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RESEARCH ARTICLE

HISTOLOGICAL OBSERVATIONS ON THE CAPSULE AND CONNECTIVE TISSUE STROMA OF MAMMARY GLAND IN MADRAS RED SHEEP (*OVIS ARIES*)

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ABSTRACT

The mammary gland in Madras red sheep was covered from outside to inside by the skin and fibroelastic capsule. The connective tissue septa branched off from capsule and grown deeper and penetrated the adipose tissue which was evident in the mammary glands of prepubertal and pubertal animals. The concentration of the elastic and reticular fibres increased in pregnant and lactating mammary glands. The adipose tissue was abundant and surrounded the small groups of ducts in prepubertal and pubertal mammary glands. In the pregnant and lactating sheep, the connective tissue became reduced and lobuloalveolar tissue was predominantly increased. The relative proportion of the interlobular tissue increased at the pubertal period but decreased during pregnant and lactating mammary glands, due to the increase in size of the glandular alveoli. The decrease in the interlobular connective tissue was due to the growth of the parenchymatous elements in these age groups of animals. In non-lactating dry animals the mammary gland showed increased amount of connective tissue elements and corpora amylacea due to regression effect.

INTRODUCTION

The mammary gland development during all stages is influenced by some factors, which certainly are the endocrine hormones that interact with the different growth factors and the epithelial and mesenchymal constituents. The final stage involves the functional differentiation process during pregnancy-associated lobuloalveolar development, followed by involution when nursing or milking ceases. Earlier studies on growth and development of mammary gland have been centered only on pregnant and lactating animals. Reports are available on histological and histochemical features of bovine and buffalo mammary glands (Uppal *et al.,* 1994). The literature on the histomorphology of the mammary gland during prepubertal, pubertal and dry animals is supposedly limited. Therefore, the present study was focused to record the microanatomy of connective tissue stroma of mammary glands during different age groups of Madras red sheep.

MATERIALS AND METHODS

A total of 30 Madras red ewes of different age groups were included in the current study. The ewes used were divided into five age groups viz. prepubertal (4 to 6 months), pubertal (7 to 18 months), pregnant (1.5 years to 2.5 years), lactating (2 to 4 years) and dry (4 to 8 years) with 6 animals in each group. The tissue samples collected from mammary glands of all these animals were fixed in various standard fixatives viz., 10% neutral buffered formalin, Zenker's fluid, Carnoy's fluid, Bouin's fluid and Telly's fixative. All tissues collected as above were processed by routine Alcohol-Benzene schedule and paraffin blocks were cut at 5-7 µm thickness for histological study. The sections were stained with standard Haematoxylin and Eosin, Masson's trichrome method for collagen and muscle fibres, Verhoeff's method for elastic fibres, Periodic acid Schiff (PAS) technique for mucopolysaccharides, Mercury-Bromophenol blue method for basic proteins, Gomori's calcium method for alkaline phosphatase activity, Gomori's lead method for acid phosphatase activity, Von Kossa method for calcium, Oil red 'O' in propylene glycol for lipids (Bancroft and Gamble, 2003).

RESULTS AND DISCUSSION

The globular mammary gland of sheep was located in the inguinal region held in position by the glandular reflections of skin and suspensory ligaments. In prepubertal animal, the mammary gland was not developed grossly. However the appearance of rudimentary teats marked its location in the inguinal region. In pregnant and lactating sheep, it was

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globular or rounded sac shaped due to the deposition of connective tissue and secretory parenchyma.

Capsule

The mammary gland in Madras red sheep was covered from outside to inside by the skin and fibroelastic capsule as has been reported in other domestic animals (Calhoun and Stinson, 1981). The epidermis of the skin showed three layers, viz. the stratum basale, stratum spinosum and the stratum corneum. The dermis had long tortuous sweat glands, voluminous sebaceous glands and few hair follicles. The capsule comprised of irregularly arranged collagen and elastic fibres. A few reticular fibres were seen intermingled with other fibres. The connective tissue septa branched off from capsule and grown deeper and penetrated the adipose tissue which was evident in the mammary glands of pubertal animals. The concentration of the elastic and reticular fibres increased in pregnant and lactating mammary glands. These observations are similar to those described earlier in sheep (May, 1970). Small arteries and veins with coiled branching were noticed in the outer part of the capsule. Calhoun and Stinson (1981) described that in domestic animals, the connective tissue was made of loose collagenous, elastic and reticular fibres with extensive plexus of blood and lymph capillaries. The adipose tissue had no definite pattern of arrangement but mostly aggregated in the inner part of the capsule. These were stained by the Sudan black B and Oil red O for neutral fats.

