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HISTOLOGY OF MAMMARY GLAND DURING LACTATING AND NON-LACTATING PHASES OF MADRAS RED SHEEP WITH SPECIAL REFERENCE TO INVOLUTION

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Abstract: The histological study was carried out on mammary glands collected from 14 Madras red sheep, 7 in lactating period 7 in non-lactation period. The connective tissue became reduced and lobuloalveolar tissue was predominantly increased during lactating period. The alveoli were laminated, filled with secretion and lined by cuboidal active secretory cells containing lipid droplets and protein granules during active lactation. The alveoli of the non-lactating sheep mammary glands showed the alveolar degeneration. Large empty vacuoles and autophagosomes in the cytoplasm of numerous apoptotic alveolar epithelial cells. Macrophages, mast cells, neutrophils and lymphocytes were found in the interalveolar areas and lumina of alveoli. The occurrence of corpora amylacea located both in the lumen of alveoli (intra-alveolar bodies) and septal connective tissue (Interstitial bodies) was frequent in mammary glands of non-lactating sheep. It was concluded that these regressive changes during non-lactating period form a physical barrier in ductulo-alveolar system for protecting the gland from bacterial infection.

Keywords: Histology, Mammary Gland, Lactation, Involution.

INTRODUCTION

Development and secretion of the mammary gland is significantly affected by the hormones of the pituitary gland. The full development of the mammary gland is reached during gestation. The final stage involves the functional differentiation process during pregnancy-associated lobuloalveolar development, followed by lactation and involution when nursing or milking ceases. Involution in dairy animals is characterized by a low rate of epithelial cell turnover and mammary gland morphological maintenance. Involution and mammary cell turnover is a critical process in the cycle of the mammary gland in dairy animals, which must undergo a dry period in order to avoid losses in milk production in their subsequent lactation cycle (Collier *et al*. 2004).

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Involutionary changes have been reported in domestic animals (Katica *et al*., 2012) mostly comparing the lactating and non-lactating dairy cows. The literature on the morphology of the mammary gland during various physiological phases in small ruminants is limited. Therefore, the current histomorphological observation is aimed to record the structural changes in the mammary glands during lactating and non-lactating phases of Madras red sheep.

MATERIALS AND METHODS

The total number of animals used in the current study were 14, divided in two groups, seven (7) during the lactating period and seven (7) during the non-lactation period. The tissue samples were collected from several sites of mammary glands from the left and right halves, immediately after slaughter of the animals. The tissue samples collected from mammary glands of all these animals were fixed in various standard fixatives. All tissues collected as above were processed by routine Alcohol-Benzene schedule and molded in paraffin blocks for light microscopy. The paraffin blocks of the mammary gland were cut by digital microtome in several serial incisions at 5-7 µm thickness for histological study.

The sections were stained with standard Haematoxylin and Eosin, Masson's trichrome method for collagen and muscle fibres, Verhoeff's method for elastic fibres, Periodic acid Schiff (PAS) technique for mucopolysaccharides, Mercury-Bromophenol blue method for basic proteins, Gomori's calcium method for alkaline phosphatase activity, Gomori's lead method for acid phosphatase activity, Von Kossa method for calcium (Bancroft and Gamble, 2003). Tissue pieces from mammary glands of two animals were cut into 1-2 millimeter thickness and fixed in 2.5% Glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 hours at 4°C and processed for transmission electron microscope (Bozzola and Russell, 1998).

RESULTS AND DISCUSSION

In the lactating sheep, the connective tissue became reduced and lobuloalveolar tissue was predominantly increased in the mammary glands. The relative proportion of the interlobular connective tissue increased at the pubertal period but decreased during pregnant and lactating mammary glands, due to the increase in size of the glandular alveoli. Gayer *et al*. (1986) also reported that the lobulo-alveolar development of ductules occurred during pregnancy and lactation which was followed by the regression of secretory alveoli at involution in pigs.

The tissues from lactating ewes showed an extensive development of glandular epithelium. The alveolar size was 106.05 ± 14.70 µm in lactating sheep but decreased significantly to 32.45 ± 1.64 µm in the mammary glands of dry sheep (Fig.1). The luminal diameter also showed the same trend as that of the alveolar size during different phases in these sheep. The

increased size of the alveoli in lactating mammary gland may be correlated with its active secretion that fills the lumen of the alveoli when compared to other age group of animals.

The alveoli were luminated and lined by cuboidal active secretory cells containing lipid droplets and protein granules with strongly basophilic nuclei. The internuclear distance in the lactating sheep was 8.38 ± 1.03 µm but reduced to 2.21 ±.0.27 µm in non-lactating sheep. In the current study, the glandular alveoli became hypertrophied and tensed with full of secretion in the mammary glands of lactating sheep.

The alveolar epithelial cells of the secreting gland contained extensive dilated granular endoplasmic reticulum found in parallel arrays. The Golgi apparatus, the site of formation of the definitive proteinaceous granules, comprised of large vacuoles and flattened sacs and microvesicles. This corroborated with the findings of Augsburger (1987) in lactating goat mammary gland, where scattered protein granules of different diameters were identified both in the alveolar lumina and in the Golgi vacuoles of the secretory cells. Lipid vacuoles were often being pinched off from the luminal surfaces, and many proteinaceous granules were also seen in the alveolar lumen and cytoplasm.

The alveoli of the non-lactating sheep mammary glands showed the alveolar degeneration. Only alveolar remnant and small ducts were seen lined with one or two layers of closely packed cuboidal epithelial cells with densely staining nuclei. The alveolar epithelial cells were seen with large empty vacuoles and autophagosomes in their cytoplasm. However, the important feature was the appearance of cells undergoing apoptosis. Some apoptotic bodies were seen within lining epithelial cells, lumina of alveoli and some were engulfed by macrophages. Macrophages, mast cells, neutrophils and lymphocytes were found in the interalveolar areas (Fig.2) as well as in the lumina of alveoli and ducts. Intraepithelial macrophages with engulfed material at different stages of degradation within their cytoplasm was a common feature.

