Histological study of the constituents that related to the immune defensive mechanism in the vagina of ewes

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Summary

In order to study the vaginal defensive mechanisms in ewes, vaginal smears and biopsies were collected from eight adult Awasi ewes. The biopsies samples were processed by the routine histological methods and stained by H & E and PAS stains, while the smears samples were stained with MB. Samples were examined under light microscope.

The resent study revealed that the vaginal wall lacks many important constituents, among these were the vaginal glands, goblet cells, muscularis mucosa and lymphatic nodules. And as the vagina was the nearest organ to the external environment and as it receives the male copulatory organ, so it is more liable organ to be infected by external pathogens. On the contrary, the vagina has special compensatory histological mechanisms i: e, its epithelium was thrown into deep folds which serve to increase the surface area and in turn raise the epithelial efficiency. The study suggested that these folds were a remnants formed as a result of a failure during embryonic development of the glands as the gland formed by invagination of the epithelium. Moreover, the vaginal wall had a thick basement membrane. It appeared segmented due to accumulation of the defensive cells on some parts of it during their migration from the blood vessels to the epithelium. Besides, the vagina contained a great numbers of defensive cells, such as neutrophils, macrophages, lymphocytes, plasma cells and mast cells. In spite of the relation of dendritic cells with immune defense, this study couldn't recognize them by using only the general stains.

On the other hand, the vaginal smears demonstrated that the vagina had many defensive cellular mechanisms among these, the process of keratinization of the vaginal epithelium; the process of sheet formation which lining the vaginal lumen; the presence of apparent junctional complexes which coalesce the cells of the sheet formation. These junctional complexes close completely the intercellular spaces leading to prevent any entrance of any foreign materials and pathogens to the underlying tissue. Some vaginal cells showed a pale foamy (vacuolated) appearance. Vacuolation was another defensive phenomena, it was indicative of increase cellular activity. Moreover, the recent study reported the process of metachromasia which is associated with cellular activity in protein synthesis, keratin, finally this study referred to the important of endogenous microorganisms which act to convert the cellular epithelial glycogen into lactic acid. The latter act to decrease the pH of the vaginal lumen and prevent the pathogenic bacteria from proliferation in the acidic environment.

It is concluded that all the above cellular constituents and mechanisms support the vagina with an adequate adaptation enable it to raise its immune defensive response.

دراسة نسيجية للتراكيب المرتبطة بالآلية الدفاعية المناعية في مهبل النعاج

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الخلاصة

لأجل دراسة الآليات الدفاعية المناعية في مهبل النعاج العواسية، تم أخذ مسحات وخزع مهبلية من ثمان نعاج عواسية بالغة. تم معاملة عينات الخزع بالطرق النسيجية الروتينية ثم صبغت بصبغتي الهيماتوكسلين والأيوسين (Hematoxylin and Eosin) وصبغة كاشف شف الدوري (Periodic Acid-Shiff reagent)، أما المسحات المهبلية فقد صبغت بصبغة المثيلين الأزرق (Methylene blue)، فحصت جميع العينات تحت المجهر الضوئي.

