

Histological and Histometrical Features of the Mammary Gland in Immature and Lactating Local Breed Ewes (*Ovis aries*)

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Abstract

Twelve samples of mammary glands (6 immature and 6 lactating ewes) were used for this study. Specimens of glands were immediately dissected and fixed in 10% formalin sol. The specimens were processed according to paraffin technique, sectioned at 5-6µm and stained by hematoxylin and eosin, Masson's Trichrom stains. In both immature and lactating ewes the mammary glands were covered by skin. In immature ewes the mammary quarter was composed of few mammary lobes that separated by very thick inter lobar adipose tissue. The lactating quarter was composed of huge mammary lobes and lobules without adipose tissue. In immature the lobe was consisted of few small lobules that composed of few of mammary alveoli while the lactating lobes had large sizes lobules and each had large alveoli which lined by tall cuboidal cells and supported by fibrous tissue and myo epithelial cells. In both immature and lactating ewes, the gland and teat cisternae were showed many of mucosal folds which lined by pseudo stratified columnar epithelium and supported by fibrous connective tissue. The teat cisterna of both immature and lactating was composed three layers; mucosal cisterna, fibro muscular layer and skin. The teat canal was lined by pseudo stratified squamous epithelium and the sub epithelial connective tissue showed well developed venous sinus and fibro muscular. Statistical analysis was revealed significant differences between the values of all parameters of immature and lactating ewes. Apparent surface areas of the lobe in immature and lactating were $122143.063 \pm 23.21 \mu m^2$ & $992541.188 \pm 33.81 \mu m^2$ respectively. Surface areas of lobule in immature and lactating were $1042.938 \pm 21.02 \mu m^2$ & $91565.977 \pm 25.60 \mu m^2$ respectively. Surface areas of alveoli in immature and lactating were $994.238 \pm 11.39 \mu m^2$ & $4584.270 \pm 19.26 \mu m^2$ respectively. Epithelial heights of alveoli in immature and lactating were $10.290 \pm 1.01 \mu m$ & $23.012 \pm 2.81 \mu m$ respectively. Thickness of inter lobar tissue in immature and lactating ewes $677.393 \pm 23.67 \mu m$ and $361.401 \pm 10.86 \mu m$ respectively. Thickness of inter lobular connective tissue in immature and lactating were $112.969 \pm 11.48 \mu m$ and $90.281 \pm 9.81 \mu m$ respectively.

Keywords: Mammary gland, Ewes, Immature, Lactating, Histological.

الصفات النسجية والنسجية القياسية للغدة اللبنية في النعاج المحلية غير البالغة والمرضع

الخلاصة

استخدمت اثنتي عشرة عينة من الغدد الثديية (6 نعاج غير ناضجة و 6 نعاج مرضعة). تم تشريح عينات الغدد على الفور بعد الذبح وتثبيتها في 10% محلول الفورمالين. تمت معالجة العينات وفقاً لتقنية البارافين، وتم تقطيعها عند مستوى 5-6 ميكرومتر وتم صبغها بواسطة ملون الهيماتوكسيلين والأيوزين وملون ماسون الثلاثي الصبغ. في كل من النعاج غير الناضجة والمرضعة كانت الغدد الثديية مغطاة بالجلد. في النعاج غير الناضجة، كان شطر الثدي مكوناً من عدد قليل من فصوص الثديية المفصولة عن بعضها بنسيج دهني ضام سميك جداً بين الفصوص. بينما تكون شطر الثدي للنعجة المرضع من فصوص وفصيصات ثديية ضخمة بدون أنسجة دهنية. في حالة النعجة غير الناضجة، كان الفص يتكون من عدد قليل من الفصيصات الصغيرة التي تتكون من عدد قليل من الحويصلات الثديية بينما كانت الفصوص في المرضعة كبيرة الحجم ولكل منها حويصلات كبيرة مبطنة بخلايا مكعبة عالية ومدعومة بنسيج ليفي وخلايا طلائية عضلية. في كل من النعاج غير الناضجة والمرضعة، ظهر العديد من الطيات المخاطية التي تصطف بواسطة ظهارة مطبقة عمودية كاذبة ومدعومة بنسيج ضام ليفي.