In the epithelium of the non-lactating sheep mammary gland, the ergastoplasm was broken into smaller fragments and vesicles and lost their parallel arrangement. Secondary lysosomes or digestion vacuoles as multivesicular bodies were identified in alveolar epithelial cells. This is in agreement with the findings of Heinz and Michel (1991) who also opined that the occurrence of different large vacuoles was the most evident sign of the involution of mammary gland in cattle.

The occurrence of corpora amylacea both in alveoli and connective tissue was frequent in non-lactating mammary glands (Fig.1&2). Corpora amylacea were observed as round, oval or

irregular cauliflower shaped concentrically laminated bodies with few droplets like structures in the centre. Their origin appeared to be cellular and the desquamated and infiltrated cells accumulate in the lumen became fragmented, degenerated and lysed to form a solid lump like structure of the corpora amylacea. These were seen in their various stages of formation and located both in the lumen of alveoli (intra-alveolar bodies) and septal connective tissue (Interstitial bodies). Gradual increases in both size and numbers of corpora from parturition to late lactation suggest that the development of these structures accelerates as lactation progresses and these structures may have a role in the involutionary process.

Few mononuclear phagocytic cells were seen in the lumen of the regressing alveoli in the dry mammary glands (Fig.3). It has been reported that the early period of mammary gland involution coincides with a period of acutely increased susceptibility to intramammary infection (Nickerson, 1989; Oliver & Sordillo, 1989) and one of the reasons could be due to the loss of the physical barrier by sloughing of the alveolar epithelium.

From the current observation, it was concluded that the sloughing of alveolar epithelial cells into the lumina of alveoli and ducts in combination with apoptotic changes progressed from late lactation to dry period. These regressive changes are important in forming the physical barrier in ductulo-alveolar system for protecting the gland from bacterial infection.

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LEGENDS TO FIGURES

Figure 1. Photomicrograph of the dry mammary gland showing reduced secretory tissue and increased stroma (CT). The alveoli (A) are shrunken with formation of corpora amylacea (CA) both in alveoli and interstitial tissue. **H&E x 400**

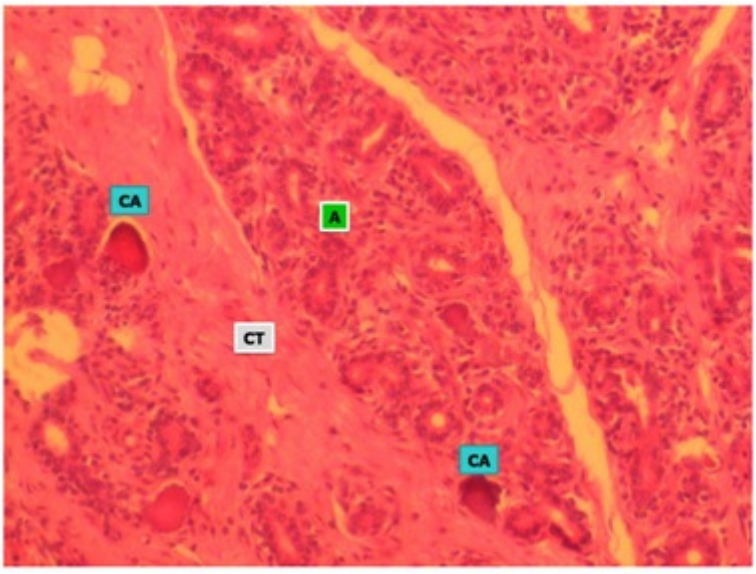

Figure 2. Photomicrograph of the dry mammary gland during involution showing mast cell (arrow) close to the blood capillary (BV). Increased connective tissue stroma (ST) and degenerating alveoli (A), ducts and corpora amylacea (CA). Semi-thin sections, **Toluidine blue x 630**

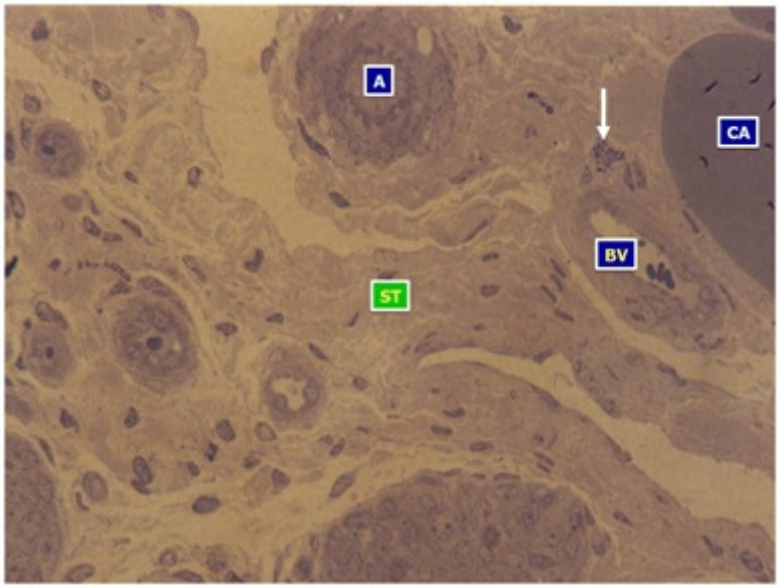

Figure 3. Electron photomicrograph of mammary gland in non-lactating sheep showing the lumen of the alveoli with macrophage containing phagocytosed materials (arrow) in the cytoplasm. N – Nucleus, L- Lumen. **TEM x 7000**