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Comparative Histo-morphometric Study of Mammary Gland in Ewes and She Goat in their Lactating and Non-lactating Stage

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ABSTRACT: The present work deals with the quantitative histological morpho-metric description of the mammary glands of ewes and she goat during their lactating and non lactating stage. During comparative analysis, significant differences was recorded during statistical analysis between the values of all parameters as, in the diameter of lobules, number of alveoli per lobule, diameter of alveoli, height and width of epithelial cells lining the alveoli and diameter of alveolar lumen in dry and lactating stage of mammary glands in both sheep as well as goat. The amount of cytoplasm decreased from lactating to non-lactating stage measured related to the mammary glands the small ruminants. during their lactating and non lactating stage There was presence of active alveoli as well as resting alveoli in lactating stage, whereas in dry stage, it was noticed that in the alveolar lumen most of the alveoli showed degenerative changes and de-squamated cells of both the small ruminants. Similarly, the nuclei of the epithelium, lining the alveoli were large and vesicular at the lactation stage whereas in non-lactating stage, nuclei were small in size, in both the small ruminants.

Keywords: Mammary gland, Ewes, She goat, Histomorpho-metry.

INTRODUCTION

Amongst the domesticated species goats were the first among the livestock species. Sheep and Goat are considered as an important source of meat, milk, skin, wool and manure. Analysis of mammary gland morphological patterns during the various stages of the reproductive cycle, facilitate the understanding of its functionality as a milk producing organ. In recent years the importance of small ruminants has grabbed tremendous attention as far as milk production is concern, hence it is essential to understand mammary gland modification pattern during lactation in the two small ruminant species, goat and sheep. The dynamic nature of the mammary gland makes it an ideal model for studying molecular regulation of development and cellular differentiation. The mammary gland, which plays a very important role by participating in the immune defense, is involved in evoking and controlling the inflammatory process, participate in bacterial opsonisation and presentation, and in recruiting leucocytes and even in direct killing of pathogens. A histo-morphometric study done over a normal udder and the findings thereof can be correlated with the abnormal lesions observed in mastitis and other health problems occurring in the udder. Data on the histo-morphometric variation in she and goat in their different physiological state is quite rare. Therefore considering the scarcity of data the present study was conducted with an aim of establishing the basic data in lactating and non-lactating ewes and she goat.

The histo-architecture of mammary gland parenchyma was almost similar in both ewes and doe. However, the difference in proportion between both of them during their different physiological stage. The mammary gland was a compound tubulo-alveolar gland. The secretory unit consisted of an alveolus and a duct surrounded by connective tissue septa (Mahdi, 2009). Mammary glands were comprised of two main tissue components: the parenchyma and the stroma. Nickerson and Akers (2011). The parenchyma contained the functional secretory and ductal tissue. The mammary stroma, or fat pad, contained the supportive tissue, including connective tissue, fibroblasts, adipose tissue, nerve tissue, and endothelial cells associated with blood vessels and lymph vessels (Dyce, 2002).

MATERIALS AND METHOD

The present work was a part of Ph.D. research. For the present study, mammary glands from 60 sheep and 60 goat were collected by categorizing as Lactating (30 each) and Non lactating stages (30 each). The samples were collected immediately after slaughter at Deonar abattoir. Chembur, Mumbai. The tissue pieces of 3-4mm thickness were collected and immediately after collection, they were washed with normal saline and fixed in 10% neutral buffered formalin (Luna, 1968). The micrometrical observations were carried out for the various components of the mammary glands such as, the 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 as per method described by Culling (1974). The micrometrical observations for the various parameters were recorded by calculating the average of 5 to 6 fields from each prepared slides these were done as mentioned by Bancroft *et al.* (2003). The staining procedure was carried out as per the methods and techniques of Singh *et al.* (1997).

RESULT AND DISCUSSION

In both the lactating sheep and goat, the mammary gland showed that, individual lobules were formed by groups of alveoli which were separated by intra-lobular connective tissue encircling them. The lobules generally appeared elongated irregularly polygonal or occasionally oval in shape. The lumen of some the alveoli were filled in with variable quantity of secretions and which were more distended due to increase in secretion as compared to empty alveoli. Alveoli were also located in the inter-lobular stroma in close proximity of duct. The alveoli were mostly round, oval or elliptical in shape and with acidophilic secretions in lactating animals. Alveoli were lined by simple Cuboidal epithelium. The lumen of the alveoli was filled with eosinophilic material. The alveoli of different lobules appeared in different stages of activity which were based on their cellular morphology and intra-luminal constituents. Nucleus was seen as round and intensely basophilic in lactating alveolus when compared to non-lactating alveolus.

