

HISTOLOGICAL STUDY ON STROMAL TISSUE IN MAMMARY GLAND AT LACTATING, INVOLUTION AND PREGNANT STAGE IN MURRAH BUFFALO

**D. Chaurasia^{1,*}, R.S. Dalvi², S.B. Banubakode², N.C. Nandeshwar², R. Churchan²,
S.P. Ingole¹ and B. Sinha¹**

ABSTRACT

The present histological study was conducted on mammary gland tissue of sixty Murrah buffaloes. The samples were categorized into three stages as lactating, nonlactating nonpregnant (involution stage) and nonlactating pregnant by ascertaining the stage of lactation, dry period and pregnancy period. Stroma was found to be comprised of interalveolar, interlobular and interlobar connective tissue. The amount of stromal tissue varied during different stages of lactation. In late pregnant and colostrum stage the interalveolar connective was minimum and alveoli were almost touching each other. The amount of stromal tissue increased from colostrum stage to ten months of lactation. It was maximum in nonlactating nonpregnant stage from one to two months (later stage of involution). Through stromal tissue blood and lymph vessels and nerve goes into the parenchymal tissue.

Keywords: mammary gland, stromal tissue, collagen fibers, Murrah buffalo

INTRODUCTION

Mammary gland stromal tissue undergoes dramatical histological changes in the various stage of lactation under the hormonal influences. The ratio of glandular parenchyma to the stromal tissue is one of the important parameter for selection of cattle as a milch breed. That's why histological study of the mammary gland is pre requisite. The name "Black Gold" has emerged as synonym for the one very popular breed of buffaloes i.e. Murrah, which serves as capital reserve or cash crops to rural folk by producing economic stability, livelihood security and social status (Balbhadra, 2013). During the past one year, a United States dairy firm had purchased Murrah buffaloes, each yielding over 25 kg milk a day, at a cost of Rs 2.5 lakh each from Haryana. This shows that rich countries will soon switch over to Murrah husbandry (Sing, 2013). There is paucity of detail literature of histological study of mammary gland stromal tissue in various stage of lactation in buffalo. So keeping in view the importance of Murrah buffalo in Indian economy, mammary

¹Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Chhattishgarh Kamdhenu Viswavidyalaya, Anjora, Durg, Chhattishgarh, India, *E-mail: durgavet2010@gmail.com

²Department of Veterinary Anatomy and Histology, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

gland as important accessory reproductive organ as well as scanty information, present experiment was proposed on the mammary gland of Murrah buffalo to study histological changes in stromal tissue of mammary gland.

MATERIALS AND METHODS

The present histological study was conducted on mammary gland tissue of sixty Murrah buffaloes. The mammary gland samples of buffaloes were collected from dairy farms nearby Nagpur in Maharashtra and Durg, Rajnandgoan and Raipur District of Chhattisgarh after their natural death. The samples were ensured for not having any pathological lesions. The samples were categorized into three stages as lactating, nonlactating nonpregnant and nonlactating pregnant by the stage of lactation, dry period and pregnancy period. Lactating stage was further categorized in five groups as: colostrum stage/ phase, three months of lactation, five months of lactation, seven months of lactation and ten months of lactation. Nonlactating nonpregnant stage was categorized in two groups as: Upto one month and from one to two month. Nonlactating pregnant stage was categorized into three stage as early pregnant stage, mid pregnant stage and late pregnant stage.

Mammary tissue of 3-5 mm thickness was fixed in 10% neutral buffered formalin fixative for histological and histochemical studies. After fixation tissue were dehydrated in alcohol, cleared in benzene and embedded in paraffin as per the method of Drury and Wallington (1980). Three to five micron thick sections were cut and stained in Haematoxylin and Eosin, Van Gieson's, Gordon and Sweets, Orcein and Masson's Trichrome stain for histological structure, collagen fibers, reticular

fibers, elastic fibers and muscle fibers respectively as per the method of Bancroft and Cook (1994).