Connective tissue stroma

The prepubertal mammary gland had only a gland cistern and teat cistern. The ductular system with their epithelial buds confined to a very limited zone close to the gland cistern. The adipose tissue was abundant and surrounded the small groups of ducts in prepubertal and pubertal mammary glands (Fig.1).

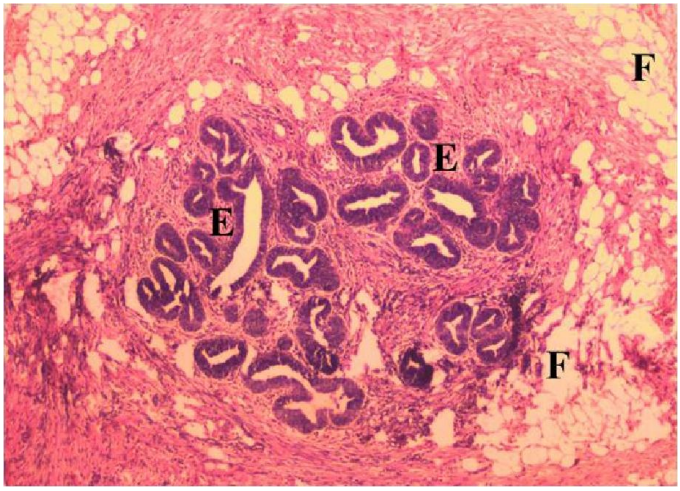

Fig. 1. Mammary gland in prepubertal sheep showing the development of ducts system with its epithelial buds (E) extending from gland cistern. The duct system is surrounded by connective tissue stroma and adipose tissue (F). H&E x 100

The connective tissue septa branched from capsule and grown deeper and penetrated the adipose tissue which was evident in these mammary glands. The adipose tissue was replaced by the growing connective tissue which was noticed in the pubertal and pregnant mammary gland. This is in conformity with the findings of Sheikh and Sultan (1977) who found that the stromal fat decreased with increase in age of the animals. The interlobar connective tissue extended from the inner face of the glandular capsule and arranged in between the lobes as reported in mammary glands of cows (Paul *et al*., 2013). It consisted of longitudinally oriented collagenous and elastic fibres as also described in buffalo (Bhatia and Sahai, 1979). The concentration of elastic fibres in the interlobular connective tissue was greater but almost absent in the intralobular connective tissue in sheep as described by Prusty (1988) in all age groups of cows. The interlobar connective tissue invested the larger ducts and showed thick walled arteries, veins and lymphatics in the mammary gland of prepubertal animal. The number and diameter of these blood vessels increased gradually in pubertal, pregnant and lactating mammary glands.

The ducts branched and followed the connective tissue to expand the area of ductular system in these age groups of sheep. In the pregnant and lactating sheep, the connective tissue became reduced and lobuloalveolar tissue was predominantly increased. The intralobular connective tissue formed the main supportive skeleton within the lobes of the mammary parenchyma (Cadar *et al*., 2012). It increased in the mammary glands of the pubertal and pregnant animals as fine collagenous fibres in between the budding alveoli. The relative proportion of the interlobular tissue increased at the pubertal period but decreased during pregnant (Fig.2) and lactating mammary glands, due to the increase in size of the glandular alveoli. Sulochana (1983) also recorded that the interlobular connective tissue during pregnancy reduced to minimum.

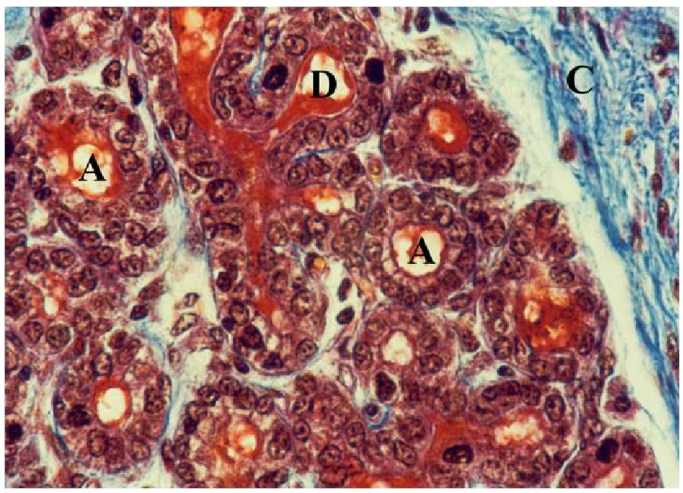

Fig. 2. Mammary gland in pregnant sheep with alveolar lumen (A) filled with fat vacuoles and acidophilic secretions. The intralobular duct with 2-3 layers of cells (D). Interlobular connective tissue with collagen fibres (C). Mallory's triple stain x 630