Average diameter of lobule (mm). The average diameter of lobule in lactating and non-lactating sheep ranged from 0.70 to 1.70 mm and 0.10 to 0.71 mm, respectively. The average of it was 1.16 ± 0.04^{a} and 0.31 ± 0.03^{a} mm. The average diameter of lobule in lactating and non-lactating goat ranged from 0.78 to 1.90 mm and 0.11 to 0.78 mm, respectively. The average of it was 1.129 ± 0.04^{b} and 0.25 ± 0.02^{b} mm. Similar observations were recorded by Sulochana *et al.* (1981), who measured lobules in goats that were 0.5 to 1.5 mm long and 0.5 mm wide. The average values of diameter of lobules showed significant difference within the species as well has in their lactating and non-lactating stages. The amount and thickness of interlobular septa and adipose tissue were found to be more in non-lactating stage than lactating stage. Significantly high difference was observed in alveolar size, alveolar luminal diameter between lactating and non-lactating groups. These observations were similar with findings of Senthilkumar *et al.* (2020) who recorded the, size of alveoli (µm) 102.15 ± 7.31, the diameter of alveolar lumen (µm) 77.83 ± 9.36 and the number of lining epithelial cells per alveoli were 13.90 ± 1.14 in lactating ewes, whereas, the respective parameters in non-lactating ewes were observed as 58.49 ± 3.73, 38.99 ± 4.01 and 16.10 ± 1.34. Similar observations were recorded by Nripendra Singh *et al.* (2022) in Barbari goats, where they stated that it ranged from 0.10 to 0.71 mm and the average of it was 0.29 ± 0.04 mm. Bhatia and Sahai (1979) found that in lactating and non-lactating buffaloes, the average number of alveoli was 70.5 and 24.52 mm in per field of the microscope. Kausar *et al.* (2001) in dromedaries had similar observations.

Both ewes and doe, showed significantly difference in the number of alveoli per lobule, in the lactating and non-lactating group. These findings are in agreement with, Paramasivan and Geetha (2014) in ewes as well as Senthilkumar *et al.* (2020). This can be subjected to the fact that lobule size is always more in lactating animals bearing more alveoli and the is always reduction in the number of alveoli per lobule during lactation, which might be due to the greater enlargement of the alveoli and lumen as compared to dry stage. In the present study, it was found that, the number and size of alveoli per lobule decreased and the parenchyma was replaced by loose connective tissue during non-lactating stage. These findings are in corroboration with the findings of Sulochana *et al.* (1981) who measured 0.5 to 1.5 mm long and 0.5 mm wide lobules in goat. Bhatia and Sahai (1979) in buffalo recorded that the average number of alveoli per field of the microscope was 70.5 and 24.52 in lactating and non-lactating buffaloes, respectively. Kausar *et al.* (2001) recorded in dromedaries. In goat species Eitedal *et al.* (2009) reported similar finding.

Average diameter of alveoli (µm). The average diameter of alveoli in lactating and non-lactating sheep ranged from 101.30 to 132.13 µm and 22.12 to 32.42 µm respectively. The average of it was 108.32 ± 0.81^{b} and 26.75 ± 0.49^{a}. These findings corroborate with findings of Parmasivan *et al.*, (2012) who recorded in sheep mammary gland that, in lactating sheep alveoli size was 39.88 ± 1.39 µm and average of it was 106.05 ± 14.70 µm, where as it decreased significantly to 32.45 ± 1.64 µm in dry stage. In the present study, the average diameter of alveoli in lactating and non-lactating goat ranged from 108.01 to 132.01 µm and 25.01 to 38.12 µm respectively. The average of it was 117.71 ± 0.96^{d} and 33.69 ± 0.38^{c} µm. The average values of diameter of alveoli showed significant difference between sheep and goat as well as between lactating and non-lactating stages. The increased size of alveoli in lactating mammary gland must be correlated with its active secretion which fills the lumen and causes distension of the alveoli when compared with other physiological state.

