Original Article

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HISTOARCHITECTURAL STUDY OF MAMMARY ALVEOLI ON LACTATION, INVOLUTION AND PREGNANT STAGE IN MURRAH BUFFALO

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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). Most of the alveoli were spherical to oval elongated in shape. In lactating stage, only small and medium sized alveoli were present. In involuting stage due to degeneration and inactiveness only small alveoli were recorded. In non-lactating late pregnant stage most of the alveoli were large sized. A highly significant statistical difference was noted among different stages of lactation in the diameter of alveoli. The active alveoli were lined by cuboidal epithelium to low cuboidal epithelium, while resting alveoli were lined by squamous epithelium with dark nuclei. The number of resting alveoli were found increased with the advancement of lactation. Up to two months of involution most of the alveoli were degenerative. A highly significant statistical difference was noted in the height of alveolar epithelium in different stages of lactation.

Keywords: *Bubalus bubalis*, buffaloes, lactation, involution, pregnant, non-lactating, mammary, alveoli

INTODUCTION

The buffalo milk has many bio-protective factors. It contains high calcium (+92%), iron (+38%) and phosphorous (+117%) than cow milk. Further, its superior whitening property renders it more suitable for the manufacture of dried ice cream mixes, dairy whiteners, edible caseins and caseinates. The Murrah is the most important Indian breed of buffalo, and is the most efficient producer of milk, not only in the India but probably in the world (National Informatics Centre, APSC, 2008). As per animal genetics training resource (2010) the Murrah buffalo is the finest genetic material of milk producing buffalo in the world. In economic terms, buffaloes are productive and efficient, especially in those agriculture countries where there is ample manpower and comparatively

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less mechanization. In India still there is a gap between production and demand of milk. The mammary alveoli is the site of milk secretion. Histoloarchitecture of alveoli changes with lactation curve. Scant literatures were available regarding study of mammary alveoli in Murrah buffalo in different stage of lactation. To consider all these factor present study was undertaken

MATERIALS AND METHODS

The present study was conducted on mammary gland of sixty Murrah buffaloes to study the histoarchitecture of mammary alveoli. The mammary gland samples of buffalo were collected from dairy farms nearby Nagpur, Durg, Rajnandgoan and Raipur District of Maharashtra and Chhattisgarh state of India after their natural death. The samples were ensured for not having any pathological lesions. The samples were categorized into three groups as lactating, involution and pregnant stage by ascertaining the stage of lactation, dry period and pregnancy period. Lactating stage was 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. Involution stage was divided into two subgroups with six samples in each: non-lactating non-pregnant stage (up to one month), non-lactating non-pregnant stage (from one to two month). Pregnant stage was divided into three subgroups with six samples in each: non-lactating early pregnant stage, non-lactating mid pregnant stage and non-lactating late pregnant stage. For histological study the tissue samples of 3 to 5 mm thickness were collected on ice in thermocol box and brought to the laboratory. Tissue samples were fixed in 10% neutral buffered formalin, processed and 4 to 7 µm thick sections were cut with the help of rotary microtome as per method of Drury and Wallington (1980). Haematoxylin and Eosin stain as well as Toluidine blue stain were done as per Singh and Sulochana (1996). The stained sections of mammary gland were observed under Nikon Eclipse 80i microscope and measurements of various components of mammary alveoli was carried out as under and analyzed (Snedecor and Cochron, 1980).

Maximum and minimum diameter of small (maximum diameter 60 to 120 µm and minimum diameter 30 to 90 µm), medium (maximum diameter 121 to 220 µm and minimum diameter 91 to 160 µm) and large alveoli(maximum diameter 221 to 360 and minimum diameter 161 to 220 µm).

Percentage number of small, medium and large sized alveoli.

Height and width of the alveolar epithelium

Diameter of nuclei of alveolar epithelium.

RESULTS AND DISCUSSIONS

During the present study, it was noticed that each lobule was composed of many alveoli. The alveoli were lined by a single layer of epithelial cells, rested on the basement membrane (Figure 1). The myoepithelial cells were found in between the secretory epithelial cells and basement membrane. These observations of the present study are in agreement with the findings reported Patel *et al.* (2007) in buffalo, Kensinger *et al.* (1982) in pig, in goat, Cadar *et al.* (2012); Parmasivan *et al.* (2013) in Sheep and Vaish *et al.* (2015) in goat. The alveoli in the lobules were separated from each other by interalveolar connective tissue (Figure 2).

