



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(6): 392-395

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Received: 25-09-2019

Accepted: 27-10-2019

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Histochemical and immunohistochemical studies in udder of madras red ewes

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Abstract

The present histochemical and immunohistochemical study was conducted in udder and teat of lactating and non-lactating adult Madras Red ewes (n=6). In lactating animals, the basement membrane of the alveoli, apical border of the alveolar epithelium, cytoplasmic secretory materials and myoepithelial cells were positive for PAS reaction. Basal lamina of the intralobular ducts, interlobular ducts and large lactiferous ducts were positive for PAS reaction. The lumen of the alveolar contents showed a positive reaction in Von-Kossa method for calcium. Immunohistochemical staining of cytokeratin AE1/AE3 revealed the strongly positive reaction in epithelial lining. Alveoli, ductular system showed high positive staining in lactating animals than the non-lactating animals which showed weak staining.

Keywords: Histochemistry, immunohistochemistry, madras red ewe, udder, teat

Introduction

The small ruminant sector plays an important role in the rural economy at the national level. They have a significant role in ensuring food and nutritional security for the families of millions of poor households. As a mean of income and employment generation for these households, rearing of small ruminants helps in alleviating poverty and regularize income distribution [1]. The mammary gland is an exocrine epithelial gland exclusive to the mammalian species that is quantitatively and qualitatively adapted to the growth requirements and behavior of each species. It shows histological similarities to other epithelial glands such as the salivary and sweat glands. It develops during pregnancy and early lactation and regresses very quickly after dry-off [2]. Cytokeratin AE1/AE3 can be used as a reasonably effective epithelial screen to search for epithelial differentiation in malignant tumour [3]. Understanding the normal histochemistry and epithelial parts of mammary gland by immunohistochemistry using suitable marker helps to identify the pathological lesions in the affected glands. The present study was conducted to establish the histochemical and immunohistochemical details of mammary gland in two different groups of adult Madras Red ewes.

Materials and methods

Histochemistry

The present histochemical and immunohistochemical study was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai. The udder and teat samples were collected immediately after slaughter of animals from the corporation slaughter house, Chennai and directly fixed in 10 per cent neutral buffered formalin, Zenker's fluid, and Bouin's fluid. Collected tissues were processed by routine Alcohol-xylene schedule and paraffin blocks were made [3]. Sections were cut at 5-7 µm thickness for histochemical study. The sections were stained with the standard histochemical techniques, such as Periodic acid Schiff (PAS) technique for mucopolysaccharides [4] and Von-Kossa method for calcium [5].

Immunohistochemistry

3 µm paraffin sections were cut and mounted on charged slides and incubated at 60-70 °C for 30 minutes. Sections were deparaffinized by two changes in xylene followed by dehydrated with absolute alcohol two changes and washed twice in distilled water. Heat mediated antigen retrieval was done using TRIS-EDTA buffer (pH 8.5 – 9.0). Sections were washed twice in distilled water for two minutes. Blocking of endogenous peroxidase was done with 3 per cent hydrogen peroxide for ten minutes. Then, they were incubated in CD3 (ready to use) primary

Antibody in a moist chamber for one hour. Polyexcel HRP (ready to use) secondary antibody was added and incubated for 12 minutes and sections were washed three times in PBS. Diaminobenzidine (DAB) chromogen solution (1ml DAB buffer + 1 drop DAB chromogen) was added and kept for two to five minutes and washed in distilled water. Counterstaining was done with Gill's haematoxylin for one minute. Sections were blued by running tap water for five minutes, dehydrated through graded series of alcohol, cleared by xylene and mounted in synthetic mountant [6].

Results

In lactating Madras Red ewes, the basement membrane of the alveoli, apical border of the alveolar epithelium and cytoplasmic secretory materials were positive for PAS reaction (Fig 1). In non-lactating ewes, the reaction is mildly positive (Fig 2). The lumen of the alveolar contents showed a positive reaction in Von-Kossa method for calcium (Fig 3). Interlobular ducts located between the lobules also showed black calcium deposits in their lumen. The intensity was found to be more in lactating alveolus than non-lactating alveolar lumen. Among the two species, the intensity was found to be more in lactating ewes than the nonlactating ewes. Basal lamina of the intralobular ducts, interlobular ducts and large lactiferous ducts was positive for PAS reaction and calcium. Myoepithelial cells were located in between the basement membrane and lining epithelium and found to be weakly positive for PAS reaction.

