Kafkas Univ Vet Fak Derg
17 (2): 223-228, 2011
DOI:10.9775/kvfd.2010.3039

RESEARCH ARTICLE

Histological and Immunohistochemical Studies on the Furstenberg's Rosette in Cows

Reşat Nuri AŞTI * Nevin KURTDEDE * Hikmet ALTUNAY **
Belma ALABAY * Asuman ÖZEN * Alev Gürol BAYRAKTAROĞLU *

* Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Ankara, TR-06110 Dışkapı, Ankara - TURKEY
** Department of Histology and Embryology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy, TR-15100 Burdur - TURKEY

Makale Kodu (Article Code): KVFD-2010-3039

Summary

The purpose of this study was to investigate the light and electron microscopic structure of Furstenberg's rosette region and to determine of uptake of macromolecules from follicle associated epithelium (FAE) of healthy cows in lactation period. In this study, 26 distal teat end taken from 10 animals in lactation period were used as material. Tissue samples were prepared for examination by light and transmission electron microscope. Indirect immunoperoxidase method was implemented to determine of cells containing IgA and IgG. Ferritin solution (25 mg/ml) was applied to distal teat end 1 h before slaughtered to show uptake of macromolecules in FAE. It was observed that Furstenberg's rosette region consists of lymphoid and nonlymphoid areas. Solitary and aggregate lymphoid follicles were seen in lymphoid areas. It was attracted attention that upper surfaces of these follicles were covered with FAE. IgA positive cells were seen in centrum germinativum of lymphoid follicles in the lymphoid region and subepithelial areas. High endothelial venules (HEV), which is characteristic feature of lympho-epithelial tissues, were observed at connective tissue which is around of the lymphoid follicles. In thin sections, the teat end tissue have been previously applied to ferritin, ferritin particles were detected in apical invaginations and pinocytotic vesicles of membranous cells (M cells). It was determined that there is Furstenbeg's rosette associated lymphoid tissue (FALT) in teat end and this region was generated spesific immune response against antigens after their uptaking and proccessing. Thus, we have reached conclusion that FALT may play a role mucosal immunity of teat end.

Keywords: *Furstenberg's rosette, FAE, FALT, Mammary gland, Cow*

İneklerde Furstenberg Rozeti Üzerinde Histolojik ve İmmunohistokimyasal Çalışmalar

Özet

Araştırma laktasyon dönemindeki sağlıklı ineklerde Furstenberg rozeti bölgesinin yapısını ışık ve elektron mikroskobik düzeylerde incelemek ve folikül ile ilişkili epitel (FAE)'den makromoleküllerin alınışını belirlemek amacıyla yapıldı. Çalışmada materyal olarak 10 adet sağlıklı hayvandan alınan 26 meme başı kullanıldı. Doku örnekleri ışık ve elektron mikroskobik incelemeler için hazırlandı. IgA ve IgG içeren hücrelerin belirlenmesi için indirekt immunperoksidaz metodu uygulandı. FAE tarafından makromoleküllerin alınışını göstermek için, kesimden 1 saat önce distal meme ucuna ferritin solüsyonu (25 mg/ml) uygulandı. Furstenberg rozetinin lenfoid ve nonlenfoid bölgelerden oluştuğu gözlendi. Lenfoid bölgede soliter ve agregat lenf folikülleri görüldü. Bu foliküllerin üstünün FAE ile örtülü olduğu dikkati çekti. Lenfoid bölgede subepiteliyal alanlarda ve foliküllerin germinal merkezinde IgA pozitif hücreler belirlendi. Lenfo-epiteliyal dokuların karakteristik özeliklerinden, yüksek endoteliyal venüller (HEV) lenf folikülerinin çevresindeki bağ dokuda gözlendi. Öncesinde ferritin uygulanmış meme başlarından hazırlanan ince kesitlerde, membranöz hücrelerinin (M cells) pinositoz veziküllerinde ve apikal invaginasyonlarında ferritin partikülleri belirlendi. Meme başında Furstenberg rozeti ile ilişkili lenfoid doku (FALT)'nun bulunduğu ve bu bölgede antijenlerin alınıp işlenerek spesifik immun yanıtın oluşturulduğu belirlendi. Böylelikle FALT'ın memebaşı mukozal savunmasında önemli rol oynabileceği sonucuna varıldı.

