Haryana Vet. (Dec., 2019) **58(2),** 209-212

Research Article

HISTOLOGICAL AND ULTRASTRUCTURAL STUDY OF ALVEOLAR LUMEN AND MYOEPITHELIAL CELLS IN LACTATING AND NON-LACTATING MURRAH BUFFALO

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Received: 11.12.2018; Accepted: 28.01.2019

ABSTRACT

Present experiment was conducted on sixty Murrah buffalo divided into three groups: lactating, involution stage/dry and pregnant stage (non-lactating early pregnant stage, non-lactating mid pregnant stage and non-lactating late pregnant stage). Alveolar lumen was filled with electron dense protein (casein micelle) and lipid droplets. It was noted that the amount of secretary material in the alveolar lumen was reduced with the advancement of lactation. The size of the casein micelle and lipid droplets was found to reduce towards the end of lactation. In non-lactating non-pregnant stage, small lumen had no secretory material, but desquamated cells were observed in alveolar lumen. In non-lactating late pregnant stage, the alveolar lumen was filled with secretory material, which consisted of electron dense predominant granular protein material and large lipid droplets of intermediate electron density between them.

Keywords: Alveolar lumen, Casein, Lactating, Lipid droplet, Non-lactating, Secretory material

As per animal genetics training resource, Murrah buffalo is the finest genetic material of milk producing buffalo in the world. Nature and amount of secretory material in the alveolar lumen reflexes the physiological stages of mammary gland parenchyma (alveoli and duct) and lactation stage. Alteration of the functional and physiological stages is the basis of continuous and constant milk secretion assurance. Further, there is a positive correlation between glandular parenchyma area and milk production (Cedar *et al.*, 2012). Oxytocin stimulates contractility of the myoepithelial cells that surround mammary alveoli responsible for the ejection of milk in the alveolar lumen. The mammary gland was an excellent model for studying the 'stem/progenitor' cells because of repeated expansion and renewal (Jamie *et al.*, 2015). Due to scanty literature, present experiment was undertaken.

MATERIALS AND METHODS

The present study was conducted on mammary gland of sixty Murrah buffaloes to know the histoarchitecture of mammary alveolar lumen and myoepithelial cells. The normal mammary gland samples of naturally dead buffaloes were collected from dairy farms nearby Nagpur district of Maharashtra and Durg, Rajnandgoan and Raipur Districts of Chhattisgarh. These samples were categorized into two groups as lactating and non-lactating/dry by ascertaining the stage of lactation, dry period and pregnancy period. Lactating stage was further divided into five subgroups with six samples in each: Colostrum stage/ phase, three months of lactation, five months of lactation, seven months of lactation, ten months of lactation. Non-lactating group was divided into involution and pregnant stage, involution stage was further divided into non-lactating non-pregnant stage (upto one month) and non-lactating non-pregnant stage (from one to two month), and pregnant stage was divided into non-lactating early pregnant stage, non-lactating mid pregnant stage and non-lactating late pregnant stage with six samples in each. For histological study, the tissue samples of 3-5 mm thickness were collected in ice box and brought to the laboratory. These samples were fixed in 10% neutral buffered formalin, processed for routine paraffin block making technique and 4 to 7 µm thick sections were cut and stained with Haematoxylin and Eosin, Masson Trichrome as well with Toluidine blue as per the procedure described by Singh and Sulochana (1996). The stained sections of mammary glands were observed under Nikon Eclipse 80i microscope and measurements of diameters of alveolar lumen and nuclei of myoepithelial cells were carried out and analysed as per Snedecor and Cochron (1980).

For ultrastructural studies, tissue samples were processed as per Bozzola and Russell (1998) and ultrastructure and status of secretory material present in the alveolar lumen and ultrastructure of myoepithelial cells was recorded. The electron microscopic work was done at Ruska Laboratory, College of Veterinary Science, SVVU, Rajendra Nagar, Hyderabad, India.