RESULTS AND DISCUSSION

During the present work, the stromal tissue was found to be comprised of interalveolar, interlobular and interlobar connective tissue (Figure 1). The amount of interalveolar connective tissue varied during different stages of lactation. The interalveolar connective tissue was dense and mainly composed of collagen fibers. Blood capillaries were predominantly seen in lactating stage in interalveolar connective tissue (Figure 2). In colostrum stage, the interalveolar connective tissue was very scanty and alveolus was almost touching to each other (Figure 2). From colostrum stage onwards, the amount of interalveolar connective tissue was increased with the advancement of lactation upto ten month (Figure 2 and 3). Comparatively, more amount of interalveolar connective tissue was observed in between resting alveoli than active alveoli (Figure 2 and 4). In nonlactating nonpregnant animals, more amount of interalveolar tissue was found (Figure 1). In the nonlactating pregnant stage, the interalveolar connective tissue was seen throughout the pregnancy (Figure 5). Intalobular duct, blood and lymph vessels and nerve were present in interalveolar connective tissue (Figure 6). These finding were in agreement with the Trautmann and Fiebtger (2002) and Riviere (2007) in domestic animals, Sordillo and Nickerson (1988) and Bragulla and Konig (2004) in cow, Sulochana *et al.* (1989) in sheep, Parmar *et al.* (1985) in goat and Nosier (1973) in camel. The blood capillaries in the interalveolar connective tissue observed in mammary gland during lactating stage could be

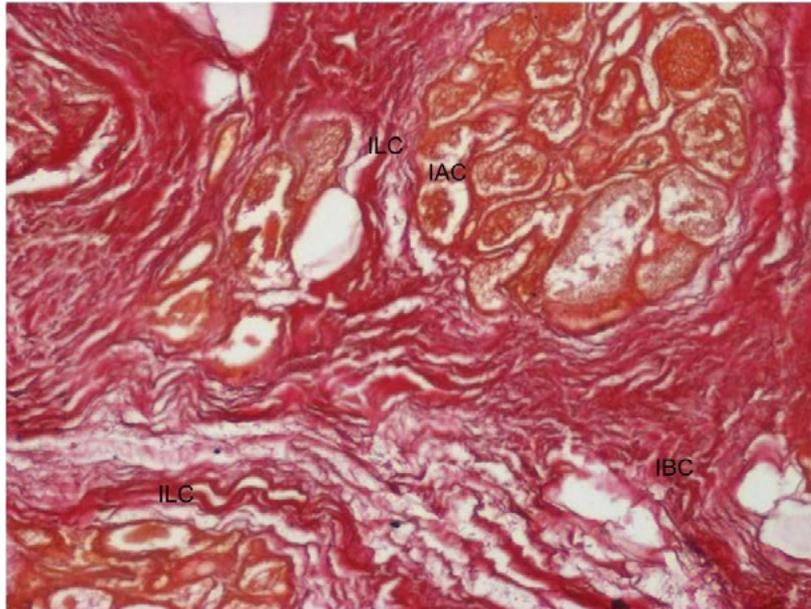


Figure 1. Photomicrograph of mammary gland of nonlactating nonpregnant upto one month stage showing interalveolar connective tissue (IAC), interlobular connective tissue (ILC) and interlobar connective tissue (IBC).

(Van Gieson's X 100)