Anahtar sözcükler: *Furstenberg rozeti, FAE, FALT, Meme bezi, İnek*

İletişim (Correspondence)
+90 312 3170315
dralevgurol@gmail.com

INTRODUCTION

Because of entering of pathogen agents from teat canal, the first barrier against these was formed in teat canal [1-3]. Sphincter existing in this region limits the entering of microorganisms by holding the canal as closed. The keratin lining of teat canal forming physical barrier against microorganisms includes antimicrobial lipids and proteins [1,4]. Thus bacterial invasion and colonization in teat canal is prevented [5,6].

Proximal region of teat canal ends in the structure that showing mucosal folds called as Furstenberg's rosette in cows. Longitudinal folds coming from teat sinus form a rosette by approaching to each other in this region [7]. Although teat canal consists of stratified squamous keratinized epithelium, Furstenberg's rosette region is covered with two layered columnar epithelium. Furstenberg's rosette region is also called as squamocolumnar junction [1,7,8]. Lymphocytes, polimorph nuclear leukocytes [9] and mononuclear phagocytes are observed in intraepithelial intervals [6,8].

It was observed that lymphocytes show considerable numerical increase while gone to Furstenberg's rosette region from teat sinus and reach the highest density in this region in normal teat tissues [7,8]. Furthermore it is explained that lymphocyte make dense aggregates in a same apperance of lymphoid follicles [10]. Plasma cells [11,12], polymorph nuclear leukocytes and mononuclear phagocytes are observed in Furstenberg's rosette region intensively [8,11]. It is explained that Furstenberg's rosette region where plasma cells exist in high concentration can be main source of immunity against bacteria [13]. Comparisons made between normal and inflamed groups brought up that the considerable increases of numbers of lymphocytes, plasma cells, polymorph nuclear leukocytes [14] and mononuclear phagocytes [5] happen in inflamed teat end tissue. However considerable decreases in density of defense cells in this region is observed in early dry period [7,8].

Mucosal lymphoid tissues form contact region with antigens and act production of antibody against antigens [15]. These lymphoid tissues consist of solitary or aggregate lymphoid follicles and specialized epithelium covering these follicles. This epithelium is called as follicle associated epithelium (FAE) or lympho-epithelium [15,16]. Membranous cells (M cells) existing in FAE are the cells specialized for uptake and present of antigens [17,18]. These cells uptake intraluminal antigens from the luminal surface and pass them from their narrow cytoplasm [17,19-21].

The purpose of this study was to display lymphoid tissue, whose existence and structure of FAE, functioning in mucosal immune system in Furstenberg's rosette region in healthy cows which are in lactation period.

MATERIAL and METHODS

Mammary gland tissue samples were obtained from Çubuk slaughterhouse. California mastitis test (CMT) was applied by taking the milk samples from 10 Holstein cows whose general condition are well for the outside appearance, have not got anatomical defects in their mammary gland and not showing inflamation symptoms. Milk samples taken in aseptic condition in the slaughterhouse were brought to Ankara University Veterinary Faculty Microbiology Department laboratory in cold chain and microbiological analyzed were made [22]. Milk samples taken from 26 mammary lobes for CMT and microbiological analysis results were negative, whose tissue samples were used as material.

A) Light microscopy: After some parts of tissue samples were fixed in 10% neutral buffered formalin in order to examine the general histological appearance, they were embedded in paraplast and triple staining method was applied to 7 µm microtome (Leica 2025, Germany) sections taken from these blocks [23].

Indirect immunoperoxidase staining method was applied to the other parts of tissue samples after 10 µm cryostat (Leica L50, Germany) sections were taken in order to determine the cells containing IgA and IgG [24]. Monoclonal anti-bovine IgA (MCA 628, Serotec Ltd, Oxford, UK) was used as primary antibody to determine IgA positive cells and anti-mouse IgG peroxidase (A9044, Sigma chemical Co, St. Louis, Mo.) was used as secondary antibody. Monoclonal anti-bovine IgG (B6901, Sigma chemical Co, St. Louis, Mo.) was used as primary antibody for IgG positive cells and anti-mouse IgG peroxidase was used as secondary antibody.

B) Electron microscopy: Taken pieces were fixed in 1% osmium tetroxide solution for 2 h as a second time after their prefixation made for 24 h in glutaraldehyde-paraformaldehyde, and they were blocked in Araldite M by passing from graded alcohols and propylene oxide. Thin sections having 300-400 Angstrom thickness were taken from the desired regions by applying of toluidine blue-pyronin. Uranil acetate and lead citrate staining [25] were applied to these sections and they were examined in a Carl Zeiss EM 9S-2 transmission electron microscope (Zeiss Oberkochen, Germany).