RESULTS AND DISCUSSION

Histology: There was a highly significant statistical difference in the diameter of alveolar lumen in different stages of lactation (Table 1). The diameter of alveolar lumen in non-lactating non-pregnant animal reduced upto two months. However, in non-lactating early to late pregnant stage, a linear increase in the diameter of alveolar lumen was observed (Table 1). A highly significant statistical difference was noted in diameter of alveolar

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Table 1

Diameter of alveolar lumen in lactating and non-lactating buffalo

Parameter	Stages of lactating buffalo					'F' value
	Colostrum stage of lactation	Three months of lactation	Five months of lactation	Seven months of lactation	Ten months of lactation	
Average diameter of alveolar lumen (μm)	98.79 ± 8.61	87.71 ± 6.33	78.01 ± 5.28	70.75 ± 4.17	64.10 ± 3.39	5.51**
	Stages of non-lactating buffalo					
	Nonpregnant		Pregnant			
	Upto one month	From one two month	Early pregnant	Mid pregnant	Late pregnant	
Average diameter of alveolar lumen (μm)	58.10 ± 2.73	38.57 ± 2.04	76.61 ± 4.64	93.54 ± 7.22	112.73 ± 8.80	25.15**

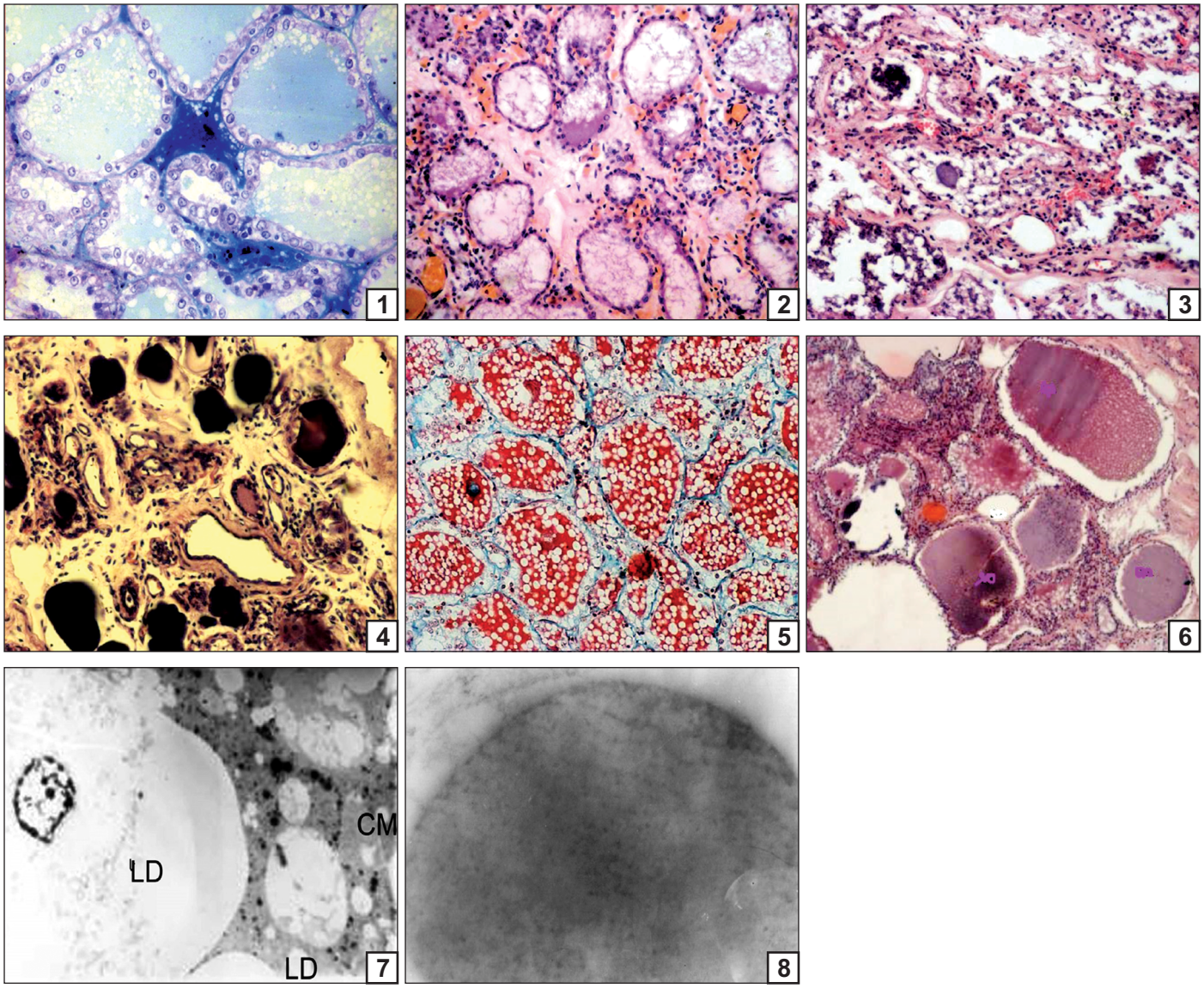
**Significant at 1% level

lumen in non-lactating group (Table 1). The diameter of alveolar lumen was found reduced with the advancement of lactation from colostrum stage to ten months of lactation (Table 1). These observations are in agreement with the findings reported by Joana *et al.* (2014) in sheep and goat that during late gestation, as well as during early to mid-lactation, mammary gland expansion occurs, with an increase in the number of epithelial cells and lumen area, which leads to increment of the parenchyma tissue, as well as a reduction of stroma, corresponding macroscopically to the increase in mammary gland volume.

The observations of the present study regarding increase or decrease in the diameter of alveolar lumen of mammary gland during different stages of lactation could be attributed to the feedback inhibitor of lactation. This inhibitor was thought to be secreted by mammary epithelial cells, which in turns inhibits further milk secretion, as its own concentration increases in the alveolar lumen. Although, the exact mechanism of how this feedback inhibitor works is not known; Hurley (2009) stated that there was no stimulation of prolactin, if milk was not removed. It resulted in acute accumulation of milk in the gland and increased intramammary pressure by activation of sympathetic nervous system leading to decreased mammary blood flow, reduced availability of hormones and nutrients to the gland.