Number of alveoli per lobule/cm. The number of alveoli per lobule/1^{cm} in lactating and non-lactating sheep ranged from 1167 to 1675 and 499 to 726 respectively. The average of it was 1449.10 ± 19.87^{a} and 624.47 ± 8.45^{a} per lobule/cm. Similar observations were recorded by Senthil kumar *et al.* (2019) in ewes. Also, Paramasivan and Geetha (2014) had recorded similar observations that, the number of alveoli per lobule was 254.77 ± 14.96 in pregnant ewes, which was seen to get reduced significantly to 108.27 ± 15.64 during the lactating stage. In the present study, the number of alveoli per lobule/cm in lactating and non-

lactating goat ranged from 1108 to 2315 and 510 to 860 respectively. The average of it was 1616.57 ± 76.79[b] and 64.80 ± 15.81[b] per lobule/cm. The average number of alveoli per lobule/cm showed significant difference between sheep and goat as well as between lactating and non-lactating stages. Nripendra Singh *et al.* (2022) reported similar findings in Barbari goat, who observed that, in goat it ranged from 526 to 860 and the average of it was 658.70 ± 18.39 per lobule/cm. Senthilkumar *et al.* (2020) in his study reported that, in an active lobule the number of alveoli ranged from 100 to 300, and this was totally dependent on the size of lobule and the physiological condition of the animal. The observation reported in the present study corroborates with that of Naik (2015) in mammary gland of Malnad Gidda breed of cow that the number of alveoli per lobule was 116.4 ± 5.97 µm.

Average diameter of alveolar lumen (µm). The average diameter of alveolar lumen (µm) in lactating and non-lactating sheep ranged from 0.85 to 1.45 µm and 0.11 to 1.09 µm respectively. The average of it was 0.99 ± 0.01[b] µm and 0.83 ± 0.03[a] µm. The average diameter of alveolar lumen (µm) in lactating and non-lactating goat ranged from 0.98 to 1.52 µm and 0.52 to 1.46 µm respectively. The average of alveolar lumen was 1.20 ± 0.03[c] and 0.94 ± 0.02[b] µm. The average diameter of alveolar lumen showed significant difference between sheep and goat as well as between lactating and non-lactating stages. These observations are in accordance with the findings of Nripendra Singh *et al*. (2022) done in Barbari goat, which ranged from 22.10 to 37.15 µm. The average of it was 30.34 ± 0.91, Mehta *et al.* (2013); Senthilkumar *et al.* (2020) also observed smaller diameter of alveoli which ranged from 0.414 × 103 to 1.490 × 103µm in different stages of hormonal induction. Jacobson (2000) also observed diameter of alveoli was 200 µm in domestic animals.

Height of alveolar epithelium (µm). The Height of alveolar epithelium in lactating sheep ranged from 10.11 to 15.88 µm and the average of it was 11.49 ± 0.18[b] µm. These findings corobarate with findings of Senthilkumar *et al*. (2020), who recorded that in lactating ewes, the height of alveolar epithelium was 9.48 ± 1.19(µm), the diameter of its nucleus was 4.07 ± 0.16 (µm) and the inter-nuclear distance (µm) was 4.77 ± 0.56. Where, in non-lactating sheep, the present study confirmed the height of alveolar epithelium was recorded as 3.02 to 1.10 µm and he average of it was and 5.71 ± 0.34[a] µm. These findings are in accordance with the readings taken by Senthilkumar *et al*. (2020), in non lactating ewes where in the height of alveolar epithelium (µm), the diameter of its nucleus (µm) and inter-nuclear distance (µm) the values were 11.15 ± 0.72, 5.27 ± 0.43 and 3.35 ± 0.49 respectively. The findings related with height of alveolar epithelium (µm) in lactating and non-lactating goat ranged from 14.10 to 18.1 µm and 4.02 to 10.61 µm respectively. The average of it was 16.27 ± 0.12[d] µm and 7.26 ± 0.26[c] µm. The average values of height of alveolar epithelium showed significant difference between sheep and goat as well as between lactating and non-lactating stages. Nripendra Singh *et al.* (2022) reported similar findings, where the height of alveolar epithelium (µm) in Barbari goat ranged from 3.02 to 10.60 µm and the average of it was 6.58 ± 0.42. The average height of alveolar cells was more in active gland than in less active gland. The average values of height of alveolar epithelium showed significant difference between sheep and goat as well as between lactating and non-lactating stages.

