeeding, „inaction". The size of the gland reduces, it significantly preserves the glandular structure and the volume is much larger than during puberty.

The function depends on the amount of the binding fat and particularly glandular elements. The size of mammary gland does not necessarily follow its activity. Its function is associated with the sexual life, therefore, the overall biological cycles repeat in case of gestation**.** During the period of involution, epithelial cells degenerate gradually, alveolar ducts are covered with cubic epithelium; cellular plasmas show higher levels, and dark parts of casein may be seen in alveoli, intersticium and lactiferous ducts (Corpora amylacea), (*Eurell, Frappier, 2006)*. Early involution of the mammary gland is characterized by a high level of lymphocytes, which in many cases leads to inflammatory process (*Tatarczh, Philip, Lee, 1997*). In addition to B lymphocytes being present during gestation, also granulated lymphocytes are present in non-gravid ewes (*Lee et al., 1989*).

Until puberty, the sheep mammary gland is composed of the connective tissue, a small number of ducts and alveoli. The full development of the mammary gland is reached during gestation. Secretion of the mammary gland is significantly affected by the hormones of the pituitary gland. Lactocytes are affected by LTH hormone, due to which this hormone is called prolactin. Besides prolactin, the function of the mammary gland is also affected by STH, and indirectly by TTH.

Material and methods

Samples required for the research were taken in the field conditions, in Central Bosnia Canton, (Dubska pramenka is the indigenous sheep bread, village Dub, the area around Travnik) (Koco Porcu, *Marković, 2006*), and the research was conducted at the Faculty of the Veterinary Medicine of the University of Sarajevo. The assay of the histological characteristics of the mammary gland of Dubska pramenka was conducted during the dry period and lactation. The total number of

examined animals is 16, divided in two groups, eight (8) during the dry period and eight (8) during the lactation period. The samples of the mammary gland were taken from several sites, during both examined periods, from the left and right halves of the udder, being careful not to damage the active part of the gland - parenchyma.

The samples were stored in plastic containers with lids, filled with 10% formalin, until moulded in paraffin blocks. Moulding in paraffin blocks was done in a way that the samples of the mammary gland were fixed in 70% alcohol for two days, then in 96% alcohol for one day and in the end, in 100% alcohol for one day. From this procedure, the samples were transferred to a solution made of 100 % alcohol and toluol for two hours and then to toluol for four hours. The prepared samples were left in paraffin I for five hours and in paraffin II for twelve hours, until the moulding procedure in paraffin blocks was complete. The paraffin blocks with moulded samples of the mammary gland were cut by digital microtome in several serial incisions of 0,5 to 1,5 micron depth. The incisions were placed on glass slides, stained with hematoxilyn - eosin and azan, covered with cover glass, and glued with Canada balsam.

Histological examinations were done with light microscope with magnification at 100, 200 and 400 times. Microscopic examination included the entire preparations of the mammary gland, in order to get a complete picture of the organs examined during the period set forth. The assay results were presented using descriptive interpretation of the histological preparations, taking into account that comparative description of the histological preparations is representative of our research.

Research results

From a descriptive perspective, the active part of the mammary gland-parenchyma has shrank during the dry period (Image 1). It is surrounded by a somewhat thicker layer of the binding tissue with visible lymphatic and fat cells, adipocytes, at somewhat higher levels. Lobularity is well-expressed, and lobar ducts covered with two-layer high prismatic epithelium are present in intersticium (Image 2). The acini - active parts of the gland, contain lactocytes, which are in the form of high prismatic or low prismatic cells (Image 3.) and squamous cells can also be seen. Inside the sacks, there is a thick secretion and abundance of *corpora amylacea*. The shrinkage of diameters of glandular sacks is visible during the dry period in relation to the lactation period (Image 4). Apoptosis i.e. apoptic cells are visible in alveolar epithelium.