يتكون خزان الحلمة سواء في النعاج غير الناضج أو المرضع من ثلاث طبقات؛ الطبقة المخاطية، الطبقة العضلية الليفية والجلد. كانت قناة الحلمة مبطنة بظهارة حرشفية مطبقة وأظهر النسيج الضام تحت الظهارة جيوب وريدية ونسيج عضلي ليفي متطورة بشكل جيد. أظهر التحليل الإحصائي فروق معنوية بين قيم جميع معاملات النعاج غير الناضجة والمرضعة. كان قطر الفص في غدة النعاج المرضعات وغير الناضجة 23.21 ± 122143.063 المايكروميتر و 33.81 ± 992541.188 المايكروميتر على التوالي. كانت أقطار الفصيصات في المرضعات وغير الناضجة 21.02 ± 1042.938 المايكروميتر و 25.60 ± 91565.977 المايكروميتر على التوالي. كانت أقطار الحويصلات اللبنية في المرضعات وغير الناضجة 11.39 ± 994.238 المايكروميتر و 19.26 ± 4584.270 المايكروميتر على التوالي. كانت ارتفاعات الظهارة للحويصلات اللبنية في المرضعات وغير الناضجة 1.01 ± 10.290 المايكروميتر و 23.012 ± 23.012 المايكروميتر على التوالي. سماكة النسيج الفصي في النعاج غير الناضجة والمرضعة 23.67 ± 677.393 المايكروميتر و 10.86 ± 361.401 المايكروميتر على التوالي. كانت سُمك النسيج الضام بين الفصيصات في حالة المرضعات وغير الناضجة 11.48 ± 112.969 المايكروميتر و 9.81 ± 90.281 المايكروميتر على التوالي.

Introduction

The mammary gland is an exocrine epithelial tissue specified to the mammalians which is adapted to the growth requirements in each species', the mammary gland develops during pregnancy and early lactation, and regresses quickly after dry-off (1). Recognizing of the indigenous breed special factors, especially the structure of mammary gland such as (Teat characteristics) they influencing milking and has direct influencing on the milking management (2). General the mammary glands are located below the caudal part of the abdomen between the thighs and divide into two equal quarters and each is corresponded a gland. Also the skin which covered the mammary gland has specific features as a thin, shriveled and it is tightly bound down and naked. The mammary gland is fixed by strong groups of the fasciae that enclose the mammary gland (3). Gland is composed of highly branched duct system that conducted with each other by connective tissue septum and the mammary alveoli (secretory units) drain into small ducts that combine with others until, all drain into dozen of wide lactiferous ducts. Lactiferous ducts are converge on a large sinus situated in the lower part of the glandular cistern of each quarter and finally lead into the teat cisterna and

canal (4). The several articles have explained some of the factors affecting milk production, especially lactation stage are very important (5) in camel,(6) in cattle. (7) has explained the relationship has between the external and internal udder features, teat parameters characters with machine milked of dromedary camels. (8) have also investigated the correlation between the udder anatomical traits, alveolar and cisternal milk with machine milking performances of dairy camels. On the other hand other articles have described only the morphological traits (8, 9 & 10), all these studies reported a large differences among various species. The current study was suggested to recognize the histometrical features of mammary gland during the immature and lactating periods in local breed ewes, in order to provide important data about that of indigenous breed.

Materials and Methods

Twelve samples of mammary glands of healthy ewes (6 immature & 6 lactating) were used for this work. The sample were removed immediately from carcasses and infused with 10% formalin throughout the teats orifices. Each sample was trimmed into small specimens which were involved (teat canal, teat cistern, gland cistern, parenchyma and skin of gland and skin of teat). All specimens were fixed in neutral buffer formalin 10%. The specimens were

processed with routine paraffin technique. The paraffin blocks were sectioned serially at (5-6) μm by rotary microtome. The prepared tissue sections were stained with the Hematoxylin and Eosin stain and Masson's Trichrom stain (11). The Histometrical measurements included the diameters of the mammary lobes, lobules and alveoli, thickness of inter lobar connective tissue, thickness of inter lobular connective tissue and the heights of the alveolar epithelial cells. The tissue sections were examined by light microscopy and microphotography has been done by Future Win Joe microscopic camera, then images have analyzed and scored by using Fiji image analyzer system (12). The statistical analysis was done by using SPSS (Version-24) and data were represented by $\text{mean} \pm \text{Se}$. One way analysis of Variance (ANOVA) was used to detect the differences.