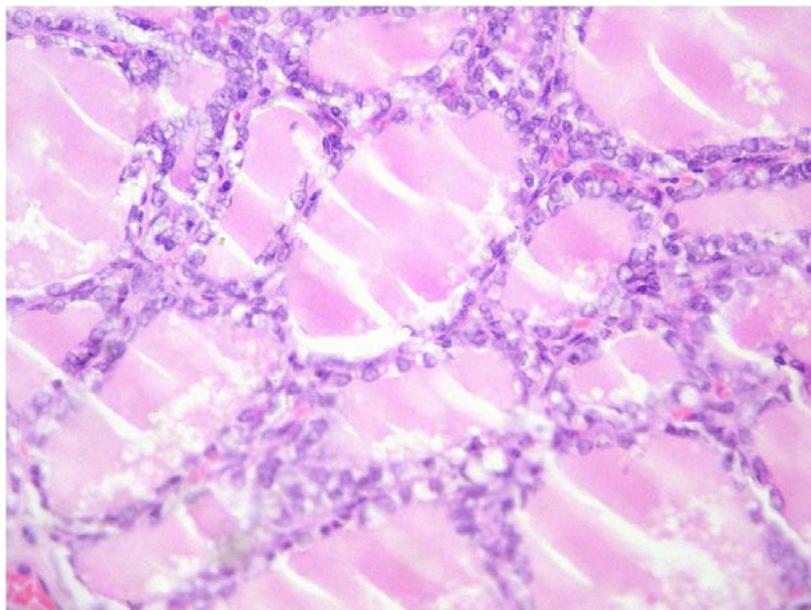


Figure 2. Photomicrograph of mammary gland of colostrum stage of lactation showing active alveoli (A), interalveolar connective tissue (IAC) and blood capillaries (BC).

(Haematoxylin and Eosin X 400)

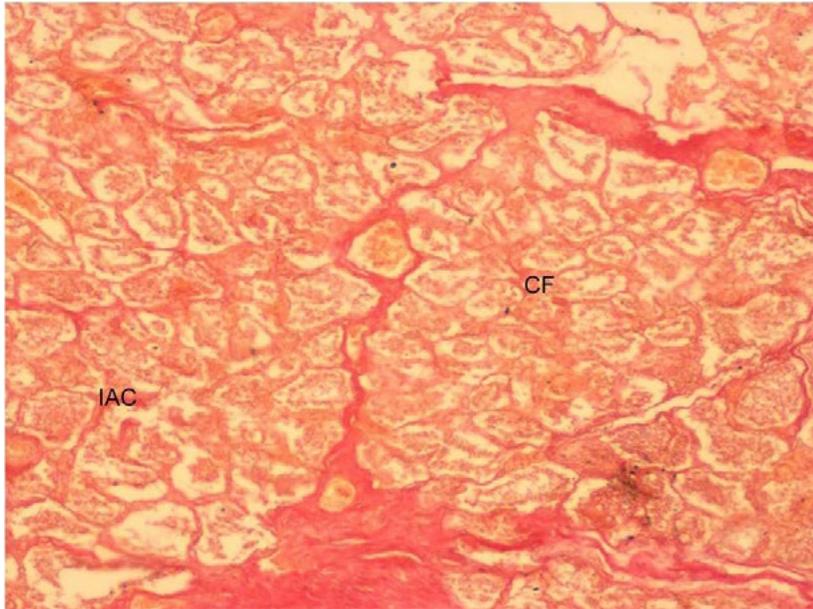


Figure 3. Photomicrograph of mammary gland of ten months of lactation showing collagen fibers (CF) in interalveolar connective tissue (IAC), and interlobular connective tissue (ILC).

(Van Gieson's X 100)