كشفت الدراسة أن المقاطع النسيجية لجدار المهبل تميزت بخلوها من بعض التراكيب المهمة مثل الغدد المهبلية، الخلايا الكأسية، عضلية المخاطية والعقيدات اللمفاوية. علاوةً على ذلك فأن موقع المهبل الأقرب إلى المحيط الخارجي ولكونه الممر لدخول القضيب يجعله أكثر الأعضاء عرضة لدخول الجراثيم هذا من ناحية، ومن ناحية أخرى كشفت الدراسة أن المهبل أنفرد بأمتلاك وسائل دفاعية تعوضه عن التراكيب المفقودة أعلاه حيث أمتلكت ظهارته طيات عميقة تعمل على زيادة المساحة السطحية للظهارة وبالتالي تزيد من كفائتها، وتعتقد الدراسة أن تلك الطيات هي بقايا جنينية تكونت من أنبعاج داخلي للظهارة خلال مراحل تكون الغدد. كذلك أمتلك جدار المهبل غشاء قاعدي سميك لكنه يبدو وكأنه متقطع (Segmented) بسبب كثرة الخلايا الدفاعية المترسبة على بعض أجزاءه خلال هجرتها من الأوعية الدموية تجاه الظهارة. أمتلك المهبل كذلك خلايا دفاعية مثل العدلات (Neutrophils)، الخلايا البلعمية (Macrophages)، الخلايا اللمفية (Lymphocytes)، الخلايا البلازمية (Plasma cells) والخلايا البدينة (Mast cells). برغم علاقة الخلايا التشجرية (Dendritic cells) لمناعة الجسم فأن الدراسة لم تستطع من تمييزها لأنها تحتاج إلى صبغات مناعية خاصة. من ناحية أخرى فأن المسحات المهبلية أظهرت آليات تميز بها المهبل وكانت سبباً في زيادة وسائله الدفاعية مثل عملية تقرن (Keratinization) خلايا الظهارة وعملية التراصف الخلوي (Cellular sheet formation) التي تعمل على تكوين صفيحة (Sheet) متراصة تبطن تجويف المهبل أضافة لوجود أرتباطات خلوية معقدة (Junctional complexes) بارزة تعمل على ربط الخلايا ببعضها وغلق المساحات بين الخلوية وبالتالي منع دخول المواد الغريبة والجراثيم من تجويف المهبل إلى النسيج التحتاني. تميزت بعض خلايا المسحات المهبلية بالمظهر الرغوي (Foamy) وهي ظاهرة (Vacuolation) التي تعتبر دليلاً على النشاط الخلوي، كذلك بينت الدراسة وجود حالات التحول اللوني (Metachromasia) التي تعتبر هي الأخرى أحدى حالات النشاط الخلوي في تصنيع البروتين (الكيراتين). أشارت الدراسة كذلك لأهمية وجود الجراثيم المتوطنة (Endogenous microorganisms) كوسيلة دفاعية حيث تقوم بتحويل الكلايكوجين المتكون في خلايا المهبل الظهارية (خلال هجرتها من القاعدة إلى السطح) إلى حامض اللاكتيك الذي يعمل على خفض الأس الهيدروجيني للمهبل وبالتالي عمل وسط حامضي يمنع تكاثر الجراثيم فيه.

تستنتج الدراسة الحالية أن الآليات والخلايا المتنوعة أعلاه عوضت المهبل عن بعض النواقص التركيبية الموجودة به وزودته بالتكيف اللازم ومكنته من رفع أستجابته المناعية.

Introduction

Fortunately nature provided mechanisms whereby living organisms are sustained in the presence of pathogens. Since the vaginal mucosa lacks glands, it undergoes compensatory different mechanisms. That include cellular infiltration (1 and 2). Many of these cells (neutrophiles and macrophages) migrate from the lamina propria to the epithelium, other cells (lymphocytes, plasma cells and mast cells) deposits in the lamina propria (3). Under the influence of estrogens, the epithelial cells synthesize and accumulate glycogen as migrate toward the surface (4). Previous studies have clearly investigated the cytological changes which occur in the vaginal epithelium of several animals species during the successive stages of the estrus. In ewes, changes in the vaginal smears were studied by (5 and 6). The immune function in the reproductive tract is important for health and maintains a high rate of fecundity (7). Cells of the immune system function to prevent establishment of infection from microorganisms, and to clear cancerous or damaged cells in the host (8).