Ferritin solution (25 mg/ml, F4543, Sigma chemical Co, St. Louis, Mo.) which had been prepared in 0.15 M $NaCl_2$ solution at 37°C was applied to Furstenberg's rosette region by entering from teat canal after the milk inside the 4 mammary lobe of 2 animals was emptied 1 h before the slaughtered. Standard electron microscopic procedure was applied to tissue samples. Contrast staining was not implemented to these sections to distinguish ferritin particles easily. However, slightly was stained to the ones whose photographs to be taken [21].

RESULTS

Anatomically lengthwise folds coming from teat sinus to teat end forms a rosette is called as Furstenberg's rosette *(Fig. 1, arrows)*.

Light Microscopic Findings

It was observed that teat end canal was covered stratified squamous keratinized epithelium on the light microscopy in cows. It was seen that Furstenberg's rosette region is lined with two layered epithelium. It attracted attention that numbers of lymphocytes, plasma cells, polymorph nuclear leukocytes and macrophages increase towards to Furstenberg's rosette region. In some cases solitary and aggregate lymphoid follicles *(Fig. 2, L)* were encountered together with lymphocyte infiltration, although lymphocyte infiltration were seen in the most of the cases. In thin sections showed that Furstenberg's rosette was covered with single-layered squamous epithelium in lymphoid region *(Fig. 3, arrows)*.

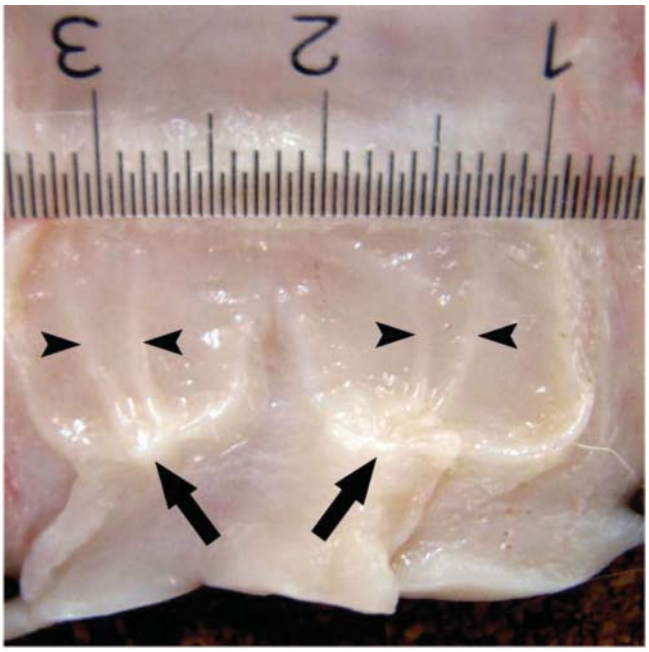

Fig 1. Macroscopic appearence of Furstenberg's rosette. Arrows: Furstenberg's rosette region, arrowheads: lenghtwise folds of teat sinus

Şekil 1. Furstenberg rozetinin makroskobik görünümü. Oklar: Furstenberg rozeti bölgesi, okbaşları: meme başı sinusunun boyuna kıvrımları

IgA positive cells (immunoblasts and plasma cells) were determined *(Fig. 2, arrows)* in the centrum germinativum (CG) of the lymphoid follicles and subepithelial region (S) of lymphoid areas and just bottom of the epithelium in nonlymphoid regions *(Fig. 4, arrows)*. Although IgG positive cells were not exist in lymphoid follicles, they were seen in very low number in the nonlymphoid areas.

Electron Microscopic Findings

It was seen that epithelial M cells containing short, thick and rare microfolds *(arrows)* on apical surfaces and whose cytoplasm became flattened, were present in the FAE. It was determined that these cells carry many pinocytotic vesicles in apical cytoplasm and have close relation with lymphocytes (L), macrophages and polymorph nuclear leukocytes (P) *(Fig. 5)*.

Ferritin uptake from epithelial cells in nonlymphoid region was not observed. Ferritin particles were seen at the apical surface in invaginations *(Fig. 6, arrowhead)* and pinocytotic vesicles (arrows) of FAE cells in lymphoid areas. Furthermore ferritin particles were observed inside the phagocytic vacuoles in macrophages having close relation with FAE cells. High endothelial *(arrows)* venules (HEV), which are characteristic feature of lympho-epithelial tissues were seen in connective tissue around the lymphoid follicles *(Fig. 7)*.