In the present study, lactating mammary gland was lined by the simple cuboidal epithelium. In these, epithelial cells, Cytokeratin AE1/AE3 expression were strong in cytoplasm than the nucleus. Epithelial cells were showed high positive reaction than the connective tissue septa (Fig 4). Similarly, lactating gland showed more intense staining than the non-lactating gland (Fig 5). Intralobular, interlobular ducts and myoepithelial cells were also expressed cytotkeratin AE1/AE3 staining. The teat skin was lined by stratified squamous keratinized epithelium, which showing five different layers such as stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum (Fig 6). Sebaceous glands, nerve fibre endings, corpuscles, sweat glands were present in dermis region. Sweat gland epithelium was also highly positive for cytotkeratin AE1/AE3 expression (Fig 7).

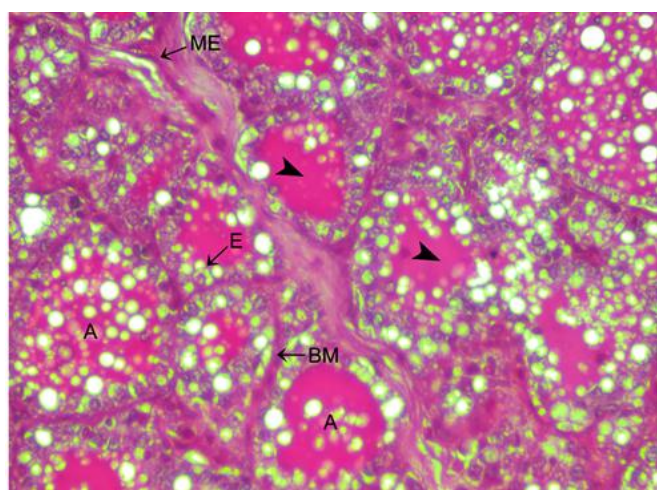


Fig 1: Photomicrograph of the mammary gland of the adult lactating ewe showing the alveoli (A), myoepithelial cells (ME), basement membrane (BM) and alveolar secretions (arrow heads) showing positive reaction for mucopolysaccharides. PAS x 400

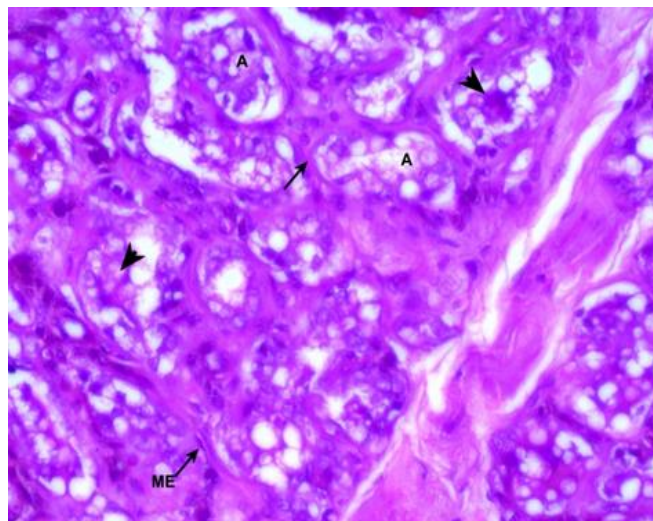


Fig 2: Photomicrograph of the mammary gland of the adult non-lactating ewe showing the alveoli (A), myoepithelial cells (ME), basement membrane (arrow) and alveolar secretions (arrow heads) showing low positive reaction for mucopolysaccharides. PAS x 400