Width of alveolar epithelium (µm). The width of alveolar epithelium (µm) in lactating sheep ranged from 10.07 to 31.00 µm and the average of it was 24.64 ± 0.95[a] µm and in lactating goat 13.11 to 34.43 µm the average of it was 23.85 ± 1.18[b]. Width of alveolar epithelium in lactating stage was significantly more than that measured in non-lactating stage. Where as in non-lactating sheep the width was recorded as ranged to be from 5.23 to 11.62 µm and average of it was 8.69 ± 0.33[a] µm, where as in non-lactating goat it ranged from and 4.09 to 12.12 µm and average of it was 7.71 ±0.39[b] µm. Senthilkumar *et al.* (2019) recorded similar findings in Madras red ewes. Nripendra Singh *et al* (2022) recorded in his study conducted in Barbari goat, where he stated that, the width of alveolar epithelium (µm) in Barbari goat ranged from 4.08 to 12.12 µm and the average of it was 8.16 ± 0.51µm. The average values of width of alveolar epithelium showed significant difference between sheep and goat as well as between lactating and non-lactating stages. The present recording recorded even coraborate with findings of, Senthilkumar *et al.* (2020), wherein he recorded that the width of alveolar epithelium (µm) of lactating and non-lactating goat, ranged from 13.11 to 34.43µm and 4.09 to 12.12µm respectively. The average of it was 23.85 ± 1.18 and 7.71 ±0.39 µm.

Diameter of nuclei (µm). The diameter of nuclei in lactating sheep ranged from 0.92 to 1.13 µm and the average of it was 1.03 ± 0.01[a]. Where as in non-lactating sheep it ranged from and 0.26 to 0.78 µm and the average of it was 0.55 ± 0.02[a] µm. The findings are in agreement with the findings of, observations recorded by Paramasivan and Geetha (2014) who studied the mammary gland of Madras Red Sheep and noted that the inter-nuclear 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 present study, the diameter of nuclei in lactating and non-lactating goat ranged from 0.92 to 1.18 µm and 0.26 to 0.81 µm respectively. The average of it was 1.07 ± 0.01[b] and 0.51 ± 0.02[b] µm respectively. The average values of diameter of nuclei showed significant difference between sheep and goat as well as between lactating and non-lactating stages. These findings are in accordance with findings of Naik (2015) who studied the micrometry of mammary gland of Malnad Gidda breed of cow, where in the records mention that the nuclear diameter and inter-nuclear distance were 2.41 ± 0.07 µm and 2.98 ± 0.21 µm.

Average diameter of myoepithelial cells (µm). The average diameter of myoepithelial cells in lactating sheep ranged from 0.66 to 0.79 µm. The average of it was 0.73 ± 0.04[b] µm. Whereas the average diameter of

myoepithelial cells recorded in the present study, in lactating goat ranged from 0.69 to 0.86 µm. The average of it was 0.78 ± 0.001[a] µm. The average values of diameter of myoepithelial cells showed significant difference between sheep and goat as well as in their lactating and non-lactating stages. These findings are in accordance with the findings of Paramasivan and Geetha (2014) who recorded similar findings in Madras red Ewes.

Average diameter of intra-lobular duct (µm). The diameter of intra-lobular duct in lactating sheep ranged from 65 to 98 µm, and average of it was 81.90 ± 1.28[a] µm and in non-lactating one it was 29 to 61µm and the average of it was 43.83 ± 1.20[b] µm. The diameter of intra-lobular duct in lactating and non-lactating goat ranged from 59 to 99 µm and 33 to 66 µm respectively. The average of it was 75.37 ± 1.34[c] and 47.90 ±1.67[c] µm respectively. The average values of diameter of intra-lobular duct showed significant difference between sheep and goat as well as between lactating and non-lactating stages.

Average diameter of inter-lobular duct (µm). The average diameter of inter-lobular duct in lactating sheep ranged from 51 to 198 µm and the average of it was 156 ± 0.63[a] µm and for non-lactating sheep it was ranging between 49 to 86 µm where as the average of it was 71.43 ± 1.71[a] µm. The average diameter of inter-lobular duct in lactating and non-lactating goat ranged from 108 to 267 µm and 61 to 89 µm respectively. The average of it was 158.30 ± 5.21[b] and 71.50 ± 1.41[b] µm. The average values of diameter of inter-lobular duct showed significant difference between sheep and goat as well as between lactating and non-lactating stages. Similar observations are reported by Senthilkumar *et al*. (2020) in lactating and non-lactating goat ranged from 108 to 267 µm and 61 to 89 µm. The average of it was 158.30 ± 5.21 and 71.50 ± 1.41 µm. The findings of the present comparative study even corroborate with the findings of Nripendra Singh *et al.* (2022) who reported that in Barbari goat, the diameter of inter-lobular duct in Barbari goat ranged from 58 to 86 µm and the average of it was 72 ± 1.85 µm.

Table 1: Statistical analysis of micrometrical observations of various parameters of lactating and non-lactating mammary gland of Sheep and Goat.