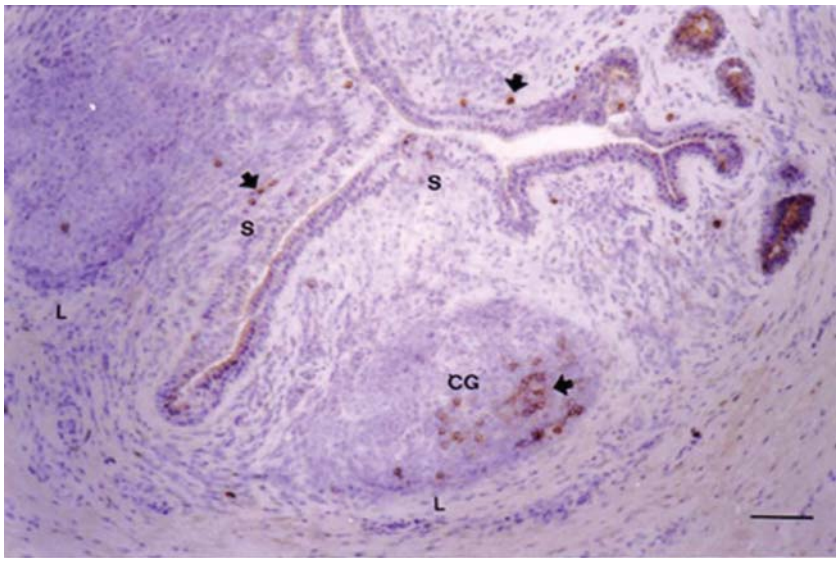

Fig 2. Photomicrograph of lymphoid region of the Furstenberg's rosette. L: lymphoid follicle, CG: centrum germinativum, S: subepithelial area, arrows: IgA positive cells, Immunoperoxidase staining, Bar: 75 µm

Şekil 2. Furstenberg rozetinin lenfoid bölgesinin fotomikrografı. L: lenfoid folikül, GC: sentrum germinativum, S: supepiteliyal bölge, oklar: IgA pozitif hücreler, İmmunoperoksidaz boyaması, Bar: 75 µm

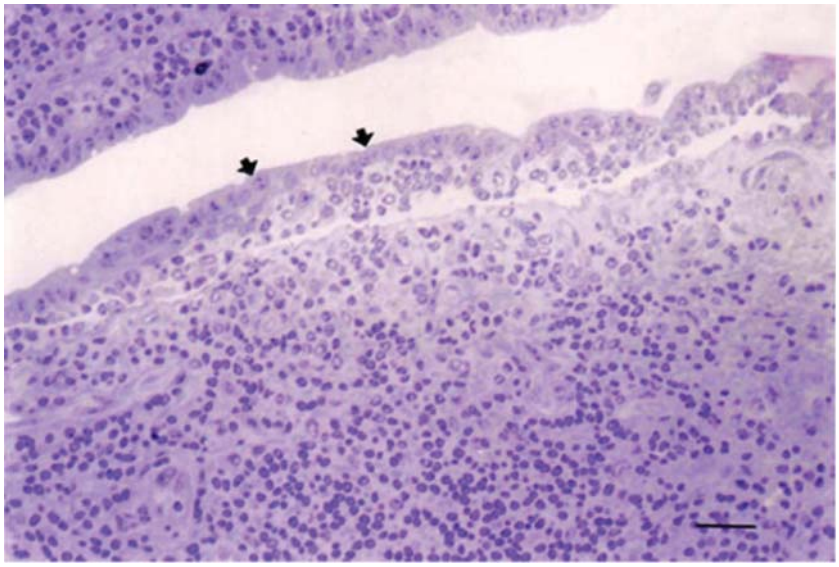

Fig 3. Photomicrograph of lymphoid region of the Furstenberg's rosette. Arrows: FAE, Toluidine blue-pyronin staining, Bar: 24 µm

Şekil 3. Furstenberg rozetinin lenfoid bölgesinin fotomikrografı. Oklar: FAE, Toluidin blue-pironin boyaması, Bar: 24 µm