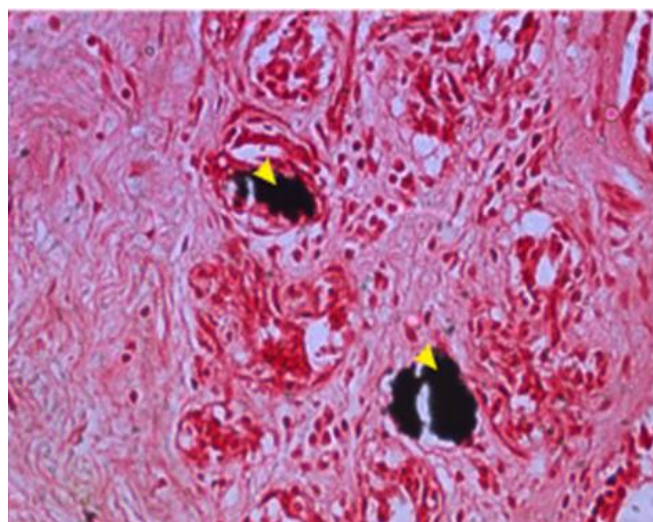


Fig 3: Photomicrograph of the mammary gland of the adult non-lactating ewe showing the alveoli and duct containing calcium deposits (arrow heads) Von-Kossa method x 400

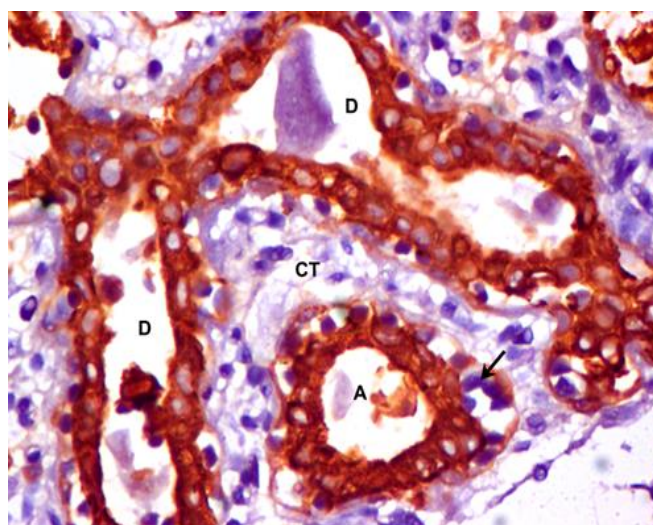


Fig 4: Photomicrograph of the mammary gland of adult lactating Madras Red ewe showing alveoli (A), duct (D) and alveoli (A), positive reaction for cytotkeratin AE1/AE3 and nucleus (arrow) in mammary epithelial cells IHC(DAB) x 400.

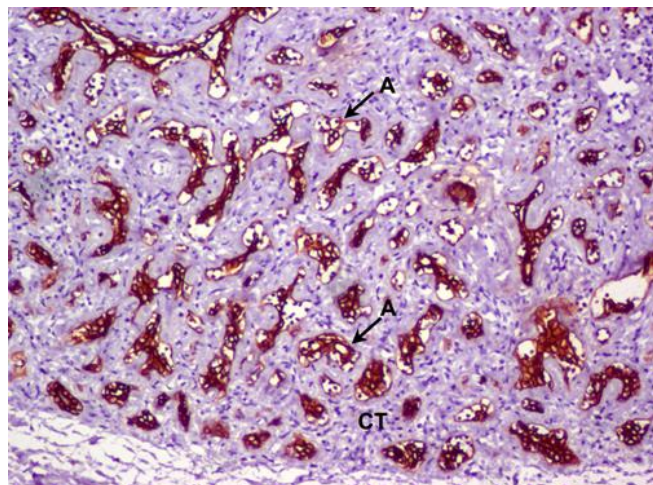


Fig 5: Photomicrograph of the mammary gland of adult non-lactating Madras Red ewe showing positive reaction for cytokeratin AE1/AE3 in alveoli (A) and connective tissue (CT) IHC (DAB) x 100.