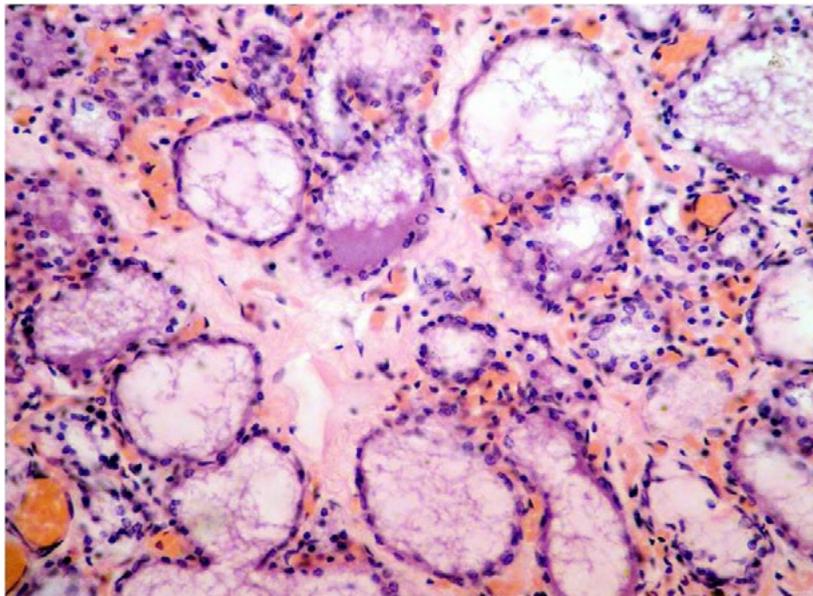


Figure 4. Photomicrograph of mammary gland of ten months of lactation showing resting alveoli (RA) and interalveolar connective tissue (IAC).

(Haematoxylin and Eosin X 400).

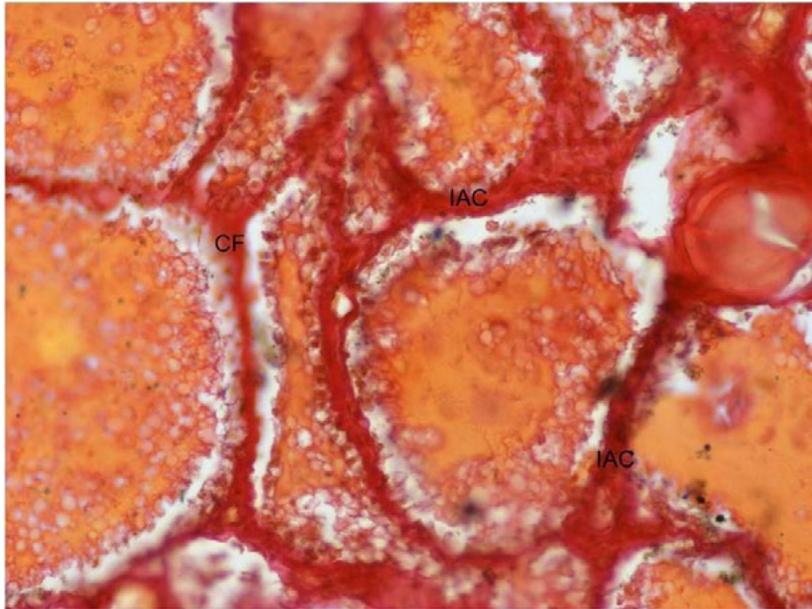


Figure 5. Photomicrograph of mammary gland of nonlactating late pregnant stage showing collagen fibers (CF) in interalveolar connective tissue (IAC).

(Van Gieson's X 400)

