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COMPARATIVE HISTOLOGICAL STUDY OF MAMMARY GLAND IN NON-DESCRIPT SHEEP DURING THE DRY PERIOD AND LACTATION

[1]*Modekar S. S.*, *PhD Scholar, Dept. of Anatomy*
[2]*Dhande P. L.*, *Professor, Dept. of Anatomy*
[3]*Meshram P. V.*, *Dept. of Pathology*
[4]*Kadam D. P.*, *Dept. of Pathology*
Bombay Veterinary College, Parel, Mumbai

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Abstract: *The importance of small ruminants to the dairy industry has increased in recent years. Various modifications occur in the mammary gland throughout various stages of lactation. Glandullamammae 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. The alveolar lobule may be squamous in shape, low or high prismatic, depending on the phase of the glandular cycle. Development and secretion of mammary gland is significantly affected by the hormones of pituitary gland. The assay of histological features of the mammary gland of non-descript sheep was studied during the dry period as well as during lactation. The total number of animals from whom the samples were collected was 20, ten (10) during the dry period and ten (10) during lactation period. The study of histological preparations of the mammary gland of sheep during the dry period and lactation shows that the parenchyma is well developed, more or less, with reduced intersticium. Histological appearance of the lactocytes resembles that of the perifollicular tissue, the presence of different cells is higher during the lactation. Well-developed lactiferous ducts with cavity, with or without secretion, but with preserved epithelium are the characteristics of both periods. The occurrence of corpora amylacea was seen both in lumen of alveoli and septal connective tissue, and was seen more in dry sheep*

Key words: *Mammary gland, Lactation, Histology.*

Introduction

The mammary gland (*Glandullamammae*) in sheep develops in pair. It is an external (exocrine) tubulo-alveolar gland with apocrine type of secretion. It originates from ectoderm. During pregnancy and lactation the growth and development of mammary gland is very extensive and controlled by pituitary and ovarian harmone (*Parmasivan et. al. 2013*). Epithelial cells of lobulo-alveolar tissue conductlobulo-alveolar 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. 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. 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). 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*).

Material and methods

The samples required for the research were procured from Deonarrabattoir, Mumbai, Maharashtra State. The assay of the histological characteristics of the mammary gland of non-descript sheep was done. A total number of examined animals is 20, divided in two groups, ten (10) during the dry period and ten (10) during the lactation period. The samples of the mammary gland were taken from healthy sheep fromseveral sites, during both examined periods, from the left and right halves of the udder.

The samples were stored in plastic containers with lids, filled with 10% formalin, until molded in paraffin blocks. Molding in paraffin blocks was done in a way that the samples of the mammary gland were fixed in alcohol. The prepared samples were left in paraffin I for five hours and in paraffin II for twelve hours, until the molding procedure in paraffin blocks was complete. The paraffin blocks with moulded samples of the mammary gland were cut by microtome in several serial incisions of 04 to 06 micron depth. The incisions were placed on glass slides, stained with Hematoxiline& Eosin, Periodic Acid Schiff's (PAS) technique for muco-polysaccharides, Gomori's method for acid and alkaline phosphatase and then covered with cover glass, and glued with Canada balsam.

Histological examinations were done with light microscope at various magnifications. 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.

Results and discussion

Histologically, the alveoli of non-lactating sheep i.e dry mammary gland showed alveolar degeneration. Only some remnants of alveoli and small duct were seen. It was lined with one or two layers of compactly arranged cuboidal epithelial cells which showed a densely stained nuclei. The alveolar epithelial cells were seen with large empty vacuoles as well as auto-phagosomes in their cytoplasm. In this stage an important feature seen was apoptosis i.e. apoptic cells were visible lining epithelial cells, lumen of alveoli and some were even engulfed by macrophages. Also in the epithelium of these dry mammary glands, the ergastoplasm was broken into smaller fragments and vesicles and lost their parallel arrangement. Secondary lysosomes or digestion vacuoles were seen in alveolar epithelial cells. This is in agreement with that of Heinz and Michel (1991) who stated that the occurrence of different large vacuoles was the most evident sign of involution of mammary gland in cattle.

(Fig 1). The corpora amylacea were and important feature seen both in alveoli and connective tissue in dry mammary gland. These were round, oval or irregular cauliflower shaped concentrically laminated bodies with few droplets like structure present at the center. Their origin is from cellular and the desquamated and infiltrated cells accumulated in the lumen and becomes fragmented, degenerated and lysed leading to formation of solid lump like structures. These corpora amylacea were of different sizes located both in alveolar lumen and in septal connective tissue. It was seen that there was gradual increase in both size and number of corpora from parturition to late lactation and their development accelerated as lactation progressed and when the mammary gland was into involution process. Similar observations were made by (*Parmasivanet al.* (2012) in sheep. Thus the alveoli and ducts in mammary gland of dry sheep showed degenerative changes and lost their shape. The lumen became obliterated in the alveoli and in the ducts. (Fig 1) The connective tissue fibers increased due to evolutionary effect.