Results and Discussion

The mammary gland of both immature and lactating ewes was covered by hairy, well glandular cutaneous tissue which supported by layer of subcutaneous fibro muscular connective tissue. The skin of mammary gland was composed of epidermis which revealed thin keratinized stratified squamous epithelium and the dermis was composed of very thick layer of dense irregular collagen connective tissue that intermingled with scattered group of sebaceous and much of sweat glands (fig.1, 2 & 3). The current results were revealed similarity between the skin of immature and lactating mammary glands, this point was similar to that reported by (7, 5, 13 & 14), the glandular structure of skin gland in ewes was similar to that was seen in the mare, bitch and queen (15 & 16) who mentioned for highly content of sweat and sebaceous glands. On the other hand, the current results revealed that the skin of mammary quarter and the wall of teats in both immature and lactating ewes had furthermore of both apocrine sweat glands and sebaceous glands, this result disagree with that mentioned by (3) who referred for neither sebaceous nor sweat glands are found in the wall of teat of lactating cow.

Immature ewe; Mammary quarter of

immature ewes was composed of few scattered mammary lobes which was intermingled by very thick layer of inter lobar adipose connective tissue and thin collagenous connective tissue (fig.4). Mammary lobe was consisted of 1-3 slightly small size lobules which separated from each other by thin fibrous connective tissue. Each mammary lobule was composed of cluster of mammary alveoli and each alveolus was lined by simple cuboidal cells which had large centrally located nuclei, intensely eosinophilic cytoplasm and surrounded by myoepithelial cells. Inter alveolar connective tissue was composed of slightly thick layer of cellular connective tissue which revealed fibroblast and lymphocytes. Intra lobular duct had a wide lumen and lined by a simple cuboidal cells (fig.5, 6, 7 & 8). This result was reported thick inter lobar adipose tissue, this point was agreed with that recorded by (17) who referred for the stromal tissue which was a mesodermal origin and is constituted by blood, lymph vessels and adipocytes. This result was suggested that the adipocytes were represented the mesenchymal cells that developed into alveolar cells during puberty and at late pregnancy. In immature ewe, the structure of mammary alveoli were similar to that described by (13 & 18) in non-lactating camel and cow, on the other hand this result was disagreed with that mentioned by (14) in non-lactating ewe and goat, also disagreed with (6) in cow mentioned for double layer of cuboidal cells. Current result showed that the simple cuboidal epithelium was in resting status in absence the effect of prolactin, growth and insulin like growth hormones. The current result revealed that the population of mammary alveoli per lobule in immature ewes was little, this result was recorded by (13) who referred that the number of alveoli was decreased in the caudal system, also this result recorded by (18) who was mentioned that in the whole udder of non-lactating animals.

Lactating ewe; The mammary quarter of the lactating ewe was composed of numerous huge mammary lobes which were separated by very thick layer of well vascular inter

lobar collagenous connective tissue those contained small sized arteries, veins and branches of autonomic nerves fibers (fig.9 & 10). This result was agreed with (6, 13, 14, 19, 20 & 21), this result suggested that the milk production required specialized connective tissue that adapted for functional status of lactating gland, so the fibroblasts were the predominant cells type which was seen in the inter lobar and inter lobular connective tissue that required for angiogenesis and fibrogenesis.