Mioepithelial cells of elongated shape are surrounding the sacks - acini. In interalveloar spaces there are blood vessels in the form of capillary. Intersticium

contains interlobular lactiferous ducts covered with high prismatic cells (Image 5), but with irregular and smaller lumen in relation to the lactation period. Lymphocytes, neutrophils are present in interalveloar spaces, in the lumen of alveoli and ducts. Blood vessels are visible in mealy irregular intralobular connective tissue. The assay of the histological structure of the mammary gland of Dubska pramenka during the lactation period has shown that the connective tissue was underdeveloped and in the form of narrow threads between the lobules, while the active part of the gland - parenchyma was well developed (Image 6). Fat cells, adipocytes, found in the connective tissue septa are at a minimum level. Among lobules there are sacks - acini of different dimensions, smaller or larger, which also contain more or less secretion (Image 7). The smaller acini are built of milk-producing cells, lactocytes, which are in the form of high prismatic cells, with darker cytoplasm and large, clearly defined round shaped nucleus (Image 8).

Among the acini, there are different cells, lymphocytes, plasmocytes, and fibroblasts. Some lobules contain glandular acini that are larger in size and that also contain high prismatic lactocytes, however, they have lighter cytoplasm and basally placed nucleus. Among such acini there is a smaller infiltration of cells. Mioepithelial cylinder-shaped cells are clearly visible around the acini. Lactiferous ducts are covered with high prismatic cells, with or without any contents. The contents inside lactiferous ducts are of somewhat liquid consistency.

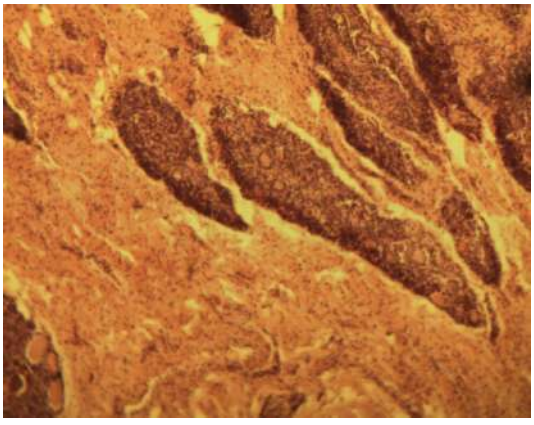

Figure 1. Mammary gland during dry period - Parenchima (oc. 10X, obj. 40X, hematoxylin-eosine)

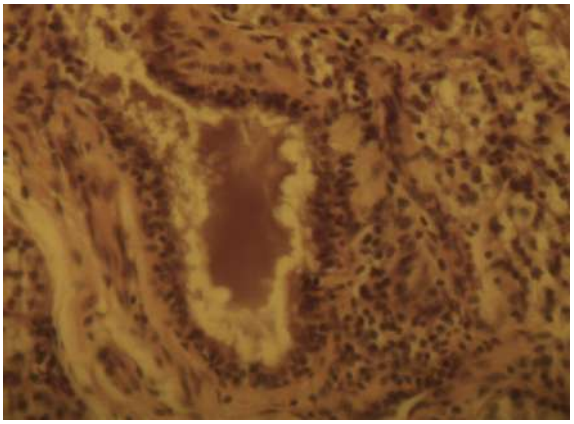

Figure 2. Mammary gland in the dry phase – lobar channel – layer of high prismatic epithelium (oc. 10X, obj. 40X, hematoxylin-eosine

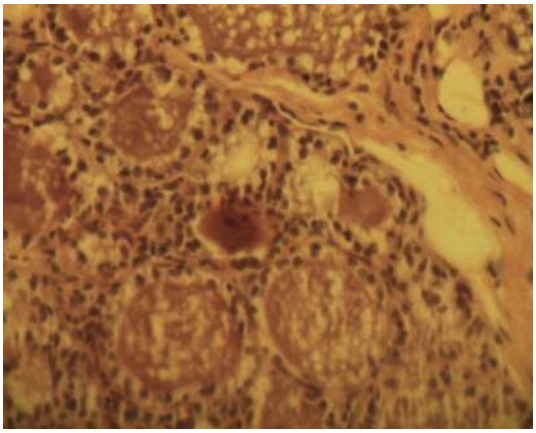

Figure 3. Mammary gland in the dry phase – Corpora amilacea (oc. 10X, obj. 40X, hematoxylin-eosine)