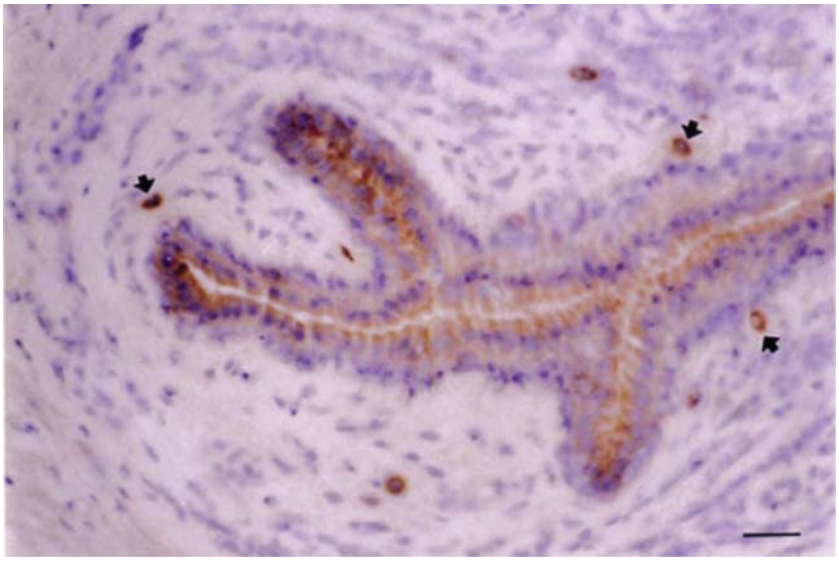

Fig 4. Photomicrograph of nonlymphoid region of the Furstenberg's rosette. Arrows: IgA positive cells, Immunoperoxidase staining, Bar: 23 µm

Şekil 4. Furstenberg rozetinin nonlenfoid bölgesinin fotomikrografı. Oklar: IgA pozitif hücreler, İmmunoperoksidaz boyaması, Bar: 23 µm

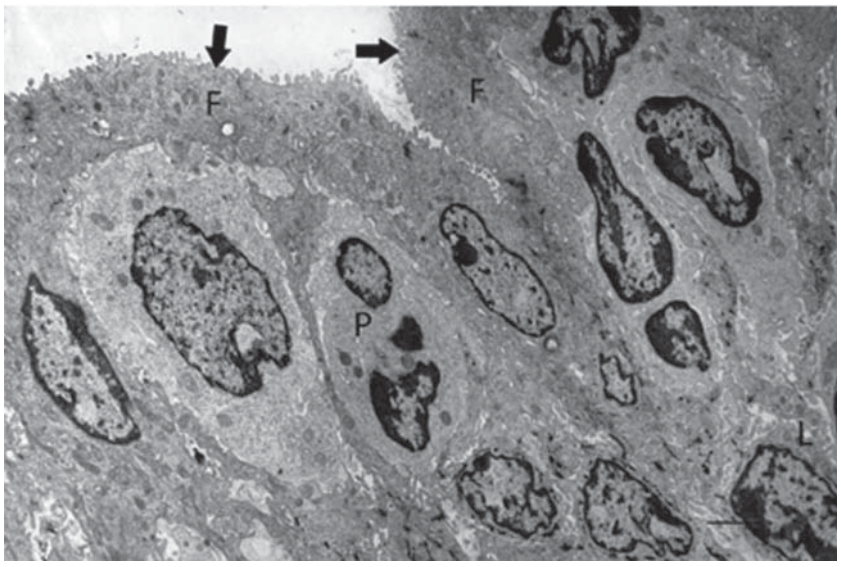

Fig 5. Electronmicrograph of FAE of lymphoid region in the Furstenberg's rosette. F: FAE, L: lymphocyte, P: polymorph nuclear leucocyte, arrows: microfolds, Bar: 0.9 µm

Şekil 5. Furstenberg rozetinde lenfoid bölgenin FAE'sinin elektronmikrografı. F: FAE, L: lenfosit, P: polimorf nüklear lökosit, oklar: mikrofoldlar, Bar: 0.9 µm