Mammary lobe was consisted of several large size lobules which separated from each other by thin layer of fibrous inter lobular connective tissue and each lobule has composed of cluster of large mammary alveoli which revealed secretory activities (fig.11 &12). Each mammary alveolus was composed of tall simple cuboidal cells which were contained large nucleus and foamy cytoplasm. These cells were rested on basement membrane and followed by clear single layer of myo epithelial cells (fig. 13). The epithelial type which were seen in the lactating mammary alveoli were described by (14) in lactating ewe and she goat, (6) lactating cow, and (13 & 18) in lactating she camel which was modified for milk production. On the other hand the presence of myo epithelial cells within the stromal tissue of inter alveolar connective tissue during immature and lactating period was reported by (21, 22 & 23). The increase in the size of mammary lobes and lobules during lactating period were associated with hormonal effects (24, 25 & 26). This result was suggested that the stromal cells are the supporting connective tissue which was the main cellular components plays a significant part of the lactating udder, so the myo epithelial cells had supporting role and represented the contractile system which is involved in the ejection of milk from secretory units.

Glandular cisterna was larger sinus and had wide irregular shape lumen. The mucosa of cisterna showed irregular mucosal surface which was lined by pseudo stratified columnar epithelium which supported by marked dense layer of fibrous connective

tissue , then followed by a cellular loose connective tissue containing mainly lymphocytes and fibrocytes (lamina propria), very thick layer of well vascular fibro muscular connective tissue (fig.14, 15 & 16). The structure of teat cisterna was continuity of that seen in glandular cisterna which composed of three layers; (i) Marked mucosal cisterna folds (Epithelium & cellular lamina propria), (ii) fibro muscular layer & (iii) skin. The teat canal had the same structure that seen in teat cisterna except that the orifice of canal showed mucosal folds which lining by thick pseudo stratified columnar epithelium and the sub epithelial connective tissue showed well developed venous sinus and fibro muscular (fig.17). This result suggested that the gland cisterna was huge sinus that adapted for milk storage, so the presence of sub epithelial myo epithelial cells which mentioned by (6) in cisterna could not beneficial, usually the myo epithelial present to support the secretory units of most exocrine glands and never seen at the end parts of duct system.

The current study showed that, the wall of the teats canal of left and right quarters was similar in both immature and lactating mammary glands; this result was different to that recorded by (13) in camel. There were three layers that forming the wall of teat, this result was agreed with results of (6) in cow, (5& 13) in camel and (14) in small ruminants, this result was suggested that such wall construction is so important for that the teat do its function completely, because this part did the main function so, adapted for milking. There was a venous plexus seen at the wall of the teats canal which formed cavernous like tissue and this result was mentioned by (6, 17 & 27). On the other hand the mucosa of teats canal was covered by stratified squamous epithelium, such result was similar to that reported by (6, 13, 14 & 28) this result suggested that this type of epithelium was the first line of defense and protection from the effects of outer environment and also from pathogens (17 & 29).

The statistical analysis of the estimated data in the mammary glands of the immature and

lactating ewes was revealed significant differences at ($P < 0.05$) between the mean values of all parameters of the immature and lactating ewes table.(1), this result agreed that reported by (19). These results was suggested that the increase in parameters values of lactating ewe were associated with replace of adipose tissue with large sized mammary lobes, also the lobules revealed marked generation of large diameter mammary alveoli, on the other hand these increases also affected the thickness of inter lobular connective tissue.

Conclusion

The current results revealed that the immature mammary gland was a mass of adipose tissue with little inert glandular tissue and covered by thick skin. The lactating mammary glands were active huge mass of secreting glandular tissue with revealed thin skin.

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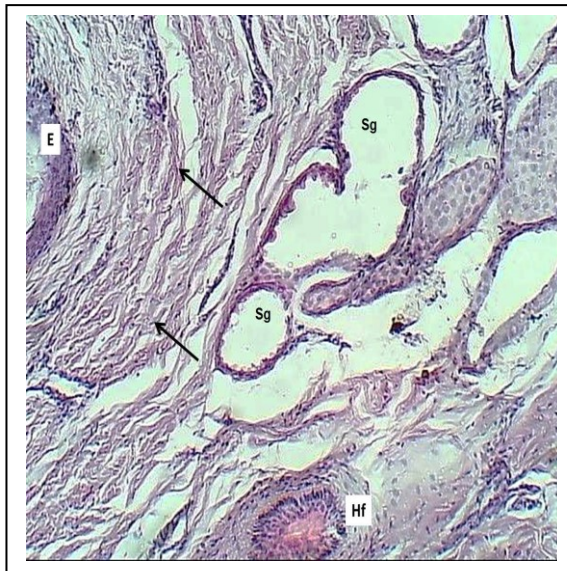


