

*Original Research***Effect of Lactation on Mammary Gland in Madras Red Ewe –An Histomorphometric Study****S. Senthilkumar<sup>1\*</sup>, T. A. Kannan<sup>2</sup>, Geetha Ramesh<sup>3</sup> and D. Sumathi<sup>4</sup>**<sup>1</sup>Department of Veterinary Anatomy, Madras Veterinary College, Chennai-600 007, Tamil Nadu, INDIA<sup>2</sup>Department of Veterinary Anatomy, Madras Veterinary College, Chennai-600 007, Tamil Nadu, INDIA<sup>3</sup>Department of Veterinary Anatomy, Madras Veterinary College, Chennai-600 007, Tamil Nadu, INDIA<sup>4</sup>Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai-600 007, Tamil Nadu, INDIA**\*Corresponding author:** [senthilkumarvetpg@gmail.com](mailto:senthilkumarvetpg@gmail.com)

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**Abstract**

*The objective of this study was to explore the histomorphometric investigation in the mammary gland of indigenous sheep breed “Madras Red”. The present study was conducted in adult lactating and non-lactating (n =6 each) Madras Red ewes. The mammary gland in ewes was a compound tubulo-alveolar gland surrounded by a connective tissue capsule. The alveoli were the principal secretory unit lined by simple cuboidal epithelium. Well-developed alveoli, filled with secretions and lipid droplets were observed in lactating animals. The non-lactating alveoli contained obliterated lumen without lipid droplets. The connective tissue stroma was well developed in non-lactating animals. Alveolar and intralobular ducts were lined by simple cuboidal epithelium. Interlobular and large lactiferous ducts were lined by bilayered cuboidal epithelium. Significant difference was observed in the length of lobule, breadth of lobule, number of alveoli per lobule, alveoli size, alveolar luminal diameter, nucleus diameter, thickness of interalveolar septa and thickness of interlobular septa.*

**Key words:** Epithelium, Histomorphometry, Madras Red Ewe, Udder**How to cite:** Senthilkumar, S., Kannan, T., Ramesh, G., & Sumathi, D. (2020). Effect of Lactation on Mammary Gland in Madras Red Ewe - An Histomorphometric Study. International Journal of Livestock Research, 10(1), 40-47. doi: 10.5455/ijlr.20191111041245**Introduction**

Madras Red sheep (*Ovis aries*) is meat type breed, native to the Kanchipuram and Madras districts of Tamil Nadu. They have white markings or patches on the forehead, inner aspect of the thigh, abdomen, upper and lower limbs, the sides of the body, back and tails in both the sexes. These animals are active, alert, strong, long and lean bodied animal with a straight top line. Their udder is medium in size and demarcated from the body with well-defined teats (Raman *et al.*, 2003). Anatomical knowledge of the mammary gland at different stages is desirable, to understand the background information in the physiology, pathology,

surgery, medicine, livestock production and management and genetics. Since the mammary glands are very prone for traumatic injury, infection and other diseases, the basic anatomy plays a crucial role in understanding to access the damage of tissue and approach in treating and restoring the normal condition of the udder and teat (Yogita *et al.*, 2018). A histomorphometric study about healthy udder can be correlated with the abnormal lesions observed in mastitis and other health problems occurring in the udder. Hence the present study was conducted with the aim of establishing the basic data in lactating and non-lactating Madras Red ewes.

## Materials and Methods

The udder samples of adult Madras Red ewes (n=12) were collected immediately after slaughter from corporation slaughter house, Chennai. After collection, samples were directly fixed in 10 per cent neutral buffered formalin, Zenker's fluid, and Bouin's fluid. Collected tissues were processed by routine Alcohol-xylene schedule and paraffin blocks were made (Luna, 1968). Sections were cut at 5-7  $\mu$ m thickness for histological study. Haematoxylin and Eosin (H&E) method for the routine histological study was applied for staining. Microscopic images were captured using the Leica microscope (CH9345 Heerbrugg). Micrometry was done using the Leica application suite (LAS V4.4).