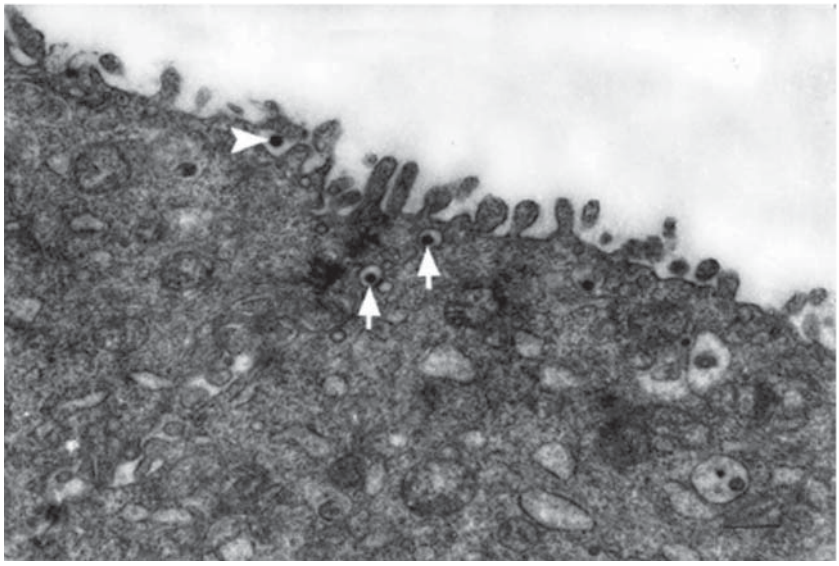

Fig 6. The uptake of ferritin particles in to M cells. Arrowhead: ferritin particule in apical invagination, arrows: ferritin particules within pinocytotic vesicles, Bar: 0.8 µm

Şekil 6. M hücrelerine ferritin partiküllerinin alınımı. Okbaşı: apikal invaginasyonda ferritin partikülü, oklar: pinositotik veziküllerde ferritin partikülleri, Bar: 0.8 µm

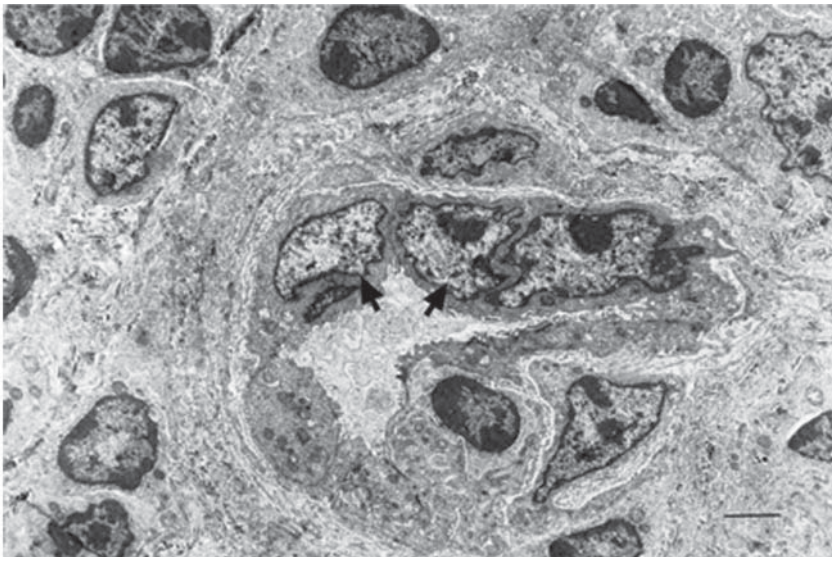

Fig 7. Electronmicrograph of high endothelial venul in lymphoid region of the Furstenberg's rosette. Arrows: endothel cells, Bar: 1.2 µm

Şekil 7. Furstenberg rozetinin lenfoid bölgesinde yüksek endoteliyal venüllerin elektronmikrografı. Oklar: endotel hücreleri, Bar: 1.2 µm

DISCUSSION

It is reported that teat sinus ends in the region showing mucosal folds which is called as Furstenberg's rosette [7]. Although teat canal consist of stratified squamous keratinized epithelium, Furstenberg's rosette region is covered with two layered columnar epithelium [1,8,9,26]. The findings obtained from this study are similar to the findings of the researchers.

It is informed that lymphocytes show considerable numerical increase while gone to Furstenberg's rosette region from teat sinus and reach the highest density in Furstenberg's rosette region [7,8]. Furthermore, it is noticed that structures resembling to lymphoid follicles were seen in addition to lymphocyte infiltrations and these may be source of immune response in Furstenberg's rosette region [10]. In the study, solitary and aggregate lymphoid follicles including centrum germinativum, lymphocyte infiltrations were seen in Furstenberg's rosette region.