Figure 1: Section of skin of mammary gland (immature ewe) shows: epithelium (E), collagen bundles (Arrows), sweat glands (Sg) & hair follicles (Hf). H&E stain.100x

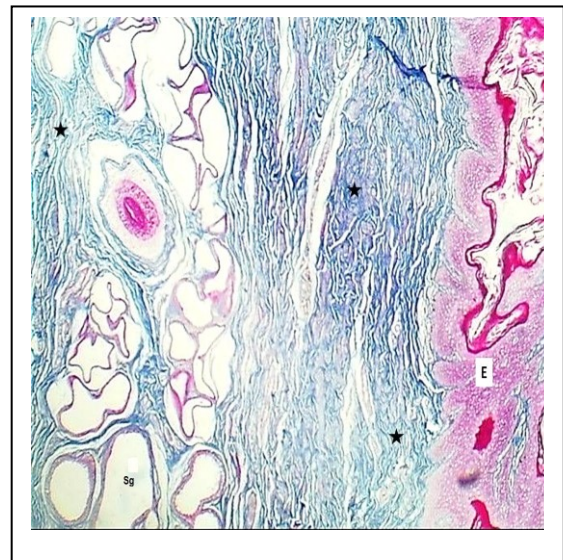


Figure 2: Section of skin of mammary gland (lactating ewe) shows sweat glands (Sg) supported by fibrous connective tissue (Asterisks). H&E stain 40x



Figure 3: Section of cutaneous tissue (lactating ewe) shows: Sweat gland (Sg), nerve (N), collagen bundles (Black arrows) & blood vessels (Red arrow). Masson trichrom stain.200x

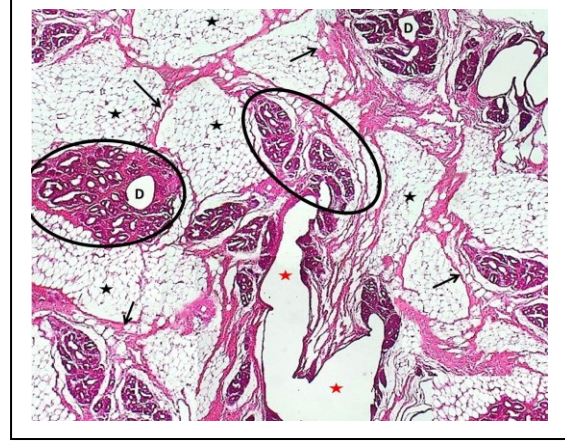


Figure 4: Section of immature mammary quarter shows: adipose connective tissue (Black asterisks), scattered small lobes (Black oval shape) & thin inter lobar connective tissue (Black arrows), intra lobular duct (D) & inter lobar milk duct (Red asterisks) . H&E stain.40x

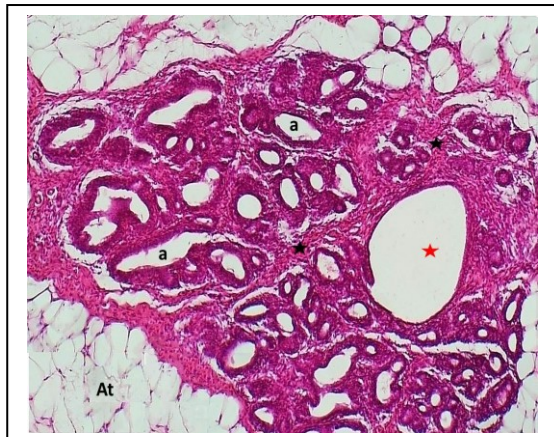


Figure 5: Section of mammary lobule (immature ewe) shows: adipose connective tissue (At), thick intra lobular collagenous connective tissue (Black asterisks), & intra lobular duct (Red asterisks). H&E stain.100x

