



*Original Research*

## Histopathology of Lactating, Non-Lactating and Mastitis Buffalo Mammary Gland Tissue

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### Abstract

*At the level of the mammary alveoli, copious milk production depends on the proliferation of mammary epithelial cells and the biochemical and structural differentiation of these cells after parturition. The present histological study was conducted on mammary gland tissue of buffaloes. The samples were categorized into three stages as lactating, nonlactating (involution stage) and mastitis. Stroma was found to be comprised of interalveolar, interlobular and interlobar connective tissue. The amount of stromal tissue varied during different stages of lactation. It is believed that production differences within or between dairy breeds are also determined by differences in the capacity of alveolar cells to differentiate or to maintain an adequate state of differentiation. Mastitis that lead to losses in mammary function are directly related to disruption of alveolar cell integrity, sloughing of cells, induced apoptosis, and increased appearance of poorly-differentiated cells. Thus the elevated neutrophil migration evoked to fight inflammation can inadvertently rendered alveolar epithelial cells non-secretory. A challenge to future researchers will be to devise mastitis treatments and therapies that prevent and/or repair damage to alveolar structure and maximize subsequent secretory cell differentiation for that it is necessary to understand the histomorphology of mastitis in buffalo as they has been an integral part of livestock agriculture for over the period of 5000 years and also an indispensable source of employment to the marginal farmers and landless labourers in many countries of Asia.*

**Key words:** Apoptosis, Buffalo, Lactating, Mammary Gland, Mastitis, Non-Lactating

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### Introduction

In Asia, buffalo has been an integral part of livestock agriculture for over the period of 5000 years producing animal draft power, milk, meat and hides. In the global dairy scenario, India has the distinction of being the



largest milk producing nation, which can be mainly credited to buffaloes for achieving and maintaining this unique status of the total milk production. The domestic buffalo (*Bubalus bubalis*) is a multipurpose livestock species in many countries of the world, particularly in South Asia, the Mediterranean region of Europe and South America, and is an indispensable source of employment to the marginal farmers and landless labourers. Apart from meat and skin, the production of buffalo milk in the Asia-Pacific region exceeds 45 million tonnes annually, of which over 30 million tonnes comes from India. According to one report, the world population of buffalo contributing to meat and milk productions is greater than 130 000 000 animals. High-yielding Indian buffalo breeds, such as Murrah, can yield as much as 4000 L of milk over 300 days. Buffalo, as the major livestock species for milk production, contribute significantly to the economy of many countries in Asia. Improved buffalo milk production and treatment for the production diseases like Mastitis could significantly enhance the economy and living standards of farmers in countries where buffaloes predominate.

Mastitis is inflammation of parenchyma of mammary gland characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. Mastitis is the major cause of economic loss to dairy industry globally. Annual losses in the dairy industry due to mastitis amount approximately to 2 billion dollars in USA and 526 million dollars in India (Varshney and Naresh, 2004, Sharma *et al.*, 2007, Tiwari *et al.*, 2013). Mammary gland stromal tissue undergoes dramatical histological changes in the various stage of lactation. The ratio of glandular parenchyma to the stromal tissue is one of the important parameter for selection of cattle as a milch breed. That is why histological study of the mammary gland is prerequisite.

There is paucity of detail literature of histological study of mammary gland stromal tissue in various stage of lactation, non-lactation and mastitic stage. So keeping in view the importance of buffalo in Indian economy, mammary in buffalo gland as important accessory reproductive organ as well as scanty information, present experiment was proposed on the mammary gland of buffalo to study histological changes in stromal tissue of mammary gland during lactating, non-lactating and mastitic stage. The present study was carried out to study the morphological changes associated with lactating, non-lactating and mastitis in buffalo mammary gland tissue.

### Materials and Methods

The present histological study was conducted on mammary gland tissue of Murrah buffaloes in the department of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai-400012. The mammary gland samples of buffaloes were collected from adult buffaloes immediately after slaughter from Deonar Abattoir, Mumbai. The samples were categorized into three stages as lactating, nonlactating and mastitic.