The alveoli were varying in their shape

mostly from spherical to oval elongated (Figure 1, 2, 3 and 4). However, occasionally rectangular, irregularly triangular and polygonal shaped alveoli were also noted during the present study in lactating as well as non-lactating stage. Similar observations were recorded by Weber *et al.* (1955) in lactating cow, Patel *et al.* (2007) in lactating and non-lactating buffalo. The findings recorded in the present study regarding presence of irregular folds in the alveolar wall and stretched alveolar wall were supported by Cowie and Buttle (1980) in cow. They stated that the alveolar wall was found wrinkled in freshly milked mammary gland, while the alveolar wall was found stretched in the animals, where mammary glands were filled with secretion.

On the basis of diameter, the alveoli were categorized into small, medium and large (Table 1 and 2). In lactating stage, only small and medium alveoli were recorded (Figure 1, 3, Graph 1 and Table 1). The percentage of small alveoli showed increasing trend, while medium alveoli showed decreasing trend from colostrum stage to ten month of lactation (Graph 1). In involution stage, only small alveoli were recorded, since the mammary gland remains inactive during this stage (Figure 4 and Table 2). In non-lactating, early pregnant stage, although, only small and medium alveoli were present, but mid pregnant and late pregnant stage revealed presence of large alveoli (Figure 2, Graph 1 and Table 2). This observation regarding increase in the size of alveoli towards the mid and late pregnant stage could be attributed to hypertrophy of alveoli and retention of secretory material in the lumen of alveoli under the influence of progesterone, estrogen and placental hormone.

A linear decrease in the diameter of alveoli from colostrum stage to ten months of lactation was recorded during the present study (Table 1). A highly significant statistical difference was noted among different stages of lactation in the diameter of alveoli (Table 1). In involuting animal, the diameter of alveoli was found reduced up to two month stage (Table 2). In non-lactating pregnant animal, small, medium and large alveoli showed linear increase in the diameter from mid pregnancy to late pregnancy (Table 2). A highly significant statistical difference was observed in alveolar diameter in non-lactating stage. In contrast to our present findings, Weber *et al.* (1955) recorded lower diameter in parturient cow and higher diameter in ten month lactating cow. The observations reported Jacobson (2000) in cow are in agreement with the present findings.

On the basis of microscopic structure the alveoli were identified as active, resting and degenerated alveoli. The active alveoli were lined by cuboidal epithelium up to seven months of lactation, while in ten months of lactation, the epithelial lining was cuboidal to low cuboidal (Figure 1 and 3). In involution stage, the lining epithelium of alveoli was cuboidal or low cuboidal to squamous and was less active. Throughout the gestation from early to late pregnant stage, alveoli showed cuboidal epithelium (Figure 2 and 5). The resting alveoli were recorded during three months to ten months of lactation stage. The number of resting alveoli were found increased with the advancement of lactation. The resting alveoli were lined by squamous epithelium with dark nuclei. The number of degenerative alveoli were very small and lining epithelial cells were flattened and increased up to two months involution stage. The observations of the present work regarding various types of alveoli corroborate with findings reported by Weber *et al.* (1955); Reid and Chandler (1973) in cow and Panchal and Vyas (2005); Patel *et al.* (2007) in buffalo. They reported variations in the type of epithelium during active and resting stage of lactation. The variations observed in the type of epithelium, could be attributed to the series of physiological changes occurring due to hormones in the mammary gland during lactating and non-lactating stage.

In lactating animal, the height and width of alveolar epithelium showed reducing trend from colostrum stage to ten months of lactation (Table 1). A highly significant statistical difference was noted in the height of alveolar epithelium, while there was no significant statistical difference in the width of alveolar epithelium in different stages of lactation (Table 1). The height and width of alveolar epithelium was reduced in involution stage upto two month (Table 2). However, in non-lactating pregnant animal, there was increase in height and width of epithelium throughout the pregnancy from early to late pregnant stage (Table 2). A highly significant statistical difference was recorded in the alveolar epithelial height and width in non-lactating stage (Table 2).