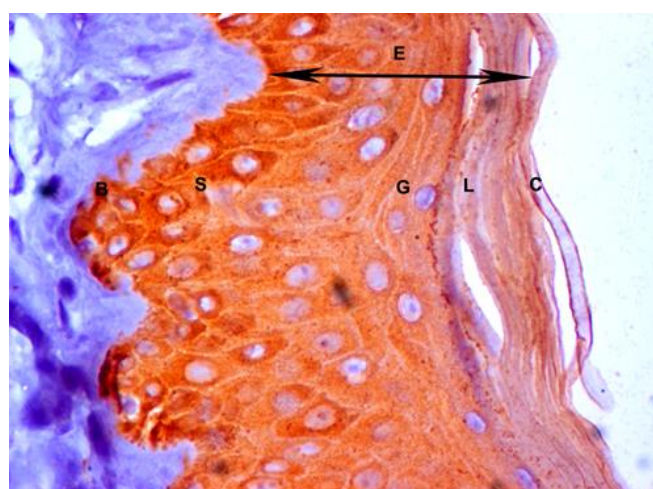


Fig 6: Photomicrograph of the teat skin epithelium (E) in Madras Red ewe showing Stratum basale (B), stratum spinosum (S), stratum granulosum (G), stratum lucidum (L) and stratum corneum (C) layers. IHC (DAB) x 400

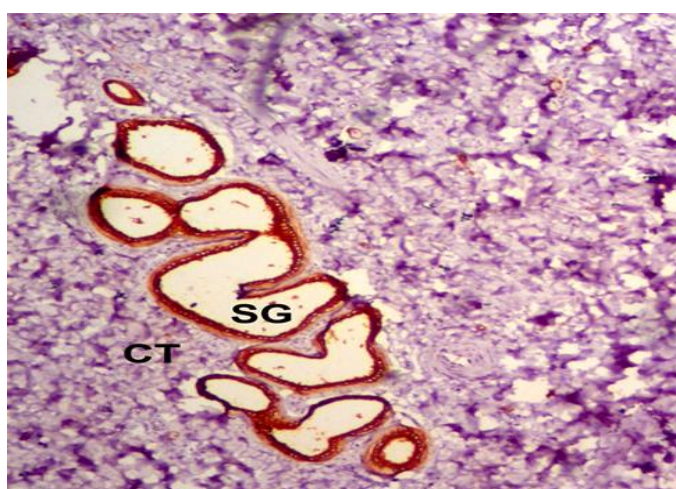


Fig 7: Photomicrograph of the sweat gland (SG) and connective tissue (CT) in teat skin of Madras Red ewe showing positive reaction for cytokeratin AE1/AE3. IHC (DAB) x 400

Discussion

In lactating Madras Red ewes, the basement membrane of the alveoli, apical border of the alveolar epithelium, duct system and cytoplasmic secretory materials were positive for

mucopolysaccharides such as glycogen. The results in the present were in accordance with the findings of Reid and Chanler (1973) [7]. This may be attributed to the fact that mammary tissue associated with the high energy demand for lactose production. Basal lamina of the intralobular ducts, interlobular ducts and large lactiferous ducts were positive neutral mucopolysaccharides and calcium, whereas myoepithelial cells were found to be weakly positive for PAS reaction. It is in total accordance with the findings of Naik (2015) [8].

Qualitative assessment for the presence of calcium by Von-Kossa method showed high intensity reaction within the alveolar lumen in lactating ewes than she-goat. This might be due to increased calcium content in sheep milk (193 mg / 100 gm) than goat milk (134 mg / 100 gm). Basal lamina of the ducts was positive for PAS reaction and calcium. A similar findings were reported by Naik (2015) [7] in Malnad Gidda cows. The, epithelial cells of alveoli, ducts and myoepithelial cells showed cytokeratin AE1/AE3 expression more in normal lactating mammary glands. Epithelial cells were showed high positive reaction than the connective tissue septa. The results were in accordance with the findings of Hirayama *et al.*, (2003) [9] in mammary gland of mares, Aydogan and Metin (2013) [10] in mammary gland of dogs.

Conclusion

Present study on mammary gland and teat of Madras Red ewe revealed the presence of epithelium in the mammary gland alveoli, ductular system and teat skin by an epithelial marker CK AE1/AE3. Histochemical staining for mucopolysaccharides and calcium revealed their expression more in lactating mammary gland and less in non-lactating mammary gland. This basic study will help in future to study more histochemical and immunohistochemical aspects in udder and teat of various sheep and goat breeds of dairy importance.

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