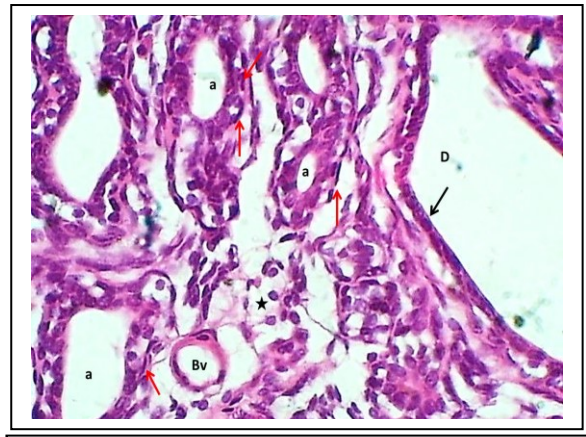


Figure 6: Section of mammary lobule (immature ewe) shows: mammary alveoli (a), blood vessel (Bv), myoepithelial cells (Red arrows), simple cuboidal epithelium (Black arrow) of intra lobular duct (D), inter alveolar loose connective tissue (Asterisk). H&E stain.400x

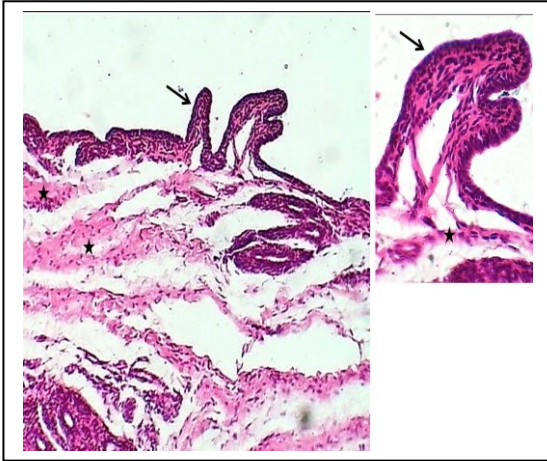


Figure7: Section of gland cisterna (Immature ewe) shows: Mucosal fold (Arrow) supported by fibrous connective tissue (Asterisks) . H&E stain 10&400x

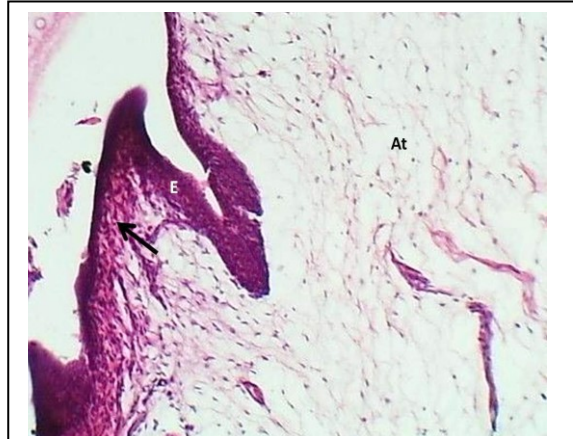


Figure 8: Section of teat canal (immature ewe) shows epithelium (E) supported by fibrous connective tissue (Black arrow) & adipose tissue (At). H&E stain .10x

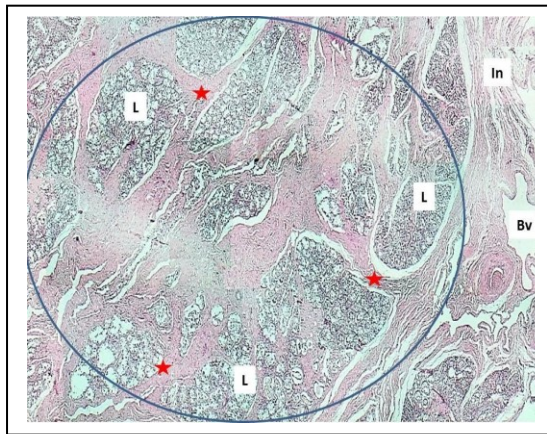


Figure 9: Section of mammary quarter (lactating ewe) shows mammary lobes (Oval shape areas), mammary lobules (L), inter lobular connective tissue (Asterisks) and thick inter lobular vascular connective tissue (In) containing small artery & vein (Bv). H&E stain. 40x