The observations recorded in the present study indicated that there were variations in the height and width of epithelial cells during lactating as well as in non-lactating stage. The findings of the present study regarding increase in the height and width of epithelial cells of alveoli of mammary gland in non-lactating pregnant stage were in agreement with the observations recorded by Carrol (1980); Cowie and Buttle (1980) in cow. They stated that during pregnancy, there was continuous and prolonged effect of estrogen and progesterone that stimulate the active growth of alveoli. Similarly, the decrease in the height and width of epithelium observed during colostrum to ten month of lactation period as well as in involution stage might be due to systemic and local factors. The level of systemic galactopoietic hormone responsible for active growth of mammary gland reduces with advancement of lactation as stated by Hurley (2009). Similarly, during non-lactating stage, there was increase in the local factor [transforming growth factor- ß1 (TGF- ß1)], which was responsible for reduction in epithelial growth.

The cytoplasm of epithelium was homogenous, eosinophilic and foamy in lactating stage. The foamy cytoplasm was seen mostly in apical region (Figure 1 and 2). At places, apical blebs were seen in the epithelium of alveoli during lactating stage. In involution stage, cytoplasm was less and only at some places, foamy appearance was seen up to one month (Figure 6). In involution stage from one to two month, foamy appearance was rarely seen and most of the alveoli showed a degree of degeneration in the alveolar epithelium (Figure 4). In non-lactating, pregnant stage throughout pregnancy, the cytoplasm of epithelium was mostly foamy (Figure 1 and 5). The foamy appearance of cytoplasm noted during present study could be attributed to the presence of lipid droplet in varying amount. Similar observations were recorded by Weber *et al.* (1955); Holst *et al.* (1987); Sordillo and Nickerson (1988) in cow, Cadar *et al.* (2012) in sheep and Hluchy and Bolcso (2011) in pig. In contrast, to findings of the present study in non-lactating pregnant animal, Sulochana *et al.* (1989) in sheep found fat globule in the alveolar epithelium from one hundred and twentieth day of gestation in sheep.

During the present work, it was noted that nuclei in the alveolar epithelial cells in colostrum stage were large and vesicular. The central area of nuclei was light with dark nucleoli and peripheral area (Figure 1). The shape of nuclei was spherical. The spherical shaped nuclei were present in the active alveoli during three months, five months and seven months of lactation (Figure 7). While at ten months of lactation, the active alveoli showed spherical and flat nuclei. In resting alveoli, the

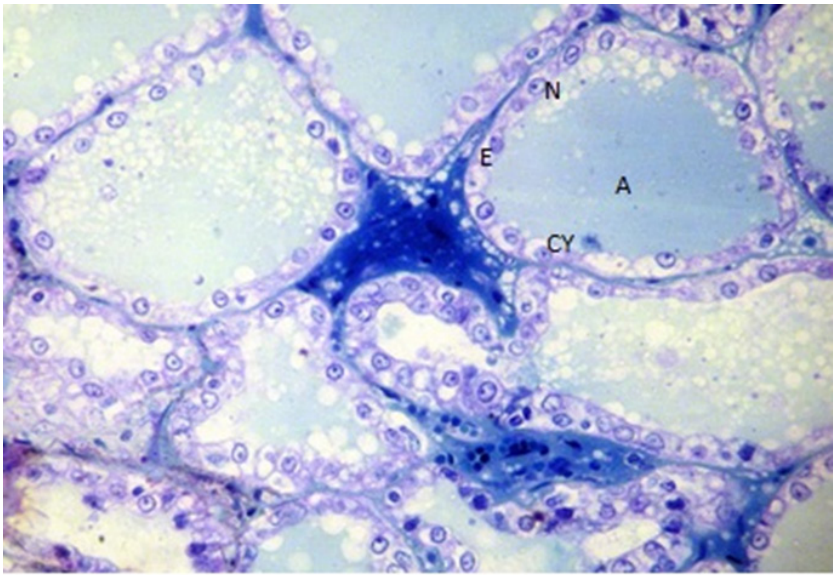

Figure 1. Photomicrograph of mammary gland of colostrum stage of lactation showing active alveoli (A) lined with cuboidal epithelium (E), foamy cytoplasm (CY) and vesicular nuclei (N). (Toluidine blue X 400).