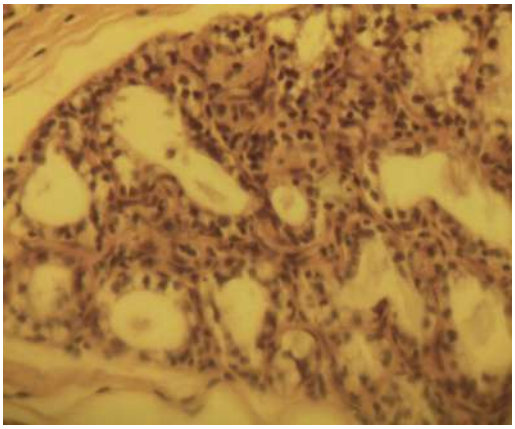

Figure 4. Mammary gland in the dry phase – decreased diameter of glandular acini. (oc. 10X, obj. 40X, hematoxylin-eosine)

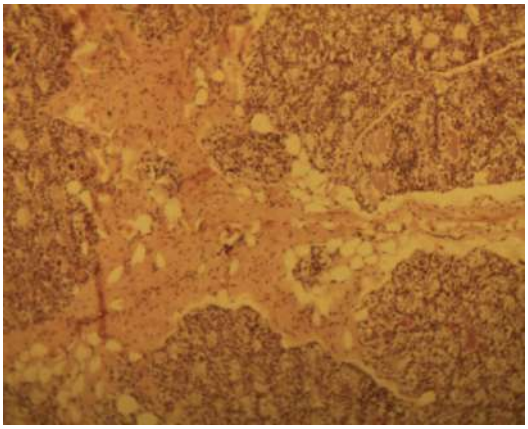

Figure 5. Mammary gland in the dry phase – different cells in the interalveolar space (oc. 10X, obj. 40X, hematoxylin-eosine)

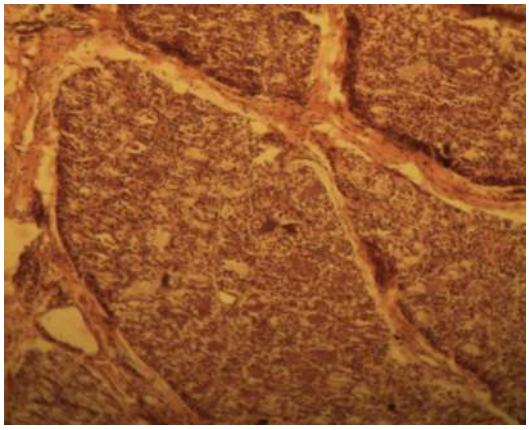

Figure 6. Mammary gland during lactation phase – connective tissue is reduced (oc. 10X, obj. 40X, hematoxylin-eosine)

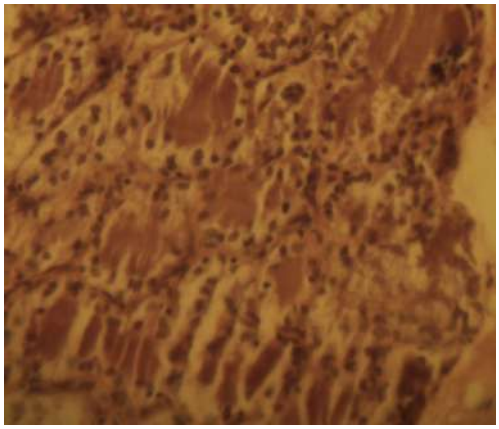

Figure 7. Mammary gland during lactation phase – secretion within acini (oc. 10X, obj. 40X, hematoxylin-eosine)