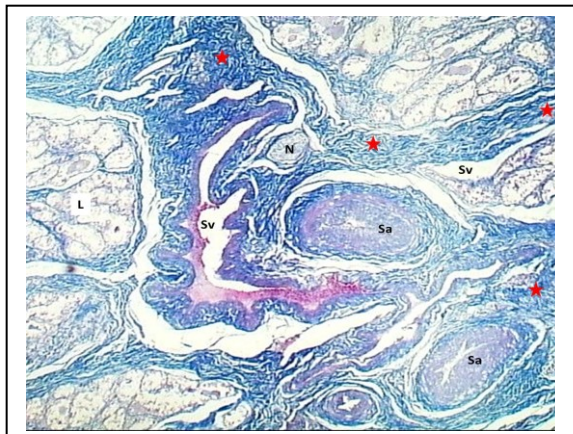


Figure 10: Section of inter lobular connective tissue (lactating ewe) shows; lobules (L), small size artery (Sa), small vein (Sv), collagenous connective tissue (Asterisks) & nerves (N). Masson's trichrom stain. 40x.

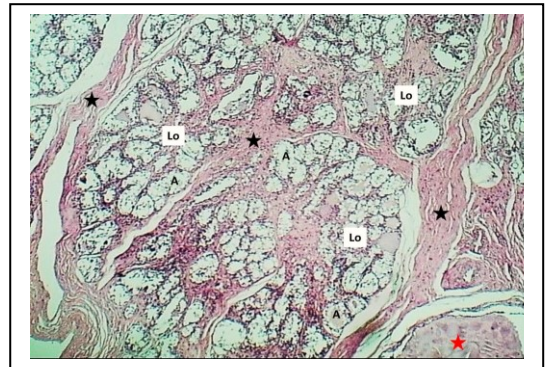


Figure 11: Section of mammary lobe (lactating ewe) shows; lobules (Lo), mammary alveoli (A), inter lobular small vein collagenous connective tissue (Red asterisks) & inter lobular milk duct (Red asterisk). H&E stain.40x

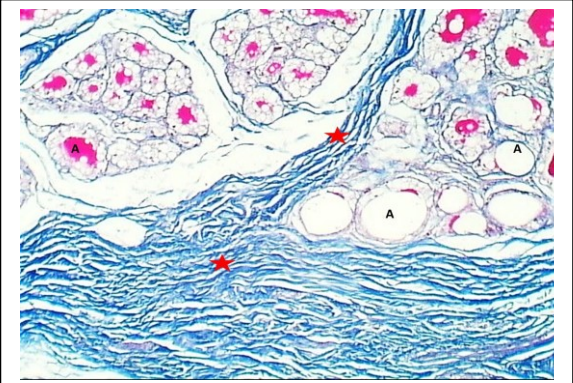


Figure 12: Section of mammary lobe (lactating ewe) shows; mammary alveoli (A), inter lobular collagenous connective tissue (Red asterisks). Masson's trichrom stain. 100x

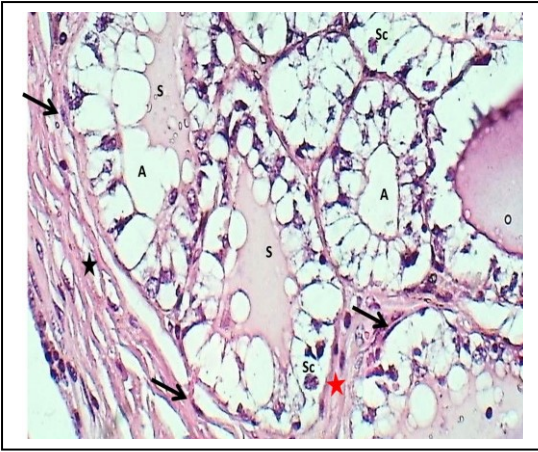


Figure 13: Magnified section of mammary lobule (Lactating ewe) shows; inter lobular connective tissue (Black asterisk), inter alveolar connective tissue (Red asterisk) , mammary alveoli (A), milk secreting cells (Sc) milk secretion (S) & myo epithelial cells (Arrows). H&E stain. 400x