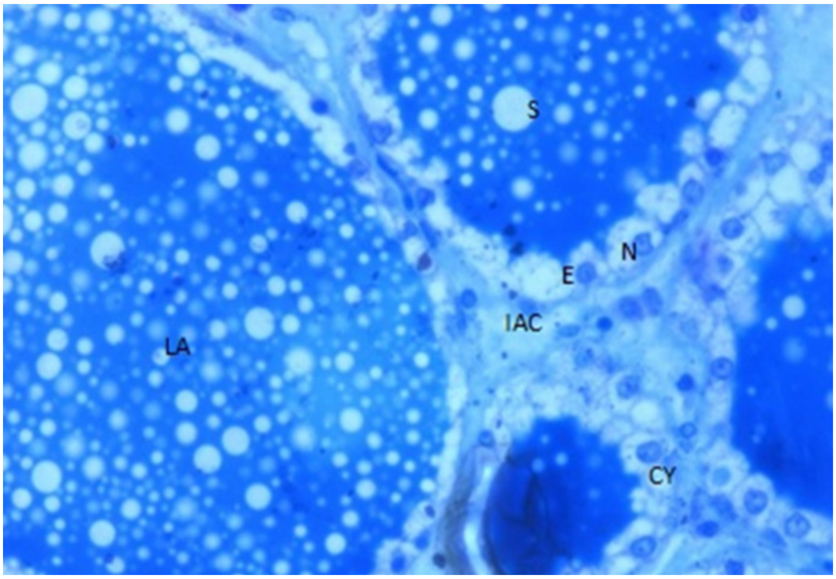

Figure 2. Photomicrograph of mammary gland of non-lactating late pregnant stage showing large sized alveoli (LA), cuboidal epithelium (E), vesicular nuclei (N) and foamy cytoplasm (CY) and interalveolar connective tissue (IAC). (Toluidine blue X 400).

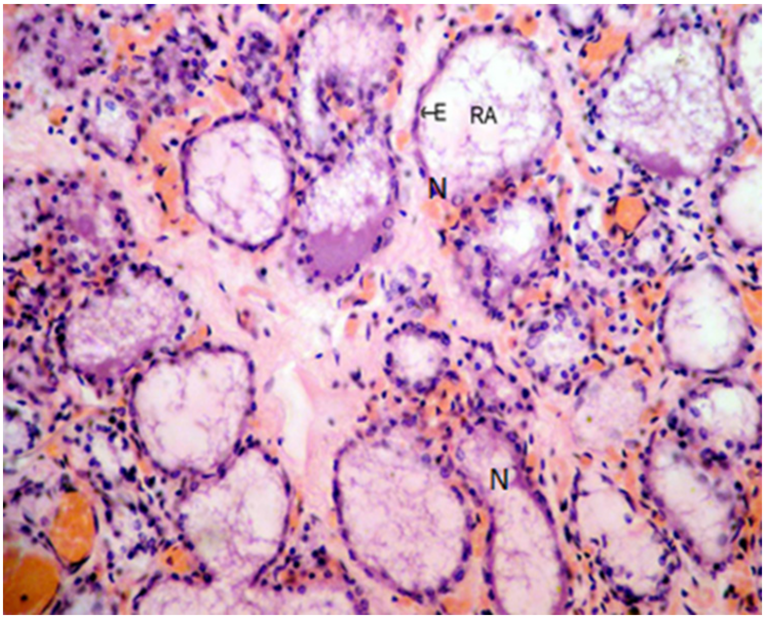

Figure 3. Photomicrograph of mammary gland of ten months of lactation showing resting alveoli (RA), epithelium (E), and nuclei (N) (Haematoxylin and Eosin X 100).