The alveolar lumen were categorized on the basis of extent of secretion as: the alveolar lumen fully filled with secretion, partially filled with secretion, partially filled with secretory material and desquamated cells, fully filled with desquamated cells and fully empty. In contrast to present finding, Hluchy and Bolcso (2011) observed three types of alveoli that represented various stages of secretion. In lactating stage upto three months of lactation, most of the alveolar lumen was found fully filled with secretion (Fig. 1). There were very few alveoli, which were empty or partially filled with desquamated cells. In ten months of lactation stage, the alveoli were found filled with scanty secretory material and desquamated cells (Fig. 2). In non-lactating non-pregnant animals, upto one month, most of the alveoli were found degenerating and had desquamated cells in their alveolar lumen (Fig. 3). Secretory material in the alveolar lumen was scanty lightly eosinophilic and foamy. Some alveolar lumen was empty and in very few corpora body was present. In contrast to present finding, Holst *et al.* (1987) observed no evidence of extensive sloughing of alveolar epithelial cells from basement membrane during twenty to thirtieth day of involution in dry cow. While, in non-lactating non-pregnant stage from one to two month secretory material was scant with desquamated cells. Corpora amylacea in the alveolar lumen was common finding (Fig. 4). In agreement with present findings, Patel *et al.* (2007) in lactating buffalo found that lumen of the alveoli showed varying proportion of luminal secretory material, desquamated cells and corpora amylacea. In non-lactating early pregnant stage, the alveolar lumen showed prominent lipid droplets (Fig. 5). In mid to late pregnant stage, lumen of most of the alveoli was filled with eosinophilic foamy secretory material. In late pregnant stage, slightly basophilic secretory material was noted in the lumen of alveoli (Fig. 6). These observations in pregnant stages corroborated the findings of Morales (1977) who found fat globules in alveolar lumen in six months of gestation.