Sr. No.	Parameters	Species	Stage	Minimum	Maximum	Average ± SE	CV	F' Value
1.	Average diameter of lobule (mm)	Sheep	Lactating	0000.70	0001.70	001.160 ± 0.04[a]	34.99	**012.0356***
			Non-lactating	0000.10	0000.71	000.307 ± 0.03[a]		
		Goat	Lactating	0000.78	0001.90	001.129 ± 0.04[b]		
			Non-lactating	0000.11	0000.78	000.254 ± 0.02[b]		
2.	Average diameter of alveoli (µm)	Sheep	Lactating	0101.30	0132.13	108.32 ± 0.81[b]	7.050	**2713.02***
			Non-lactating	0022.12	0032.42	026.75 ± 0.49[a]		
		Goat	Lactating	0108.01	0132.01	117.71 ± 0.96[d]		
			Non-lactating	0025.01	0038.12	033.69 ± 0.38[c]		
3.	Number of alveoli per lobule/ 1cm³	Sheep	Lactating	1167.00	1675.00	1449.10 ± 19.87[a]	23.050	**127.65***
			Non-lactating	0499.00	0726.00	624.47 ± 8.45[a]		
		Goat	Lactating	1108.00	2315.00	1616.57 ± 76.79[b]		
			Non-lactating	0510.00	0860.00	664.80 ± 15.81[b]		
4.	Average diameter of alveolar lumen (µm)	Sheep	Lactating	0000.85	0001.45	000.99 ±0.01[b]	20.664	**017.92***
			Non-lactating	0000.11	0001.09	000.83 ± 0.03[a]		
		Goat	Lactating	0000.98	0001.52	001.20 ± 0.03[c]		
			Non-lactating	0000.52	0001.46	000.94± 0.02[b]		
5.	Height of alveolar epithelium (µm)	Sheep	Lactating	0010.11	0015.88	011.49 ± 0.18[b]	15.330	**276.59***
			Non-lactating	0003.02	0001.21	005.71 ± 0.34[a]		
		Goat	Lactating	0014.21	0018.21	016.27 ± 0.12[d]		
			Non-lactating	0004.02	0010.61	007.26 ± 0.26[c]		
6.	Width of alveolar epithelium (µm)	Sheep	Lactating	0010.07	0031.00	024.64 ± 0.958[a]	29.630	**111.77***
			Non-lactating	0005.23	0011.62	008.69 ± 0.33[a]		
		Goat	Lactating	0013.11	0034.43	023.85 ± 1.18[b]		
			Non-lactating	0004.09	0012.12	007.71 ± 0.39[b]		

CONCLUSIONS

The udder in ewe and she goat composed of similar basic histological structure. The histo-architecture of mammary gland of small ruminants showed connective tissue stroma which divided the parenchyma into visible lobes and lobules. Lobules contained several alveoli with their intra-lobular duct system. Each alveolus was separated by inter-alveolar connective tissue. However, no distinct lobulations were seen in the non-lactating mammary gland. Lobes were separated inter-lobar connective tissue. Alveoli were lined by single layer of epithelium. Myo-epithelial cells were present, in between the epithelium and basement membrane. The concentration of the elastic and reticular fibers increased in lactating mammary glands which were less in goat as compared to sheep. In non-lactating stage in both goat as well as sheep the fibro-architectures of the capsule resembled to that of lactating goats as well as sheep. The elastic fibers were thicker in non-lactating goats.

A significant change was recorded in the diameter of lobules, number of alveoli per lobule, diameter of alveoli, height and width of epithelial cells lining the alveoli and diameter of alveolar lumen in dry and lactating stage of mammary gland in both sheep as well as goat. Active alveoli and resting alveoli were noticed in lactating stage, whereas in dry stage most of the alveoli showed degenerative changes and desquamated cells in the alveolar lumen. The amount of cytoplasm

decreased from lactating to non-lactating stage. The nuclei of the epithelium, lining the alveoli were large and vesicular at the lactation stage in both the small ruminants whereas in non-lactating stage, nuclei were small in size. Hence this comparative histo-morphometric 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.

FUTURE SCOPE

Small ruminant dairy products are a vital part of the economies of many countries. Sheep and Goat are fetching remarkable importance now a days, as far as human health point of view, the importance of goat's milk in human nutrition lies not only in the biological value of its nutrients but also in its characteristics of hypoallergenicity, which makes it a differentiated food so the present comparative investigations will defiantly contribute to a better description of mammary gland development during different physiological stages for these two poorly studied species. As histo-morphometric study about healthy udder can be correlated with the abnormal lesions observed in mastitis and other health problems occurring in the udder. Data on comparative studies related to producing animals is scars, so the present comparative study will be useful for further studies as well.

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