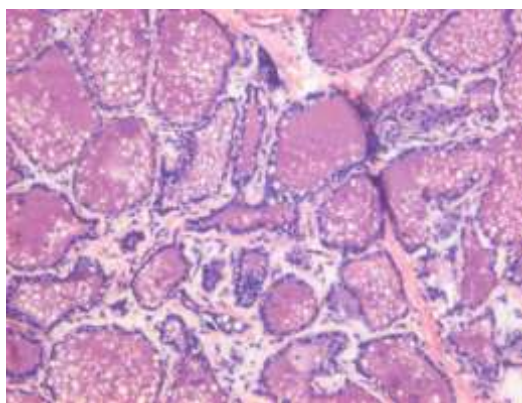
### **Histopathological Investigations**

At slaughter, the lactating, non-lactating and mastitis udder was incised quickly and mammary tissue of 3-5 mm thickness was fixed in 10% neutral buffered formalin fixative for histological studies. After fixation tissue were dehydrated in alcohol, cleared in benzene and embedded in paraffin as per the method of Drury and Wallington (1980). 3-5 micron thick sections were cut and stained with standard Haematoxylin and Eosin method (Singh and Sulochana, 1996).

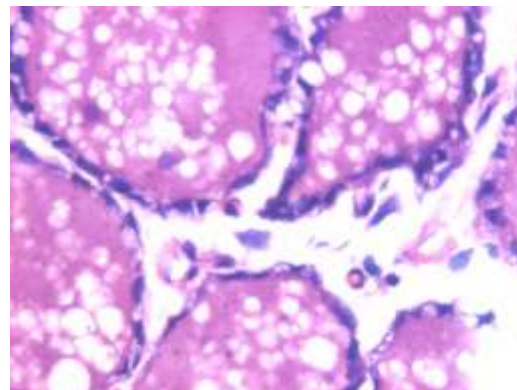
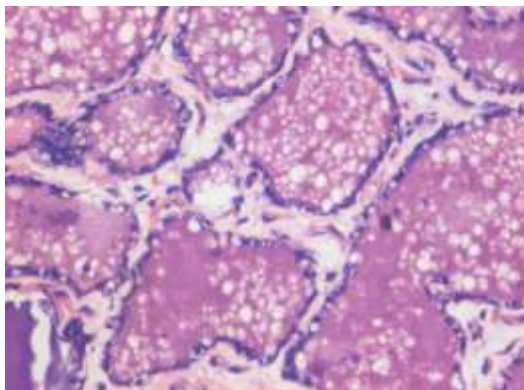
### **Results and Discussion**

During the present work, it showed that the mammary gland is composed of the stroma and parenchyma. The stroma is made up of connective tissue. The amount of connective tissue varied during different physiological stages of lactation and in disease condition. The parenchyma is composed of secretory units and the ramifying ductular system. The secretory unit of the mammary gland consists of an alveoli and alveolar ducts arising from them. Several groups of secretory units form lobules separated by connective tissue septae. The secretory tissue in the mammary gland is organized into lobules and each lobule contains about 150-220 microscopic alveoli that is also observed by Panchal and Vyas, 2005. The alveoli are sack like structure where milk is synthesized and secreted. An alveolus is the discrete milk-producing unit. A single layer of secretory epithelial cells lines the lumen of the alveolus. The epithelial lining is surrounded by contractile myo-epithelial cells.

The mammary gland of a lactating animal is a compound tubuloalveolar gland (Samuleson, 2006). Its component parts are the capsule, the interstitial framework with vessels and nerves, the parenchyma, and the lactiferous ducts. The parenchyma consist of secretory tubules with alveoli, capillary nets, and connective tissue. The stromal tissue was found to be comprised of interalveolar, interlobular and interlobar connective tissue (Fig. 1). The interlobar connective tissue was found between lobes (from Fig.1 and Fig.2).



**Fig. 1:** Tissue section of lactating mammary tissue 10X

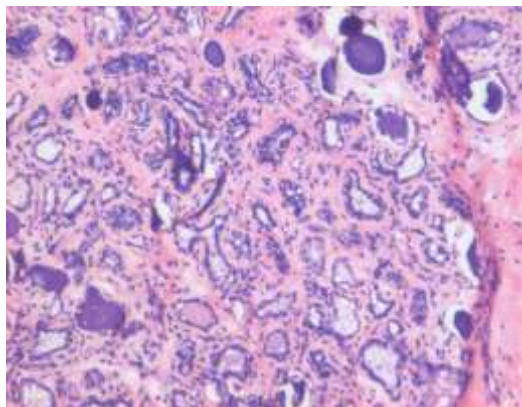


**Fig. 2:** Tissue section of lactating mammary tissue 20X. a. Active alveoli with milk secretion, b. Interalveolar connective tissue, c. alveolar myoepithelial cells surrounding the alveoli