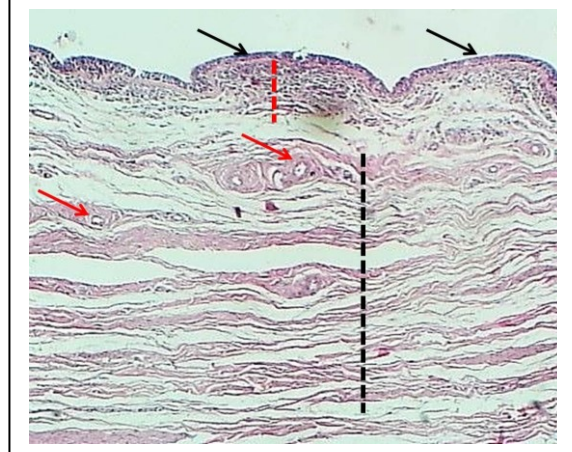


Figure 14: Section of glandular cisterna (Lactating ewe) shows; irregular mucosal surface (Black arrows), lamina propria (Red dash line), vascular fibro muscular tissue (Black dash line) & blood vessels (Red arrows). H&E stain. 40x

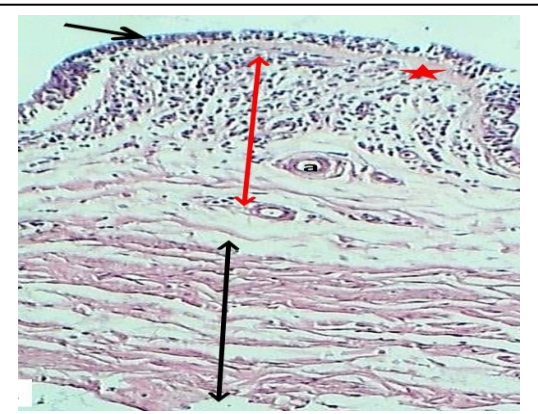


Figure 15: Section of cisternal mucosal fold (Lactating ewe) shows; epithelium (Black arrows), (E), sub epithelial collagen layer (Asterisk), lamina propria (Red double head arrow) fibro muscular layer (Black double head arrow) . H&Estain. 100x

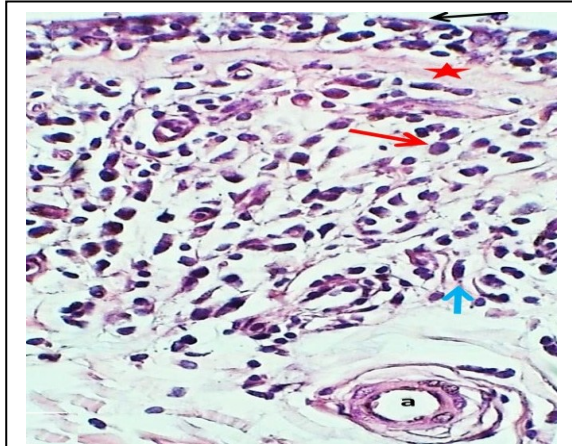


Figure 16: Section of cisternal mucosa (Lactating ewe) shows; epithelium (Black arrows), (E), sub epithelial collagen layer (Asterisk), lamina propria with lymphocytes (Red arrow) & fibrocyte (Blue arrow), & arteriole (a) . H&Estain. 400x

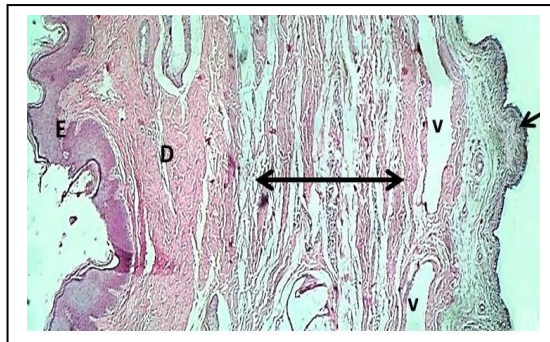


Figure 17: Section of teat canal (Lactating ewe) shows; skin epidermis (E) dermis (D) cutaneous sweat and sebaceous gland (g) fibro muscular connective tissue (Black double head arrow), & mucosal folds (arrows) , venous plexus (V) & orifice (Or.) H&E stain.40x

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