The vaginal epithelium is of stratified squamous type and protected from microorganisms by phagocytized cells and the innate immune system (9). Phagocytosis can be stimulated by other components for antibodies produced by B lymphocyte. T-lymphocytes produced cytokines can activate phagocytes and make them more effective (10 and 11). The phagocytes occur by macrophages, neutrophils and dendritic cells (3). Lymphocytes are found in the stroma and not in the epithelium (12). Functionally the lymphocytes provides immunological defense for the body. Normally, large numbers of lymphocytes are found in the connective tissue throughout the body, the number increases at sites of tissue inflammation caused by infectious agents and foreign bodies (13 and 14). Plasma cells are derived from B lymphoctes and are responsible for the synthesis of the antibodies, to neutralize harmful effects caused by antigens. They are round or oval cells having a granular cytoplasm with eccentric "cart-wheel" nucleus. They are commonly present in mucosal and submucosal coats of the vagina. Plasma cells are prominent constituent of loose connective tissue where antigens are most capable of entering the body. The plasma cells have only limited migratory ability (15). Mast cells are oval or round cells with small, centrally located nuclei. They are found beneath the epithelium in areas rich in connective tissue and around blood vessels. Mast cells function in inflammatory response, innate immunity and tissue repair (16).

Vacuolation facilitate metabolism perform functions such as storage, ingestion, digestion, excretion and expulsion of excess water (17, 18 and 19). Recent investigations indicate that keratinocytes produce immunogenic molecules and probably active in the immune process (20). Evidence also shows that these cells are capable of producing several interleukins, colony-stimulating factors, interferon's, tumor-necrosis factors as well as platelets and fibroblast-stimulating growth factor (3 and 21). On the other hand, resident microflora are present in the vagina that do not lead to any clinical problems (22). Cervical secretions contain substances that inhibit bacterial growth (23). However, this

study was conducted to focus the light on the effect relation between the cellular components of the vaginal epithelial and its relation to immune system.

Materials and Methods

Vaginal tissue samples (biopsy) were collected from apparently healthy eight ewes, fixed immediately by neutral buffered formalin 10%, and routinely processed. Each specimen was embedded in paraffin, and 5-7 µm sections were prepared for staining with Hematoxylin and Eosin (H &E) and Periodic Acid-Shiff reagent (PAS) stains. This was done according to (24). Vaginal smears were collected. A clean, dry spatula after a clean, dry vaginal speculum was used. Vaginal materials was spread on the surface of a glass slide after one drop of normal saline solution was added according to the method of (25). The smears were stained directly with Methylene blue (MB) and microscopically examined. This was done according to the method of (26). Biopsy was done at the time the vaginal smears were collected, but the latter was always done first. Morphologic studies were performed using computerized camera coupled to a light microscope.

Results

The present study revealed that the vaginal wall has a special structural characteristics, it lacks many constituents like the glands and the muscularis mucosa. Accordingly a compensatory defensive mechanisms have been observed, among these were: cellular and cytological changes, leukocytic and cellular infiltration, vacuolation, keratinazation and microflora deposition (Fig. 1). On tissue samples examination, the present results found that the vaginal mucosa was thrown into numerous deep folds (Fig. 2). The wall of the vagina contains neither glands nor muscularis mucosae. The lamina propria was full of cellular elements. The cells of the basal layer were irregularly arranged. The basement membrane appeared clear in areas devoid of cellular infiltration (Fig. 3 and 4) and disappeared in area covered by cellular infiltrations (Fig. 5) so the basement membrane appeared segmented. The recent study didn't recognize goblet cells and lymphatic nodules.

In the lamina propria the present results showed that the blood capillaries stand out clearly (Fig. 6). Upward cellular migration of neutrophils and macrophages from the blood vessels toward the epithelium (Fig. 7). Cellular infiltration of lymphocytes, plasma cells and mast cells were present (Fig. 8 and 9). The exfoliated cells of the vaginal mucosa has a large number of neutrophils and other leukocytes which formed an important part of the innate immune defense in this region. Neutrophils characterized by colorless background of the cytoplasm and by the multilobed nuclei (Fig. 10 and 11). Neutrophils migrate into the connective tissue of vagina, followed by large numbers of monocytes which differentiate into macrophages. The elicited macrophages were easily distinguished than resident one because of the presence of numerous short thick pseudopodia and ingested foreign materials in