The micrometrical parameters such length of lobule, breadth of lobule, number of alveoli per lobule, alveolar size, alveolar luminal diameter, number of cells per alveoli, height of alveolar epithelium, epithelial height of interlobular duct, epithelial height of intralobular duct, nucleus diameter and internuclear distance were measured in mammary glands of both lactating and non-lactating groups. Collected data were subjected to statistical analysis by SPSS® 26.0 for Windows.

## Results and Discussion

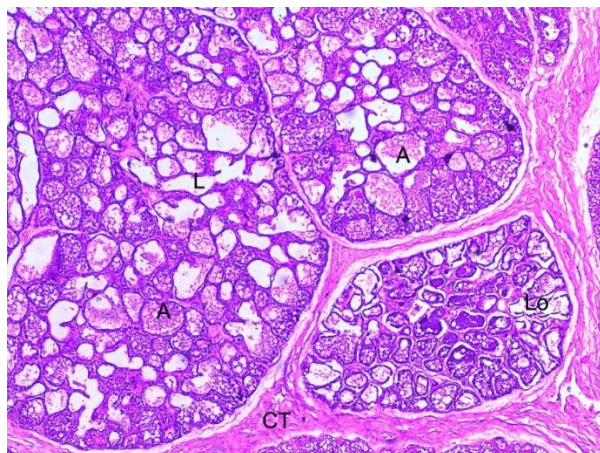
### Lobuloalveolar System

In Madras Red ewes, histological composition of mammary gland parenchyma was found to be similar. However, the difference in proportion was observed between lactating and non-lactating animals. The mammary gland was a compound tubulo-alveolar gland similar to lacrimal gland in small ruminants (Kannekanti *et al.*, 2018). Parenchyma was made up of numerous lobes and lobules consisted of tubulo-alveolar secretory units surrounded by connective tissue septa (Fig.1). Various micrometric parameters of the mammary gland of Madras Red ewes were given in Table 1. In ewes, length and breadth of the lobule ( $\mu$ m) was found to be  $1023.67 \pm 86.47$  and  $877.02 \pm 177.95$  respectively in the lactating group and  $596.77 \pm 45.07$ ,  $409.48 \pm 48.82$  in the non-lactating group. The number of alveoli per lobule were  $75.00 \pm 4.46$  and  $26.65 \pm 2.63$  in lactating and non-lactating ewes respectively.

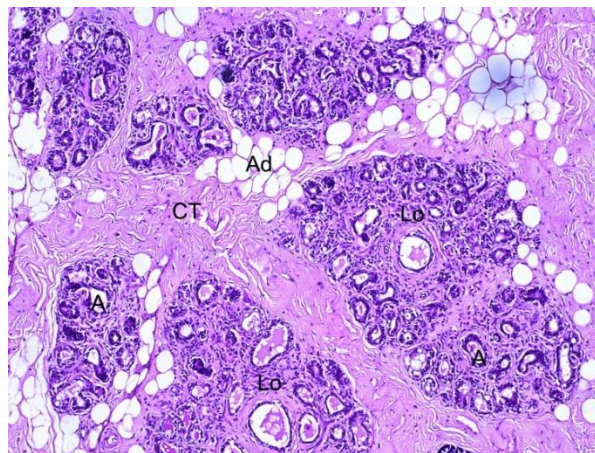
**Table 1:** Mean  $\pm$  SE of various micrometric parameters in the mammary gland of Madras Red ewes