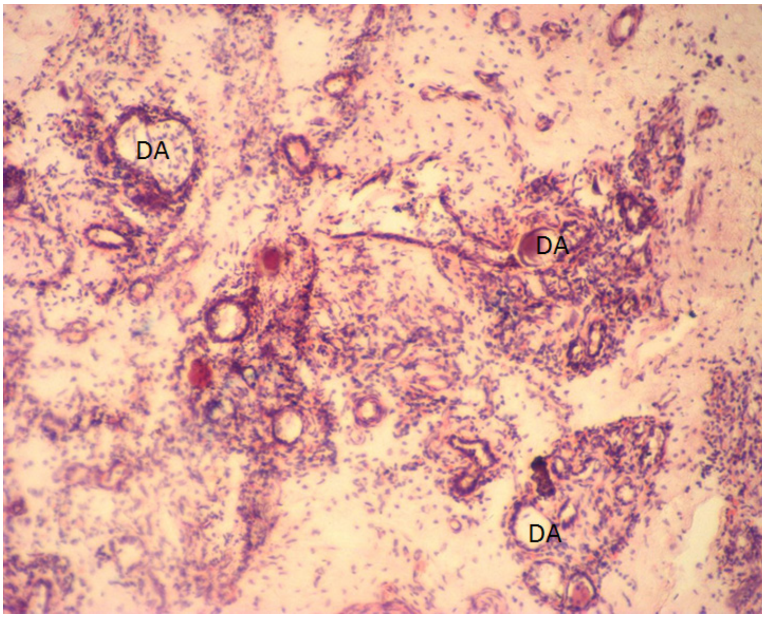

Figure 4. Photomicrograph of mammary gland of involution stage from one to two month showing small degenarative alveoli (DA). (Haematoxylin and Eosin X 100).

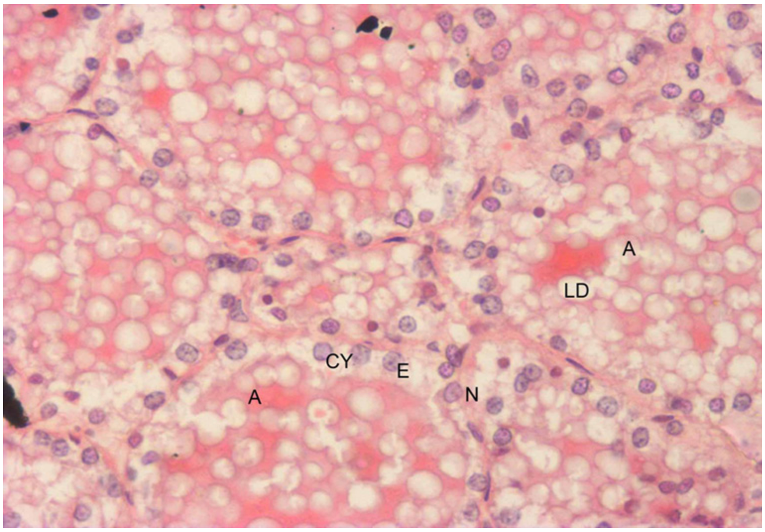

Figure 5. Photomicrograph of mammary gland of non-lactating early pregnant stage showing alveoli (A) lined with cuboidal epithelium (E), spherical vesicular nuclei (N), foamy cytoplasm (CY) and lipid droplet (LD). (Haematoxylin and Eosin X 400)

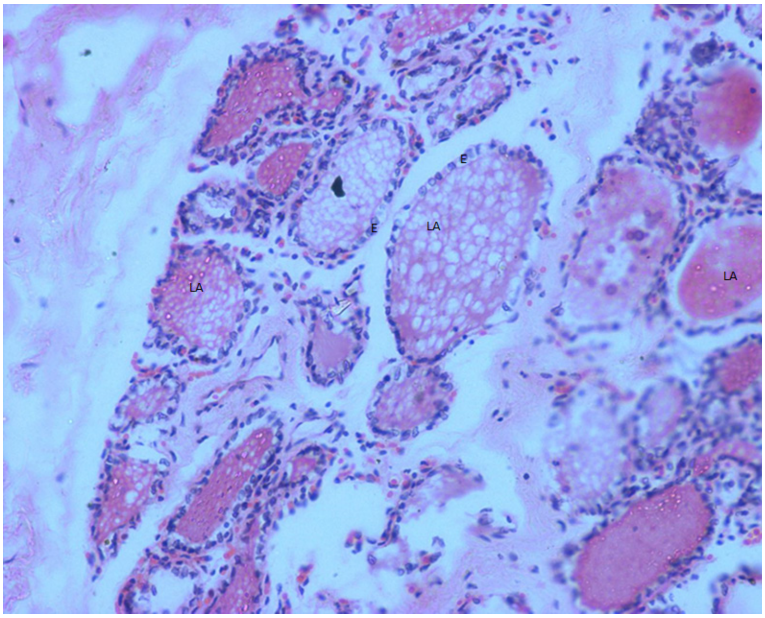

Figure 6. Photomicrograph of mammary gland of non-lactating non-pregnant up to one month stage showing less active alveoli (LA) (Haematoxylin and Eosin X 100).