It is noticed that most of the cells containing Ig in Furstenberg's rosette region have IgG [10,27]. Some other researchers [28] notified that the cells containing IgG are seen rarely but the cells containing IgA form the majority. It is informed that the source of these cells had become through changing of B lymphocytes coming from general circulation to plasma cells [6,29]. In this study, it was determined that the cells containing IgA form the majority and the cells containing IgG are seen rarely. IgA positive reaction was observed in immunoblasts in centrum germinativum of lymphoid follicles and plasma cells in lymphoid and nonlymphoid regions. These findings show that source of IgA having important role in mucosal immune response is lymphoid follicles in lymphoid region.

Characteristically mucosal lymphoid tissues settle in critical antigen entrance regions such as gut-associated lymphoid tissue (GALT) in digestive system [30,31] and

bronchus-associated lymphoid tissue (BALT) in respiratory system [32,33]. It is reported mucosal lymphoid tissues consist of solitary or aggregate lymphoid follicles and specialized epithelium called as FAE or lympho-epithelium which covers these follicles [15,34]. It is stated that M cells existing among FAE are the cells contains short, thick and rare microfolds on apical surfaces and having close relation with intraepithelial lymphocytes, polymorph nuclear leukocytes and macrophages and many pinocytotic vesicles in their apical cytoplasm [19,21]. M cells uptake intra luminal antigens and pass them from their narrow cytoplasm [17,20,35]. In the study, it was observed that epithelial cells covering the lymphoid follicles have similar ultrastructural features with membranous cells (M cells) informed by the researchers. We have opinion of that M cells are the specialized cells for uptake of antigen, like in GALT and BALT, in Furstenberg's rosette region. Because ferritin particles were observed inside the pinocytotic vesicles in cytoplasm of M cells and their close relation with macrophages.

It is reached to a conclusion that there is Furstenberg's rosette associated lymphoid tissue (FALT) in mammary gland of cow and antigens are received in this region, specific response is formed locally against these antigens and this has an important role in mucosal immun defense of teat end.

REFERENCES

1. Nickerson SC: Bovine mammary gland: Structure and function; relationship to milk production and immunity to mastitis. *Agri-practice,* 15, 10-17, 1994.

2. Zecconi A, Hamann J, Bronzo V, Moroni P, Giovannini G, Piccinini R: Relationship between teat tissue immune defences and intramammary infections. *Adv Exp Med Biol,* 480, 287-293, 2000.

3. Mavrogianni VS, Cripps PJ, Brooks H, Taitzoglou IA, Fthenakis GC: Presence of subepithelial lymphoid nodules in the teat of ewes. *Anat Histol Embryol,* 36, 168-171, 2007.

4. Sordillo LM, Shafer-Weaver KA, De Rosa KD: Immunobiology of the mammary gland. *J Dairy Sci,* 80, 1851-1865, 1997.

5. Ngatia TA, Jensen NE, Berg BB: Microscopic changes in infected bovine teats. *Br Vet J,* 147, 133-139, 1991.

6. Sordillo LM: Factors affecting mammary gland immunity and mastitis susceptibility. *Livest Prod Sci,* 98, 89-99, 2005.

7. Nickerson SC, Pankey JW: Cytologic observations of the bovine teat end. *Am J Vet Res,* 44, 1433-1441, 1983.

8. Aştı RN, Çelik İ, Kaya O: Light microscopic studies on the humoral and celluler defense system of the mammary tissues of the ewes during the lactation and dry period. *Veterinarium,* 2, 17-21, 1991.

9. Nickerson SC, Pankey JW: Neutrophil migration through teat end tissues of bovine mammary quartes experimantally challenged with *Staphylococcus aureus*. *J Dairy Sci,* 67, 826-834, 1984.

10. Collins RA, Parsons KR, Bland AP: Antibody-containing cells and specialized epithelial cells in the bovine teat. *Res Vet Sci,* 41, 50-55, 1986.

11. Nickerson SC, Pankey JW, Boddie NT: Distribution, location and ultrastructure of plasma cell in the uninfected, lactating bovine mammary gland. *J Dairy Res,* 51, 209-217, 1984.

12. Shafer-Weaver KA, Pighetti GM, Sordillo LM: Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. *Proc Soc Exp Biol Med,* 212, 271-280, 1996.

13. Nickerson SC: Immun mechanisms of the bovine udder: An overview. *J Am Vet Med Assoc,* 187, 41-45, 1985.

14. Nickerson SC, Pankey JW: Electron microscopic study of leucocytic infiltration of the mammary teat duct during infection with *Staphylococcus aureus*. *Res Vet Sci,* 38, 167-173, 1985.