The author further opined that the reduction may be explained as a result of pressure atrophy of the interlobular and intralobular connective tissue due to rapidly expanding alveoli during pregnancy and lactation in sheep. In the present study, the blood capillaries and lymphatics were observed in all the age groups as stated in domestic animals (Calhoun and Stinson, 1981). However, they showed relative increase during pregnancy and lactation. A nearly one third decrease in the

interlobular connective tissue was due to the growth of the parenchymatous elements which exerted pressure and thereby reduced the thickness of the interlobular connective tissue (Fig.3) in these age groups of animals. In contrast to the pregnant and lactating animals the mammary gland in non-lactating dry animals showed increased amount of connective tissue element (Fig.4) as has been described in dromedaries (Kausar *et al.,* 2001).

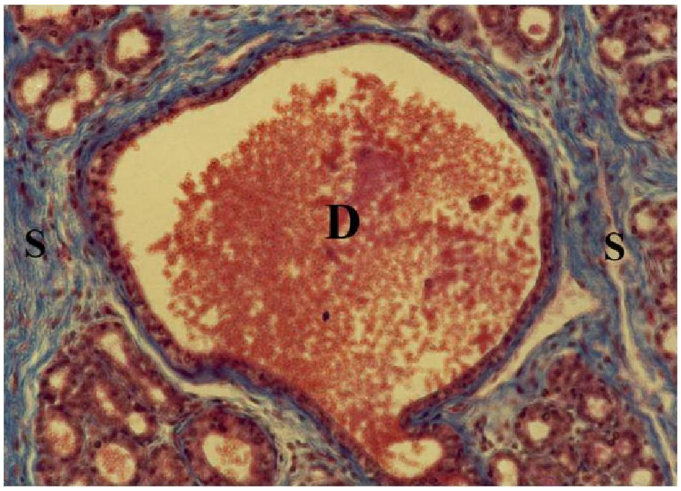

Fig. 3. Mammary gland in pregnant sheep showing the connective tissue running as interlobular and intralobular septae (S) and a large interlobular duct (D) with acidophilic secretions. Mallory's triple stain x 100

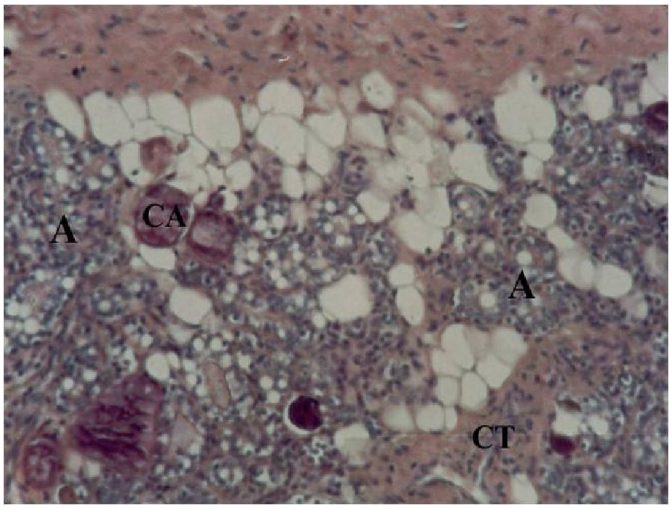

Fig. 4. Mammary gland showing the degenerating alveolar epithelium (A) and increase in connective tissue stroma (CT) with formation of interstitial corpora amylacea (CA). H&E x 630

The inter-alveolar, intra and interlobular connective tissue increased with appearance of numerous fibroblasts. The regression effect of deposition of connective tissue fibres correspondingly decreased the number of alveoli per lobule, length and breadth of lobule, alveolar diameter and luminal diameter in the mammary glands during dry periods. The corpora amylacea were seen in their various stages of formation and located both in the lumen of alveoli (intra-alveolar bodies) and septal connective tissue (Interstitial bodies). The interstitial amylacea concretions were more in the involuting mammary glands.

Conclusion

The current study on the mammary gland of Madras red sheep revealed that the connective tissue septa branched off from fibroelastic capsule. The adipose tissue was abundant and surrounded the small groups of ducts in prepubertal and pubertal mammary glands. In the pregnant and lactating sheep, the connective tissue became reduced and lobuloalveolar tissue was predominantly increased. In non-lactating dry animals the mammary gland showed increased amount of connective tissue elements and interstitial corpora amylacea.

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