The present study revealed the presence of myoepithelial cells in between the alveolar epithelial cells and basement membrane. The shape of myoepithelial cells was elongated to fusiform. Myoepithelial cells were arranged in circular manner around the alveoli (Fig. 1). The nucleus of myoepithelial cells was dark. The length and width of nuclei of myoepithelial cells in lactating stage were 11.33±1.32 μm and 4.17±0.87 μm, respectively. In



Figs. **1.** Mammary gland at colostrum stage of lactation. Alveolar lumen filled with secretory material and myoepithelial cell present between secretory cells and basement membrane (Toluidine blue × 400). **2.** Mammary gland at ten months of lactation. Scant secretory material in the alveolar lumen (H&E × 1000). **3.** Mammary gland at non-lactating non-pregnant upto one month stage. Desquamated cells and corpora amylacea in alveolar lumen (H&E × 200). **4.** Mammary gland at non-lactating non-pregnant stage from one to two month. Corpora amylacea in alveolar lumen (H&E × 100). **5.** Mammary gland at non-lactating early pregnant stage. Lipid droplet in the alveolar lumen (Masson's Trichrome × 200), **6.** Mammary gland at non-lactating late pregnant stage. Eosinophilic and basophilic secretory material in alveolar lumen (H&E × 100), **7.** Mammary gland at colostrum stage of lactation. Lipid droplets (LD) and casein micelle (CM) in alveolar lumen (× 6370), **8.** mammary gland at three months of lactation. Granular secretory proteins surrounded by three layers of membranes (× 35800).

non-lactating stage, mean length of nuclei of myoepithelial cells was 8.74±3.23 µm and width was 2.81±0.78 µm.

The higher diameter of nuclei of myoepithelial cells in lactating stage observed during present study was indicative of their active functional stage under the neurohumoral reflex of oxytocin released from hypothalamoneurohypophysial tract in response to udder stimulation as stated by Hurley (2009) in cow. The oxytocin concentration in the blood was increased within one to two minute after udder stimulation. Similarly, Carrol (1980) in cow stated that the oxytocin acts on oxytocin receptors present in myoepithelium and stimulates the myoepithelial cells to contract, which expel milk from the alveolus and result in milk ejection. In support of inactivity of myoepithelial cells of alveoli in non-lactating stage observed during present study, Hurley (2009) in cow stated that maximum oxytocin was released in response to udder stimulation, which occurred only if, the mammary gland was lactating.

ULTRASTRUCTURE: During lactating stage, the alveolar lumen was large and filled with electron dense casein micelle and lipid droplets. The same was reported by Kensinger *et al.* (1986) in pig. Casein micelle was found to predominate the luminal lipid droplets (Fig. 7). This

casein micelle was the electron dense protein material, which had granular structure. These electron dense proteins present in the lumen were surrounded by triple layer membrane in three months of lactation (Fig. 8). It was noted that the amount of secretory material in the alveolar lumen was reduced with the advancement of lactation. The size of the casein micelle and lipid droplets was found to reduce towards the end of lactation. Reid and Chandler (1973) in cow reported mosaic substructure of electron dense protein material in the alveolar lumen. However, they reported the presence of protein droplets in the alveolar lumen surrounded by three layer membranes, which was in agreement with the present observations Regarding presence of abundant secretory material in alveolar lumen in colostrum stage and subsequent reduction with advancement of lactation and empty alveolar lumen in non-lactating, non-pregnant stage, Yves *et al.* (1991) stated that the secretory activity of the mammary gland declined slowly and eventually ceased probably under the influence of decreased concentration of prolactin, growth hormone, thyroxin, insulin and oxytocin with advancement of lactation in cow.

In non-lactating, non-pregnant stage presence of desquamated cells in small alveolar lumen were observed. However, in non-lactating, late pregnant animals, the alveolar lumen was filled with large amount of secretory material, consisted of electron dense predominant granular protein material and large lipid droplets of intermediate electron density between them. Same was reported by Morales (1977) in early stage of gestation in cow. However, Kensinger *et al.* (1986) in pig reported the presence of large lipid droplets predominating.

Present study revealed the presence of myoepithelial cells between the basal part of epithelial cells lining the alveoli and basement membrane. In lactating and non-lactating stages, the myoepithelial cells were elongated in shape. The myoepithelial cells showed elongated to fusiform intermediate to densely stained nucleus. The cytoplasmic processes of intermediate electron density were extended in both directions. The cytoplasmic organelles were present in the perinuclear region. The cytoplasm was found to contain abundant myofillaments.

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