15. Franklin RM, Remus LE: Conjunctival-associated lymphoid tissue: evidence for a role in the secretory immune system. *Inv Opthalmol Vis Sci,* 25, 181-187, 1984.

16. Knop N, Knop E: Ultrastructural anatomy of CALT follicles in the rabbit reveals characteristics of M-cells, germinal centres and high endothelial venules. *J Anat,* 207, 409-426, 2005.

17. Bockman DE, Cooper MD: Pynocytosis by epithelium associated with lymphoid follicles in the bursa Fabricius, appendix, and Peyer's patches. An electron microscopic study. *Am J Anat,* 136, 455-478, 1973.

18. Guiliano EA, Moore CP, Phillips TE: Morphological evidence of M cells in healthy canine conjunctiva-associated lymphoid tissue. *Greafes Arch Clin Exp Ophthalmol,* 240, 220-226, 2002.

19. Landsverk T: The follicle-associated epithelium of the ileal Peyer's patch in ruminants is distinguished by its shedding of 50 nm particles. *Immunol Cell Biol,* 65, 251-261, 1987.

20. Kurtdede N, Aştı RN, Altunay H, Özen A: Light and electron microscopic studies of ileal Peyer's patches and M cells in Angora goats. *Ankara Univ Vet Fak Derg,* 47, 39-49, 2000.

21. Liebler EM, Lemke C, Pohlenz JF: Ultrastructural study of the uptake of ferritin by M cells in the follicle-associated epithelium in the small and large intestines of pigs. *Am J Vet Res,* 56, 725-730, 1995.

22. Quinn PJ, Carter ME, Markey B, Carter GR: Clinical Veterinary Microbiology. pp. 327-344. Mosby-Year Book Europe: Limited-Wolfe Publishing, 1994.

23. Culling CFA, Allison RT, Bar WT: Cellular Pathology Technique. pp. 347-365, London: Butterworth, 1985.

24. Miller KD: Immunocytochemical techniques. **In,** Theory and Practise of Histological Techniques. pp. 421-465. London: Harcourt Publishers Lim., 2002.

25. Veneable JH, Coggeshall RA: A simplified lead citrate stain for use in electron microscopy. *J Cell Biol,* 25, 407-408, 1965.

26. Riedl J, Kiossis E, Muller M, Seidl S, Stolla R, Hermanns W: Endoscopic, macroscopic and histologic findings in the bovine teat. 2. Furstenberg's Rosette [Endoskopische, pathologisch-anatomische und histologische befunde an der rinderzitze 2. Veranderungen im bereich der Furstenbergschen Rosette]. *Dtsch Tierarztl Wochenschr,* 111 (11): 423-429, 2004.

27. Korhonen H, Marnila P, Gill HS: Milk immunoglobulins and complement factors. *Br J Nutr,* 84, 575-580, 2000.

28. Nickerson SC, Heald CW: Cells in local reaction to experimental *Staphylococcus aureus* infection in bovine mammary gland. *J Dairy Sci,* 65, 105-116, 1982.

29. Lascelles AK: The immune system of the ruminant mammary gland and its role in the control of mastitis. *J Dairy Sci,* 62, 154-162, 1979.

30. Beyaz F, Aştı RN: Development of ileal Peyer's patches and follicle-associated epithelium in bovine foetuses. *Anat Histol Embryol,* 33, 1-8, 2004.

31. Liebler-Tenorio E, Pabst R: MALT structure and function in farm animals. *Vet Res,* 37, 257-280, 2006.

32. Anderson ML, Moore PF, Hyde DM, Dungworth DL: Bronchus associated lymphoid tissue in the lungs of cattle: Relantionship to age. *Res Vet Sci,* 41, 211-220, 1986.

33. Sato J, Chida T, Suda A, Sato A, Nakamura H: Migratory patterns of the thoracic duct lymphocytes into bronchus-associated lymphoid tissue of immunized rats. *Lung,* 178, 295-308, 2000.

34. Wolf JL, Bye WA: The membranous epithelial (M) cell and the mucosal immun system. *Ann Rev Med,* 35, 95-112, 1984.

35. Bayraktaroğlu AG, Aştı RN: Light and electron microscopic studies on conjunctiva associated lymphoid tissue (CALT) in cattle. *Revue Med Vet,* 160 (5): 252-257, 2009.