The histological structure of lactating mammary gland revealed 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 (Fig 2). Fat cells, adipocytes, found in the connective tissue septa were at a minimum level. The lobules showed different sized alveoli bearing secretary material inside. The shape and size of alveoli varies

considerably depending on the state of secretory activity. At the lactating stage, the mammary gland of sheep showed reduced connective tissue whereas the lobulo-alveolar tissue increased predominantly. In the apical regions of secretory cells, lipid droplets and apocrine secretions were seen in the lumen of alveoli. The alveoli were lined by simple cuboidal epithelium. The basement membrane and secretory substances within alveoli showed positive reaction for muco-polysaccharides. The alveolar epithelium, basement membrane and nucleus were seen positive for protein. Uniformly distributed calcium was observed in the alveolar secretion, inter-alveolar septa as well as within the cells. (*Paramsivan et al. (2012)* observed that the lumen of alveoli in the mammary gland during pregnancy and during lactating period was positive for calcium. The myo-epithelial cells were present between the lining epithelial cells and the basement membrane of alveoli possessing vesicular nuclei. Similar findings were reported by Silver (1954) who stated the myo-epithelial cells surrounded the mammary alveoli in sheep were stellate in shape. (Fig. 2)

Conclusions

The assay of the histological preparations of the mammary gland of non-descript sheep during the dry period and lactation, among all examined animals, indicates that the parenchyma in both periods is more or less well developed. The parenchyma contained glandular acini in different phases of the glandular cycle, which manifests in different appearance of the alveolar lobules and glandular cells. Protein synthesis in the lactation phase affects the appearance of the alveoli, 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 coloring. Different heights of secretory cells are the reflection of apocrine secretion performed in all examined halves of the mammary gland of the examined sheep. It is observed that in sheep, lactation is defined by occurrence of cell proliferation, a higher level of cell activity and alveoli number and increase in udder volume. The fatty vaculation and other degenerative changes in alveolar epithelial cells observed as involution progressed.

Morpho-dynamics 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.In lactating sheep, the connective tissue became reduced and lobulo-alveolar tissue was predominantly increased. In non-lactating dry animals mammary gland showed increased amount of aconnective tissue elements and interstitial corpora amylacea.

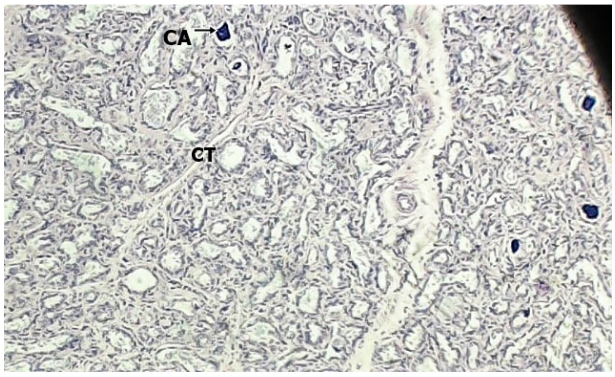

Figure 1: Photomicrograph of the sheep mammary gland during dry phase showing lobar parenchyma connective tissue stroma, shrunken alveoli and presence of corpora amylacea (CA). Decreased diameter of glandular acini. obj. 40X, hematoxylin-eosine

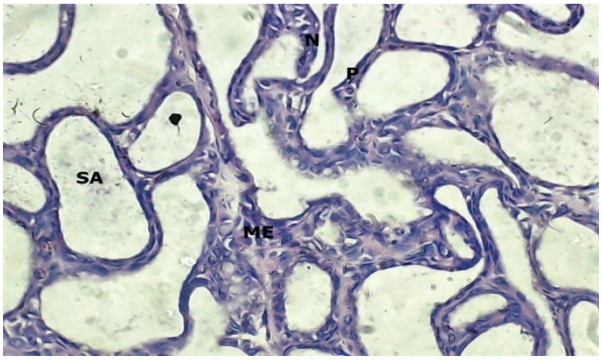

Figure 2: Photomicrograph of sheep mammary gland during lactation phase - showing secretory alveoli (SA) plasma cells (P), myo-epithelial cells (ME) secretion within acini and nucleus (N) (40X, Hematoxylin-eosine)

Discussion

The assay of the histological preparations of the mammary gland of nondescript sheep, 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 coloring. Different heights of lactocytes are the reflection of apocrine secretion performed in all examined halves of the mammary gland of the examined sheep. 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.

The mammary alveoli, because of reciprocal compression, apper polygonal in shape. They had non-homogenous aspect because some of them contain large alveoli filled with secretion, and other adjacent once contain small alveoli with almost drained lumen. The secretory cells from evicted alveoli were seen prismatic with colorless intercellular limits and large euchromatic nucleus with sizeable nucleolus. 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*). 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 normal breeding involves movement of the udder, which activate local circulation system and improves blood flows (*Lin, Li, 2005*).

Large number of plasma cells were observed in the inter-alveolar connective tissue during latepregnancy, lactataion and early involution. The foamy macrophages persisted in the alveolar and ductal lumina. (Lee & Lascelles, 1969) made similar observation in mammary gland of sheep. During the dry period the histological assay shows that the parenchyma was somewhat underdeveloped, while lactocytes expressed further activity, which is explained by the fact that their involution was not fully represented. Hence, milk biosynthesis occurs throughout the year, regardless of the phases of the glandular cycle, however, the intensity varies.

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