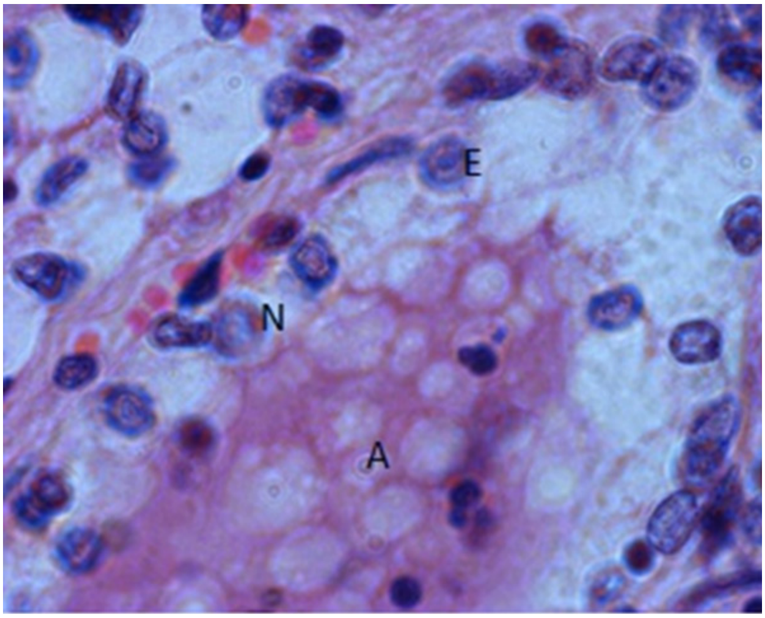

Figure 7. Photomicrograph of mammary gland of five months of lactation showing active alveoli (A) and cuboidal epithelium (E). (Haematoxylin and Eosin X 1000).

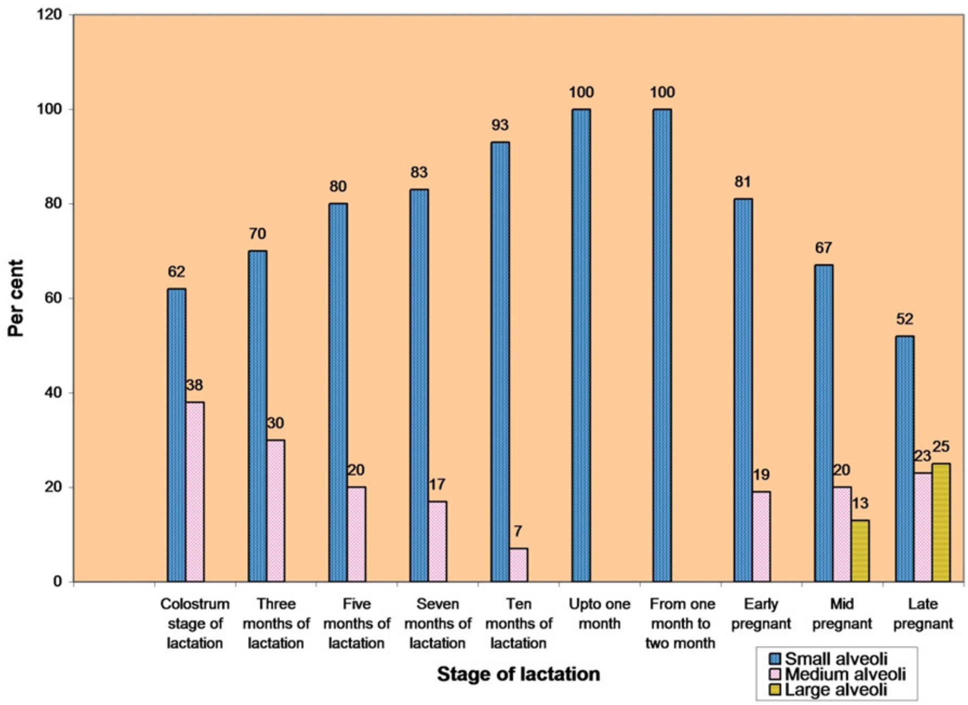

Figure 8. Percentage distribution of alveoli of mammary gland during lactating and nonlactating stage.

Table 1. Mean (± SE) values of various parameters of mammary alveoli in lactating buffalo.