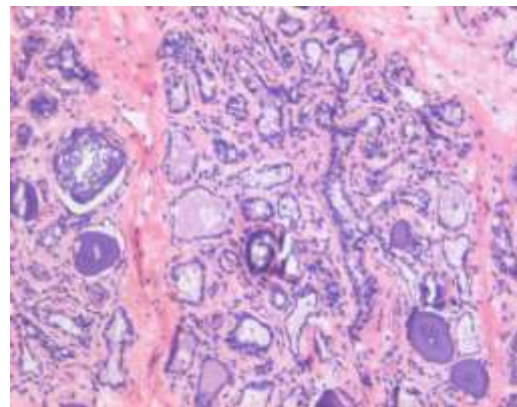
**Fig. 2:** Tissue section of lactating mammary tissue 40X showing alveolar myoepithelial cells surrounding the alveoli

Tissue section of lactating mammary tissue showed active alveoli with milk secretion, interalveolar connective tissue (Fig. 2), alveolar myoepithelial cells surrounding the alveoli (Fig. 3). This was in accordance to the Chaurasia *et al.* (2016) who have observed the same structure during lactating stage in buffalo. The alveoli were lined by simple cuboidal epithelium. The similar lining epithelium was observed in the sheep (Paramsivan *et al.*, 2013). Paul (2013) reported similar lining epithelium in desi cow. Dellman, (1993) observed alveoli were lined by a simple cuboidal epithelium that varied markedly in height during various stages of secretory activity. The shapes of the lactating gland varied form oval, oblong or elliptical in shape, which is in accordance with Mehta *et al.* (2013).

In the resting phase the mammary gland showed branched tubules with small alveoli, which are line by nonglandular epithelium. Numerous leucocytes appeared in the alveolar lumen. The interstitial connective tissue proliferates and fills in the spaces that have been formed by the reduction of the alveoli. During histomorphological study (Microstructure) of nonlactating/resting buffalo mammary tissue structure was compatible with the observations by Croom *et al.* (1976) in non-lactating mammary tissue of cattle (Fig. 3 and 4). In almost all randomly selected microscopic fields, it was observed that stroma was in abundance with scattered and restricted parenchyma. Decrease of stroma and development of parenchyma with more developed epithelial cell area is evidence of proliferation of alveolar cells in the mammary tissue. Fleming *et al.* (1986) and Croom *et al.* (1976) also reported similar changes where they stated decrease in fat pad, increase in parenchyma of mammary tissue and few epithelial cells.



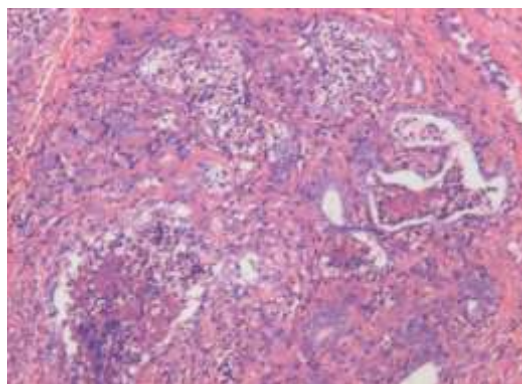
**Fig. 3:** Tissue section of resting mammary tissue 10X. Showing Inactive alveoli with small size characterized by non-secretory alveolar epithelium



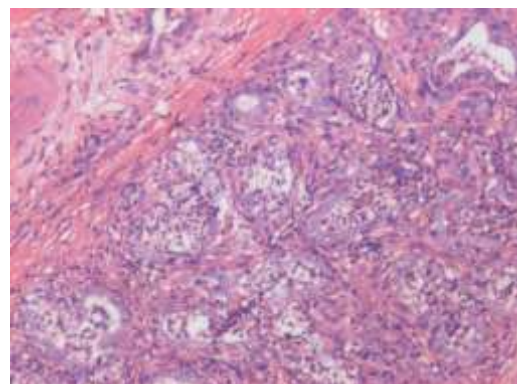
**Fig. 4:** Tissue section of resting mammary tissue 20X Abundant interlobular loose connective tissue and degenerating alveoli

Approximately there was two to three fold increase in amount of interlobular connective tissue in the nonlactating stage. These findings were in agreement with the findings of Bloom and Fawcett (1975). They opined that after few days of milk cessation, the secretion that remains in alveoli and duct, get absorbed and increases in the activity of lysosomal enzyme leading to degeneration of epithelium. This desquamation of epithelium gradually leads to collapse of alveoli and get associated with increase in stromal tissue. The findings of the present study reflect on the active glandular dynamism in the terms of alteration in the glandular complex under the influence of pituitary and gonadal hormones in tune with the physiological demands and status of the animal health.