their cytoplasm. They have kidney shaped nuclei (Fig. 8). The lymphocytes were characterized by their deep-staining round nuclei. They frequently present in the lamina propria and near the stratum basali of the epithelium. The plasma cell was another cell type that participates in host tissue defense, this cell was round to ovoid and characterized by the eccentrically placed cartwheel largely heterochromatic nuclei (Fig. 8). Mast cells were large, oval or round connective tissue cells, whose cytoplasm was filled with basophilic secretory granules, the nucleus was small spherical and centrally located. Mast cells occured near small blood vessels (Fig. 9). Dendritic cells were not detected in this study. The presence of vacuolated have been successfully identified in this work (Fig. 12). The vaginal smears examination demonstrates clearly that there were significantly increase in the process of keratinization, (Fig. 13 and 14). Besides the flattened polygonal vaginal cells were start to accumulate, coalesce and tightly fused to each other leaving no intercellular spaces forming broad sheets or packets which line the vagina (Fig 15). These cells were fused by clear junctional complexes at the periphery (Fig. 16). Fig. (17) Shows a higher magnification of the endovaginal linning exposed to a relatively high population of microorganisms. On the other hand, the present result revealed that the colour of both cytoplasm and nuclei of the exfoliated vaginal epithelial cells were gradually shifted, metachromatic, from the basic to reddish or purple colour (Fig. 18)

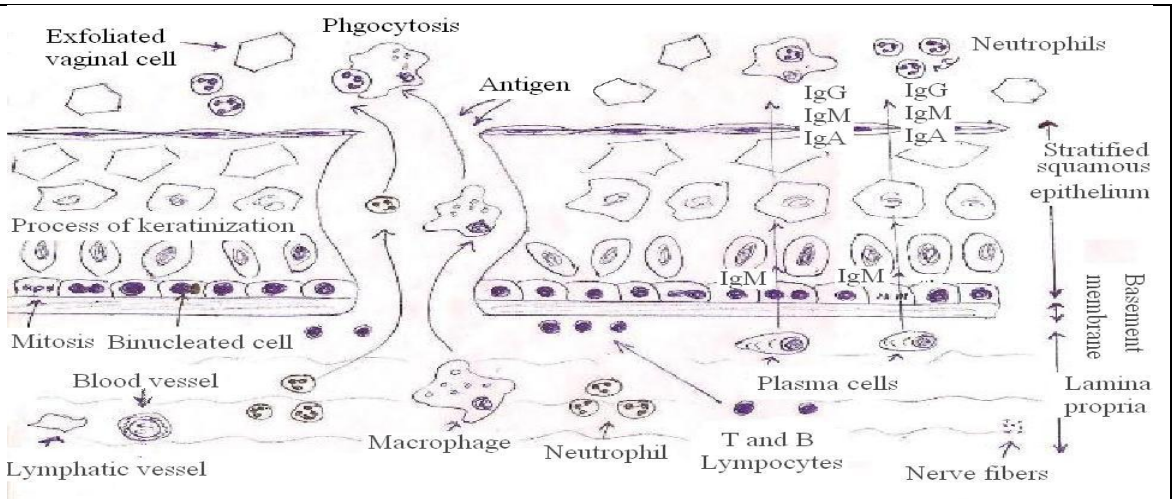

Fig. 1: Diagram of the mucosal immunity in the vagina.
(Modified from Junqueira LC & Carneiro J (2003). Basic Histology. 10th ed. A Lange Medical Book)

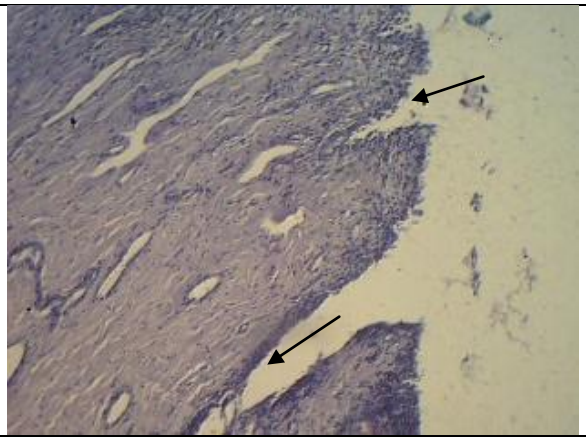

Fig. 2: Deep mucosal folds in the vagina (arrows). PAS stain. 100X.