Parameter	Stages of lactating buffalo					F-value
	Colostrum	3 months	5 month	7 month	10 months	
Maximum diameter of small alveoli (µm)	111.63±1.94	108.27±1.97	97.10±3.83	89.54±3.01	85.88±3.87	13.63**
Minimum diameter of small alveoli (µm)	86.80±1.48	83.88±2.75	75.90±4.03	68.60±4.33	57.56±4.99	10.07**
Average diameter of small alveoli (µm)	99.20±3.08	96.04±3.20	86.50±3.64	79.07±3.51	71.25±4.47	10.01**
Maximum diameter of medium alveoli (µm)	192.30±6.05	173.32±7.57	153.30±8.50	139.20±4.12	133.30±7.01	12.87**
Minimum diameter of medium alveoli (µm)	144.45±2.71	124.20±4.42	113.00±3.34	106.30±4.09	100.60±2.86	23.92**
Average diameter of medium alveoli (µm)	168.5±6.35	148.76±7.06	133.15±6.41	122.75±4.71	116.95±5.26	12.02**
Average diameter of alveoli (µm)	133.85±6.55	122.40±5.70	109.82±5.21	100.91±4.54	94.33±4.97	8.65**
Height of epithelium (µm)	10.45±0.65	9.62±0.95	8.07±0.5	6.90±0.88	6.70±0.83	3.84*
Width of epithelium (µm)	8.35±0.44	7.75±0.31	7.65±0.28	7.42±0.42	7.30±0.40	1.15NS
Diameter of nuclei (µm)	5.88±0.41	5.53±0.32	5.15±0.28	4.90 ±0.39	4.69±0.25	1.99NS

**Highly significant at 1% level

*Significant at 5% level

NS: Non significant

Table 2. Mean (±SE) values of various parameters of mammary alveoli in non-lactating buffalo (involution and pregnant stage).

Parameter	Involution stage		Pregnant stage			F-value
	Up to 1 month	From 1-2 month	Early pregnant	Mid pregnant	Late pregnant	
Maximum diameter of small alveoli (µm)	77.45±3.12	68.29±1.68	84.60±3.19	87.86±4.22	113.90±1.94	27.74**
Minimum diameter of small alveoli (µm)	47.13±4.21	42.70±3.32	64.63±3.98	75.65±3.03	85.29±1.48	28.95**
Average diameter of small alveoli (µm)	62.65±4.25	58.8±3.50	74.61±3.56	81.75±2.89	99.24±3.41	22.85**
Maximum diameter of medium alveoli (µm)	-	-	151.30±6.32	161.90±6.73	209.60±2.98	30.05**
Minimum diameter of mediuml alveoli (µm)	-	-	110.8±5.65	121.30±5.34	141.2±4.36	10.97**
Average diameter of medium alveoli (µm)	-	-	131.05±6.21	141.6±6.26	175.1±8.19	10.94**
Maximum diameter of large alveoli (µm)	-	-	-	255.80±6.07	321.52±7.11	49.33**
Minimum diameter of large alveoli (µm)	-	-	-	183.50±5.96	209.12±4.24	12.42**
Average diameter of large alveoli (µm)	-	-	-	219.55±9.29	265.30±7.11	7.78**
Average diameter of alveoli (µm)	62.65±4.25	58.8±3.50	102.83±5.73	147.63±8.26	179.88±10.33	88.00**
Height of epithelium (µm)	6.09±0.56	5.38±0.58	6.91±0.39	9.75±0.75	11.00±0.66	19.54**
Width of epithelium (µm)	6.40±4.20	6.08±0.48	6.60±0.45	8.39±0.73	10.36±0.97	7.58**
Diameter of nuclei (µm)	4.36±0.26	3.97±0.20	4.76±0.42	5.52±0.18	6.39±0.37	9.94**

**Highly significant at 1% level

*Significant at 5% level

nuclei were mostly elongated and flattened shaped (Figure 3). In involution stage, the nuclei were dark, small and spherical to flat in shape (Figure 4 and 6). In non-lactating pregnant stage, throughout pregnancy, nuclei were large, pale, vesicular and spherical in shape (Figure 2 and 5). Present study revealed that the nuclear diameter was reducing from colostrum stage to ten month of lactation in lactating animal (Table 1). A non-significant statistical difference (Table 1) was noted in the nuclear diameter during different stages of lactation (Table 1). The diameter of nucleus was decreasing up to two month in involuting animals (Table 2). In non-lactating pregnant animals from early to late pregnant stage, the diameter of nuclei showed increasing trend (Graph 15 and Table 2). There was no significant statistical difference in diameter of nucleus in different stages in non-lactating group (Table 2).

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