In the present work the histomorphological studies of sections from mastitic buffalo showed mild to moderate or severe atrophy of alveoli. The lumen of the alveoli was fully filled by the cellular exudate in udder tissue in varying amounts in a number of cases (Fig. 5). Infected mammary parenchymal tissues showed destruction of alveoli and severe fibrous tissue proliferation (Fig. 6).

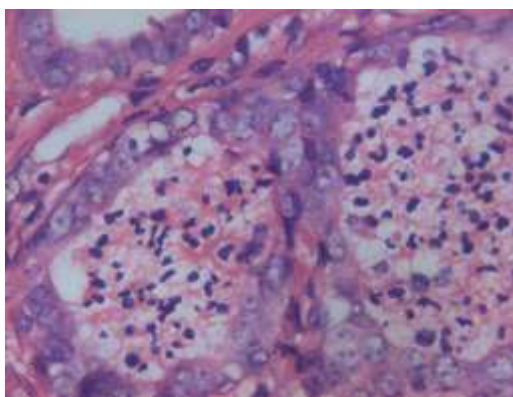


**Fig. 5:** Tissue section of Mastitic mammary tissue 10X showing cellular exudates in the lumen of the alveoli



**Fig. 6:** Tissue section of Mastitic mammary tissue 20X characterized by irregular epithelium, some of which has degenerated and sloughed into the alveolar lumen that contains some cellular debris

Mastitic udder showed significantly lower alveolar epithelial cell population, alveolar luminal diameter and number of alveoli per plate also there was irregular shape of alveoli. These changes appeared more advanced at latter stage, glandular parenchyma was losing its secretory potential. Involved parenchyma displayed reduced secretory activity, diminished luminal areas, and abundant connective tissue. The histopathological changes including number of alveoli, alveolar diameter and secretory alveolar cell population significantly decreased in mastitic animals. These results indicated pathological changes occurring in udder tissue and could be due to severe tissue damage due to different mastitis pathogens. The intensity of cellular exudates varies with the different degree of infection by pathogen which is in same accordance with Hussain *et al.* (2012) in histomorphology of mastitis cattle udder tissue.



**Fig. 7:** Tissue section of Mastitic mammary tissue 40X showing cellular exudates in the lumen of the alveoli and leukocytes and macrophages are observed in the lumen

Much of the luminal areas were filled with cellular debris and leukocytes. This migration of leukocytes, across the alveolar epithelial lining may be the cause to mechanical and/or chemical damage to the milk secretory cells as well as to the ductal cells losing its secretory activity. This is in accordance with Harmon and Heald (1982) one of the first who did *in vivo* investigations to study leukocyte migration in mammary tissues.

### Conclusion

From the present study it is concluded that during lactating stage the secretory tissue in the mammary gland is organized into lobules and each lobule contains about 150-220 microscopic alveoli that secretes milk. There is increase in the alveoli numbers, its diameter and size with oval to elliptical in shape during lactating stage to accommodate more amount of milk. There was abundant interalveolar connective tissue which provides supporting framework to the secretory alveoli, while in resting/nonlactating stage this tissue was going in involution stage towards non secretory activities of alveoli like reduced size and decreased numbers of alveoli in the lumen and few days of milk cessation. The secretion that remained in alveoli and

duct, gets absorbed and increases the activity of lysosomal enzyme leading to degeneration of epithelium. This desquamation of epithelium gradually leads to collapse of alveoli and thus increased the stromal tissue. During this two stages, lactating and nonlactation/resting the tissue does not showed any pathological lesions but during mastitis infection of the mammary tissue of buffalo showed severe to moderate degree of atrophy of secretory alveoli, the lumen was filled by the cellular exudates, the intensity of cellular exudates varied with the severity of infection by pathogens. Parenchyma showed destruction of alveoli and severe fibrous tissue proliferation with increase in lueocyte migration towards secretory alveoli epithelium causing damage to the milk secretory cells as well as to the ductal cells loosing its secretory activity.

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### Conflict of Interest

The authors declare that there will be no potential conflicts of any interest.

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