| Parameters ( $\mu\text{m}$ )           | Mean $\pm$ SE      |                     | t value             |
|--|--------------------|---------------------|---------------------|
|  | Lactating (N=6)    | Non-lactating (N=6) |                     |
| Length of lobule                       | 1023.67 $\pm$ 86.4 | 596.77 $\pm$ 45.07  | 4.86**              |
| Breadth of lobule                      | 877.02 $\pm$ 177.9 | 409.48 $\pm$ 42.82  | 2.54**              |
| Number of alveoli per lobule           | 75.00 $\pm$ 4.46   | 26.65 $\pm$ 2.63    | 8.93**              |
| Alveolar size                          | 102.15 $\pm$ 7.31  | 58.49 $\pm$ 3.73    | 5.44**              |
| Alveolar luminal diameter              | 77.83 $\pm$ 9.36   | 38.99 $\pm$ 4.01    | 4.12**              |
| Number of cells per alveoli            | 13.90 $\pm$ 1.14   | 16.10 $\pm$ 1.34    | 1.11 <sup>NS</sup>  |
| Epithelial height of alveoli           | 11.15 $\pm$ 0.72   | 9.48 $\pm$ 1.19     | 1.24 <sup>NS</sup>  |
| Nucleus diameter                       | 4.07 $\pm$ 0.16    | 5.27 $\pm$ 0.43     | 2.57*               |
| Internuclear distance                  | 4.77 $\pm$ 0.56    | 3.35 $\pm$ 0.49     | 1.90 <sup>NS</sup>  |
| Myoepithelial cell length              | 10.09 $\pm$ 0.83   | 10.25 $\pm$ 0.59    | 0.15 <sup>NS</sup>  |
| Myoepithelial cell breadth             | 1.83 $\pm$ 0.31    | 1.89 $\pm$ 0.20     | 0.163 <sup>NS</sup> |
| Epithelial height of intralobular duct | 11.92 $\pm$ 1.60   | 9.63 $\pm$ 0.94     | 1.30 <sup>NS</sup>  |
| Epithelial height of interlobular duct | 9.45 $\pm$ 1.00    | 10.19 $\pm$ 1.40    | 0.42 <sup>NS</sup>  |

<sup>NS</sup> - No significant difference between lactating and non-lactating groups ( $P \geq 0.05$ ); \*Significant difference between lactating and non-lactating groups ( $P \leq 0.05$ ); \*\*Highly significant difference between lactating and non-lactating groups ( $P \leq 0.01$ )



**Fig. 1:** Photomicrograph of the mammary gland of adult lactating adult ewe showing alveoli (A), lobe (L), lobules (Lo) and Connective tissue stroma (CT) (H&E x 100).



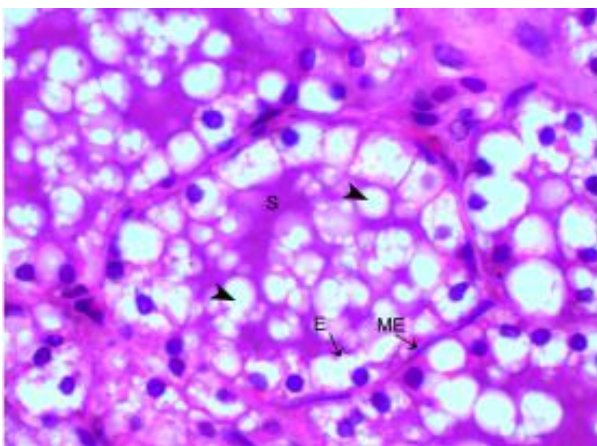
**Fig. 2:** Photomicrograph of the mammary gland of non-lactating adult ewe showing lobules (Lo), alveoli (A), adipose tissue (Ad) and connective tissue stroma (CT) (H&E x 40).

The amount and thickness of interlobular septa and adipose tissue were found to be more in non-lactating group than lactating group (Fig. 2). The arterioles were found to be more in lactating parenchyma when compared to non-lactating parenchyma. In both ewes and doe, the number of alveoli per lobule was significantly differed between lactating and non-lactating group. These findings were supported by Paramasivan *et al.* (2013) in ewes. This can be attributed to the fact that lobule size was more in lactating

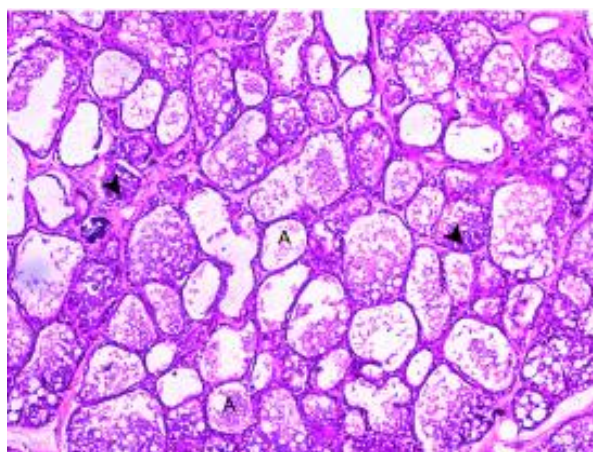
animals comprising more alveoli. Whereas, in non-lactating animals, small sized lobules had less number of degenerated and shrunken alveoli.