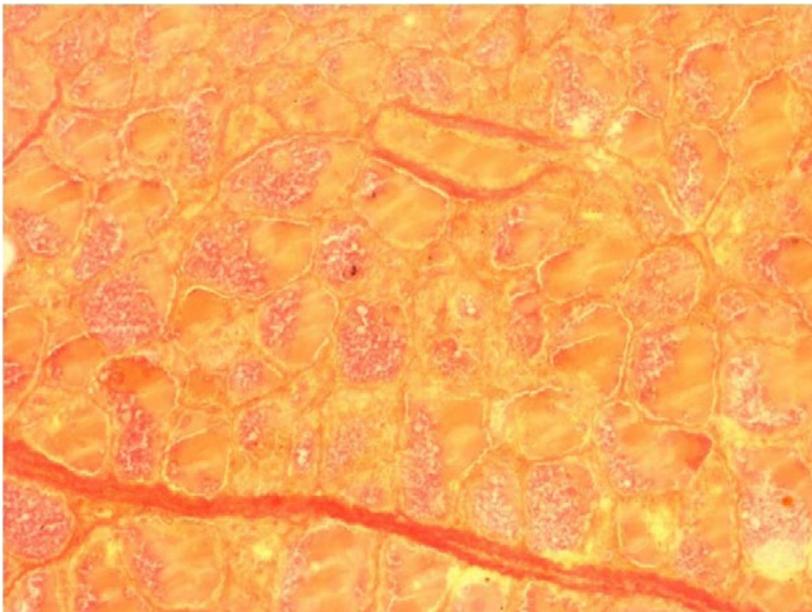


Figure 6. Photomicrograph of mammary gland of colostrum stage of lactation showing interalveolar connective tissue (IAC), interlobular connective tissue (ILC) and interlobular duct (D).

(Van Gieson's X 100)

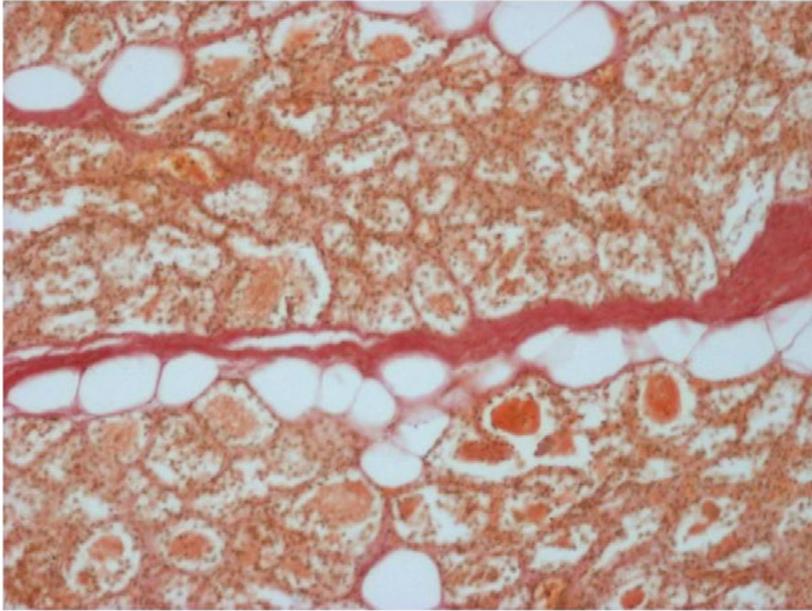


Figure 7. Photomicrograph of mammary gland of five months of lactation showing fat cells (FC) in interlobular connective tissue (ILC).

(Van Gieson's X 100)

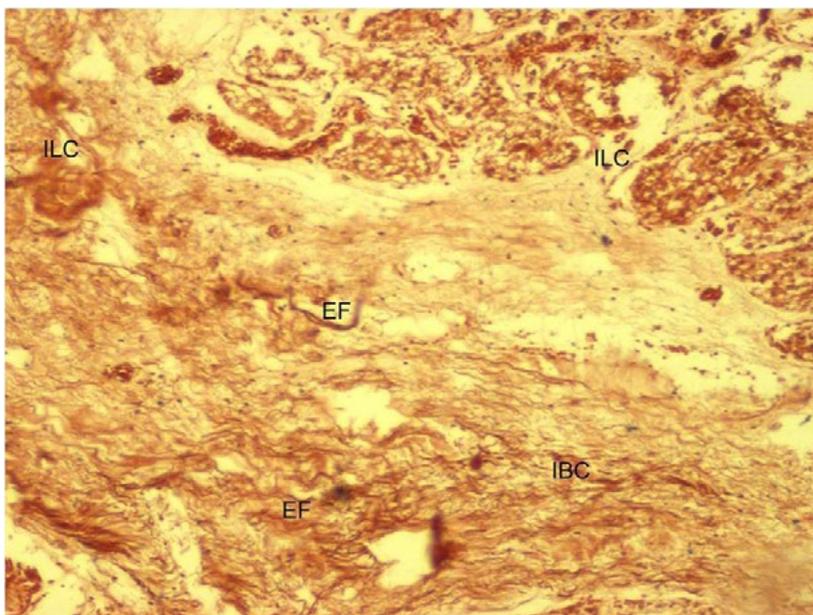


Figure 8 Photomicrograph of mammary gland of nonlactating nonpregnant one to two month stage showing elastic fibers (EF) in interlobular connective tissue (ILC) and interlobar connective tissue (IBC).