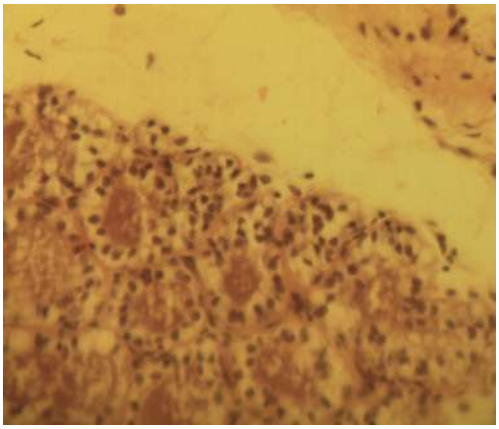

Figure 8. Mammary gland during lactation phase – high prismatic lactocytes (oc. 10X, obj. 40X, hematoxylin-eosine)

Discussion

The assay of the histological preparations of the mammary gland of Dubska pramenka during the dry period and lactation, among all examined animals, demonstrates that the parenchyma in both periods is more or less well developed with shrank intersticium. The parenchyma contains glandular acini in different phases of the glandular cycle, which manifests in different appearance of the lactocytes, glandular cells. Protein synthesis in the lactation phase affects the appearance of the lactocytes, and they are in the form of high prismatic cells of darker cytoplasm, while the accumulation of fat cells during the dry period causes light colouring. Different heights of lactocytes are the reflection of apocrine secretion performed in all examined halves of the mammary gland of the examined sheeps.

Morphodynamics of lactocytes is followed by perifollicular tissue, so some cells are at higher levels during the lactation phase i.e. at the beginning of the glandular cycle than during the dry period, when they are significantly reduced (*Hovey et al., 1999*). Both examination periods are characterized by well-developed lactiferous ducts, with vast cavities, with or without secretion and preserved epithelium (*Akers et al., 1990*). Histological condition of the mammary gland, especially its glandular cells- lactocytes, which are responsible for the biosynthesis of milk, is, undoubtedly, one of the important elements for the milk production process. If we are aware of the fact that to produce one litter of milk, for

example in cows, 400-500 liters of blood needs to circulate through udders (*Bobo*š*, Vidić, 2005*), it is then certain that the milk production is highly dependent on the conditions of blood vessels in the mammary gland in addition to the general condition of the cardio vascular system.

During vasodilation of blood vessels, the mammary gland contains more blood, that is, there is less blood during vasoconstriction. Inter-capillary pressure and the amount of blood are very important in transition of the liquid between the capillary and the surrounding tissue. All anatomic or functional change in capillary of the mammary gland can be repercuted on the parenchyma of the mammary gland. During prolonged vasoconstriction, ischemic and even necrotic conditions may appear (*Lee and Lascelles, 1969*). It could be expected that due to low air temperatures, especially during the winter months of our continental climate conditions and nomad pasture of our Dubska pramenka, which is the main cause of vasoconstriction, the productive elements of the mammary gland would suffer certain histological changes i.e. to stay underdeveloped. However, lactocytes showed no changes; they did not involute, on the contrary, their appearance reflected significant activity. This can be explained by the fact that nomad breeding involves movement of the udder, which activate local circulation system and improves blood flows (*Lin, Li, 2005*). According to some observation (*Lee, Outteridge, 1981*) low air temperatures represent negative impulse that inhibits the milk biosynthesis process. However, our examination of lactocytes as the place of the milk biosynthesis did not show any change; it showed extraordinary activity, the animals were in better condition and the negative effects of low air temperatures did not exhibit such an effect.