### Alveolar Epithelium

In Madras Red ewes, the alveoli varied from round to oval, irregularly hexagonal or pentagonal in appearance. Alveoli were lined by simple cuboidal epithelium with supranuclear vacuolation indicating the intracytoplasmic lipid accumulation (Fig. 3). Nucleus was round and intensely basophilic in lactating alveolus when compared to non-lactating alveolus. The lumen of the alveoli was filled with eosinophilic material (Fig. 4). The alveoli of different lobules appeared in different stages of activity were observed based on their cellular morphology and intraluminal contents.



**Fig. 3:** Photomicrograph of the lactating mammary gland of adult she-goat showing the presence of lipid vacuoles (arrow head), nucleus (N), myoepithelial cell (ME) and secretion (S) (H&E x 1000).

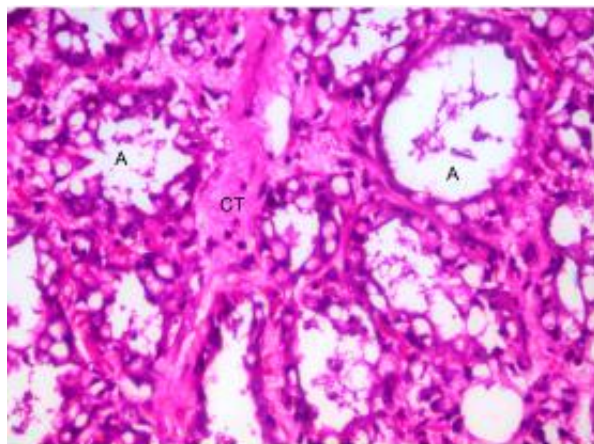


**Fig. 4:** Photomicrograph of the lactating mammary gland of adult ewe showing the lumen of alveoli (A) filled with eosinophilic material (arrow head) (H&E x 100).

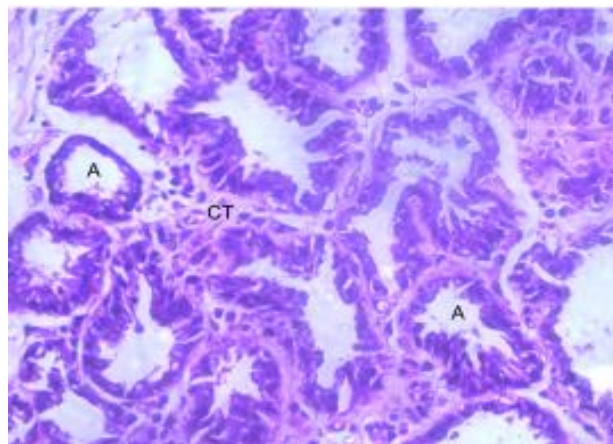
Size of alveoli ( $\mu\text{m}$ ), the diameter of alveolar lumen ( $\mu\text{m}$ ) and a number of lining epithelial cells per alveoli were  $102.15 \pm 7.31$ ,  $77.83 \pm 9.36$  and  $13.90 \pm 1.14$  respectively in lactating ewes, whereas, the respective parameters in non-lactating ewes were observed as  $58.49 \pm 3.73$ ,  $38.99 \pm 4.01$  and  $16.10 \pm 1.34$ . Highly significant difference was observed in alveolar size, alveolar luminal diameter between lactating and non-lactating groups. This is in accordance with the findings of El-Sayed *et al.* (2013) in Damascus goats. This is due to the fact that in lactating animals the alveoli were well developed and lumen filled with milk resulted in high diameter of alveoli. In the present study, number of epithelial cells per alveoli in both lactating and non-lactating groups did not differ significantly. This is in contrast to the observation made by Paramasivan (2007) in ewes. However, the present results were supported by Capuco *et al.* (1997) in cow's mammary gland that the mammary epithelial cell number did not differ between the different stages

of lactation. The author also opined that some synthetic and secretory activity was maintained even during the dry period also.