(Orcein X 100)

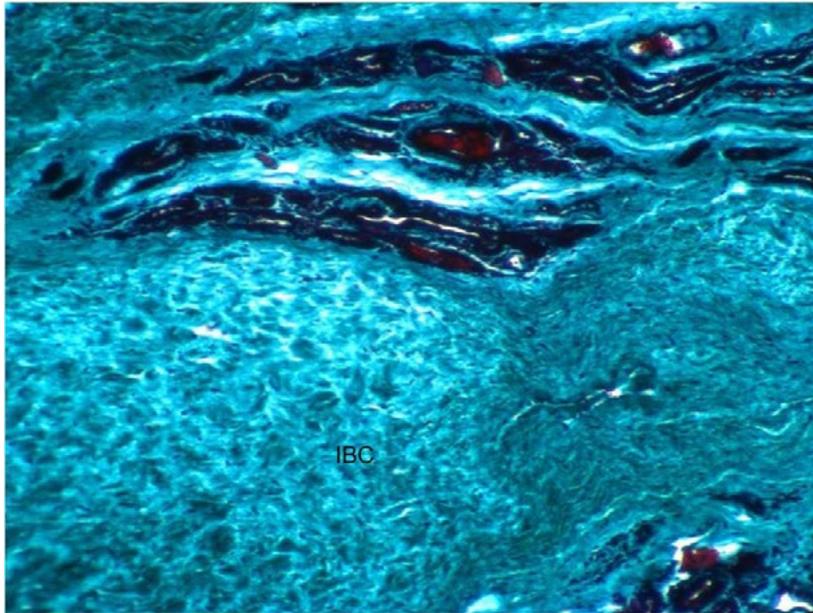


Figure 9. Photomicrograph of mammary gland of nonlactating nonpregnant one to two month stage showing interlobar connective tissue (IBC).

(Masson 's Trichrome X 100)

attributed to the higher blood supply demanded by the mammary tissue for the synthesis of milk.

Mammary parenchyma was divided into lobules by the bundles of thick dense connective tissue fibers. These fibers bundles were interlobular connective tissue present in the form of septae. Interlobular artery and vein, lymph vessels, nerves and interlobular ducts were present in the interlobular connective tissue. At places, few fat cells were seen in the interlobular connective tissue. However, in some places, fat cells were found to predominate the other connective tissue elements (Figure 7). This was in accordance to the Chaurasia *et al.* (2012) they found fully developed stromal tissue formed chiefly of the massive fat pad in prenatal period. The most abundant fibers were collagen fibers (Figure 1 and 3). The amount of collagen fibers were increased apparently with advancement of lactation from colostrum stage

to ten months of lactation. Five to eight times increased were noticed in the amount of interlobular connective tissue from colostrum stage of lactation to involution stage (Figure 1 and 6). Elastic fibers were observed in interlobular connective tissue in lactating and nonlactating stages in buffalo (Figure 8). The amount of elastic fibers was comparatively more in the nonlactating nonpregnant stage. However, present study did not show reticular fibers in the lactating and nonlactating stages in buffalo. In early pregnant stage lobulations were not distinct.

In lactating and late pregnant stage the lobes were not seen in tissue section because of large size of alveoli and lobule. The interlobar connective tissue was found between lobes (Figure 1 and 9). The interlobar connective tissue collagen fibers were more compact and dense than interalveolar and interlobular connective.

It was found that the amount of interlobar connective tissue was increased after weaning during involution stage. Approximately there was two to three fold increase in amount of interlobar connective from initial one months of involution to later period of involution (from one to two months). 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.

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