Through the udder's cold receptors, low temperatures affect hypothalamus, whose circulation system is in direct connection with adenopituitary gland, and the releasing factor of the hypothalamus (RF) under low- temperatures conditions inhibits excretion of prolactin required for milk biosynthesis (*Mutevelić et al., 2003*) . Low air temperatures affect the flow of energy, the intensity of metabolic processes is higher at low temperatures, which depends on the amount of thyroxin and triiodothyronine (*Mutevelić et al., 2003*). Reduced dairy production in cows, for example, is explained by the fact that there is a change in response of the neuroendocrine system, known as the adaptation syndrome, as discussed by. Through hypothalamus and its connection with the adenopituitary gland, which excretes ACTH, low temperatures affect the concentration of corticosteroids and glucocorticosteroids among them. They regulate the metabolism of carbohydrates, so together with the thyroid and other hormones in the feedback system they are responsible for the flow of energy.

During the dry period and at higher air temperatures, the histological assay shows that the parenchyma was somewhat underdeveloped, while lactocytes expressed further activity, which is explained by the fact that thier involution was

not fully represented. Hence, milk biosynthesis occurs throughout the year, regardless of the phases of the glandular cycle, however, the intensity varies.

Conclusions

The histological assay of the samples of Dubska pramenka's mammary under conditions of free breeding leads to the following conclusions:

* The glandular cycle of the mammary gland have continuous function throughout the year, with a varying intensity, depending of the season.

* Low air temperatures have no significant effects on lactocytes as the place of biosynthesis of milk.

* Connective tissue in the lactation phase is in the form of threads, adipocytes are reduced, and lactocytes are in the form of high-prismatic cells with darker cytoplasm.

* Layered high-prismatic epithelium of ducts and single-layered high-prismatic epithelium of alveoli is visible during the dry period, as well as lymphocytes, cellular plasma, fibroblasts and blood vessels in a mealy interlobular connective tissue.

* High air temperatures during the dry period negatively affect the parenchyma causing its shrinkage, and the amount of fat cells- adipocytes is increased, while lactocytes are in the form of low or high prismatic cells. Low air temperatures don’t have negative effects to histological characteristics of mammary gland i.e. parenchyma, milk production can even increase in favourable zoohigienic conditions.

Komparativni prikaz mlečne žlezde dubske pramenke u fazi zasušenja i laktacije

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Rezime

Glandulla mammae predstavlja sekundarnu polnu karakteristiku. Građa i struktura mlečne žlezde zavisi od funkcionalnog stanja žlezde, a na čiju funkciju utiču hormoni. Mlečna žlezda svoj puni razvoj dostiže za vreme

graviditeta, a postaje funkcionalna, sekretorna po porođaju. Laktociti mlečne žlezde mogu biti pločasti, nisko ili visokoprizmatični, zavisno od faze žlezdanog ciklusa. Pod delovanjem su LTH hormona koji se zbog toga naziva prolaktin. Na funkciju mlečne žlezde ima uticaj i STH, a indirektno i TTH. Ispitivani materijal, u uslovima terenske veterinarske prakse, uzeli smo na području Srednjobosanskog kantona. Istraživanja histoloških karakteristika mlečne žlezde dubske pramenke izvršili smo u periodu zasušenja i laktacije. Ukupan broj životinja je 16, 8 u periodu zasušenja, 8 u periodu laktacije. Nakon izrade histoloških preparata, izvršili smo deskriptivni prikaz-interpretaciju histoloških struktura. Analizom histoloških preparata mlečne žlijezde dubske pramenke u fazi zasušenja i laktacije kod svih ispitivanih životinja vidljivo je da je parenhim dobro razvijen, manje-više, sa redukovanim intersticijumom. Histološku sliku laktocita prati i perifolikularno tkivo, prisutnost pojedinih ćelija je veća u fazi laktacije-početak žlezdanog ciklusa. Dobro razvijeni izvodni kanali sa šupljinom, sa ili bez sekreta, ali sa očuvanim epitelom su karakteristika i jednog i drugog perioda. Žlezdano tkivo je dobro razvijeno, bez obzira na istraživani period, u njemu se vršila sekrecija.

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