In lactating ewes, the height of alveolar epithelium ( $\mu\text{m}$ ), the diameter of its nucleus ( $\mu\text{m}$ ) and internuclear distance ( $\mu\text{m}$ ) were found to be  $9.48 \pm 1.19$ ,  $4.07 \pm 0.16$  and  $4.77 \pm 0.56$  respectively. Whereas, in non-lactating ewes, the values were  $11.15 \pm 0.72$ ,  $5.27 \pm 0.43$  and  $3.35 \pm 0.49$  respectively. In this study, a significant difference was observed in the nucleus diameter between lactating and non-lactating groups in Madras Red ewes. A similar finding was made by Paramasivan (2007) in ewes. The lesser values in nucleus diameter in lactating animals might be due to higher synthetic activity of alveolar epithelial cells during lactation. Whereas, no significant difference was observed in internuclear distance between lactating and non-lactating groups of ewes. This is in accordance with the findings made by Paramasivan *et al.* (2016) in ewes. In lactating animals, numerous lipid vacuoles were noticed both within the alveolar cells and in the lumen of the alveoli. Lipid vacuoles were varied in size from smaller to larger droplets. The smaller lipid vacuoles were found to be more towards the basal part of the cell. Whereas, the lipid vacuoles within the cytoplasm pushed the nucleus to aside (Fig. 3). The average lipid droplet diameter was found to be more in lactating ewes ( $3.79 \mu\text{m}$ ) when compared to lactating she-goats ( $3.28\mu\text{m}$ ). In non-lactating ewes the presence of intracytoplasmic lipid vacuoles was found to be absent (Fig. 5 and 6).



**Fig. 5:** Photomicrograph of the non-lactating mammary gland in adult ewe showing the non-secretory alveoli (A) and Connective tissue stroma (CT) (H&E x 400).



**Fig. 6:** Photomicrograph of the non-lactating mammary gland in adult ewe showing the non-secretory alveoli (A) and Connective tissue stroma (CT) (H&E x 400).

### Myoepithelial Cells

In the present study, both lactating and non-lactating groups showed the presence of myoepithelial cells between the lining epithelium and basement membrane of the alveoli. They were also observed in the duct system. These cells were angular or flat or spindle-shaped with the elongated or oval vesicular nucleus. The

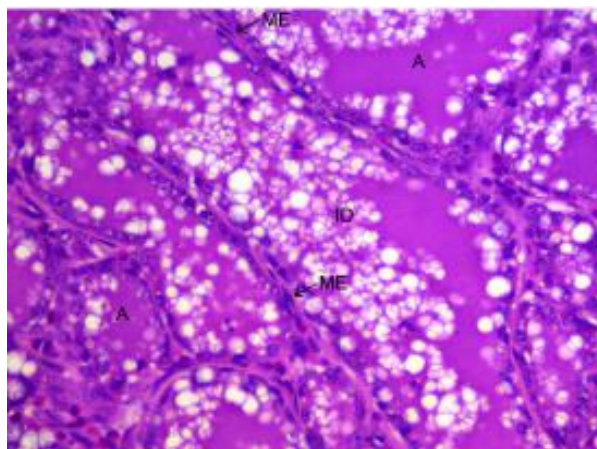
long axis of these cells was parallel to the basement membrane. A similar observation was made by (Reece, 2009) in cows. The author further opined that contraction of these cells helped in discharge of milk secretion from the alveoli and duct system. Myoepithelial cell length ( $\mu\text{m}$ ) and breadth ( $\mu\text{m}$ ) was found to be  $10.09 \pm 0.83$  and  $1.83 \pm 0.31$  respectively in lactating ewes;  $10.25 \pm 0.59$  and  $1.89 \pm 0.20$  in non-lactating ewes. In both lactating and non-lactating ewes, corpora amylacea were observed as dense bodies within the alveoli, interalveolar, interlobular and interlobar connective tissue.

### Corpora amylacea

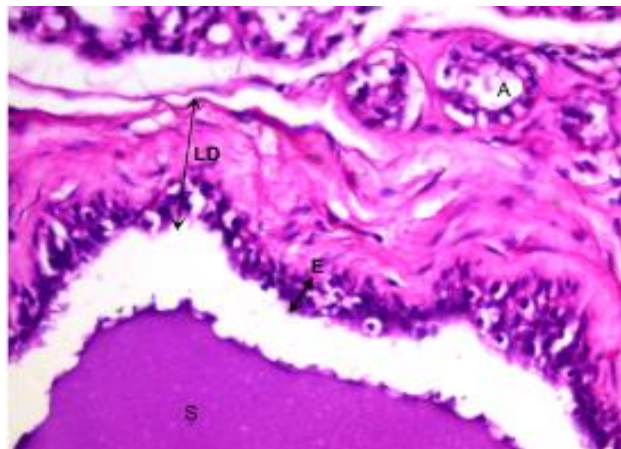
The corpora amylacea were observed as round, oval, cauliflower-shaped concentric laminated bodies with hyalinated mass at the centre. Their origin appeared to be cellular which was made of desquamated and infiltrated cells accumulated in the lumen. These desquamated epithelial cells underwent degeneration and lysis to form a solid lump-like corpora amylacea. These bodies were strongly basophilic, PAS negative and showed mild reaction for calcium. Though corpora amylacea were found in both lactating and non-lactating animals, the increased occurrence was observed in non-lactating animals, where it was found in both alveolar lumen and interstitial connective tissue. The corpora amylacea engorged the luminal spaces and clogging the small ducts leading to milk stasis and involution as reported by Nickerson *et al.* (1985) in cows.

### Duct System

In the present study, both lactating and non-lactating animals, the duct system was composed of several ducts in the following order viz., alveolar duct, intralobular duct, interlobular duct, intralobar duct, interlobar duct, and lactiferous duct which conveyed the milk from the alveoli into the gland cistern. Alveolar and intralobular ducts were lined by simple cuboidal epithelium rested upon the basement membrane (Fig. 7). Interlobular, intralobar and interlobar ducts were lined by two layers of cuboidal cells. This is in accordance with Reece (2009) in cows and Atyia (2009) in small ruminants. However, Nickel *et al.* (1981) observed that the interlobular ducts were lined by one or two layered columnar epithelium. Small dilatations were observed along the course of the ductular system. The amount of connective tissue component increased as the order of duct increases. Longitudinal smooth muscle fibres were also observed within the wall of these ducts. Myoepithelial cells were also observed in the duct wall. The lactiferous duct was the largest of the duct system which was lined by bilayered cuboidal epithelium (Fig. 8).



**Fig. 7:** Photomicrograph of the mammary gland of adult lactating ewe showing the alveoli (A), Intralobular duct (ID) and Myoepithelial cells (ME) (H&E x 400).



**Fig. 8:** Photomicrograph of the mammary gland of adult non-lactating ewe showing the alveoli (A), bilayered cuboidal epithelium (E), lactiferous duct (LD) and secretion (S) (H&E x 400).

These lactiferous ducts empty into a lactiferous sinus or gland cistern immediately proximal to the base of the teat. The gland cistern was lined by stratified cuboidal epithelium. The epithelial height of intralobular and interlobular ducts ( $\mu\text{m}$ ) was found to be  $11.92 \pm 1.60$  and  $9.45 \pm 1.00$  and  $9.63 \pm 0.94$ ,  $10.19 \pm 1.40$  respectively in lactating and non-lactating ewes. In this study, no significant difference was observed in epithelial height of intralobular and interlobular ducts between lactating and non-lactating ewes. This is in contrast to the findings of Paramasivan (2007) in ewes.

### Conclusion

Histomorphometric study revealed statistically significant difference in the length of lobule, breadth of lobule, number of alveoli per lobule, alveoli size, alveolar luminal diameter, nucleus diameter, thickness of interalveolar septa and thickness of interlobular septa between lactating and non-lactating animals. The histomorphometric study about a manually milked, meat type sheep breed such as Madras Red will be helpful in future to compare it with various stages and cycles and also with other specific sheep and goat breeds.

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