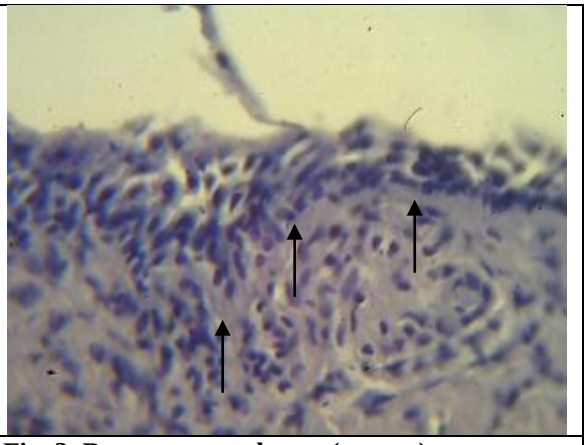

Fig. 3: Basement membrane (arrows). PAS stain. 400X.

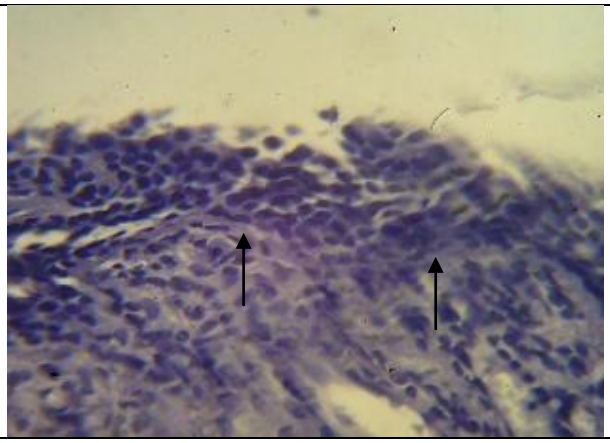

Fig. 4: Basement membrane (arrows). PAS stain. 400X.

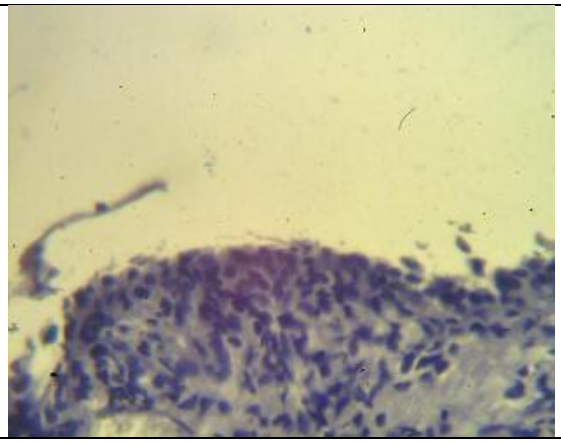

Fig. 5: Absent of Basement membrane. PAS stain. 400X.

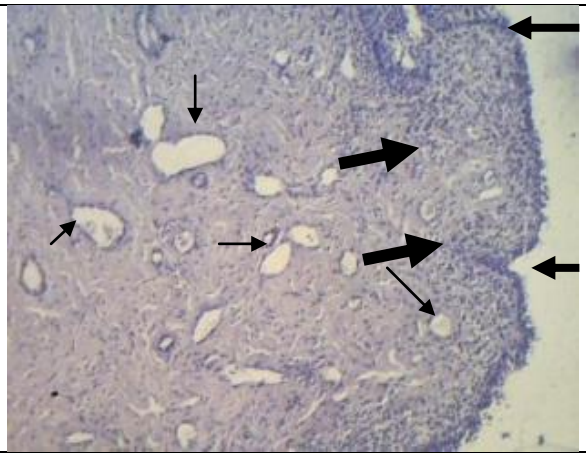

Fig. 6: Blood vessels (small arrows), with upward cellular (large arrows) migration. Mucosal folds (medium arrows). H & E stain. 100X.

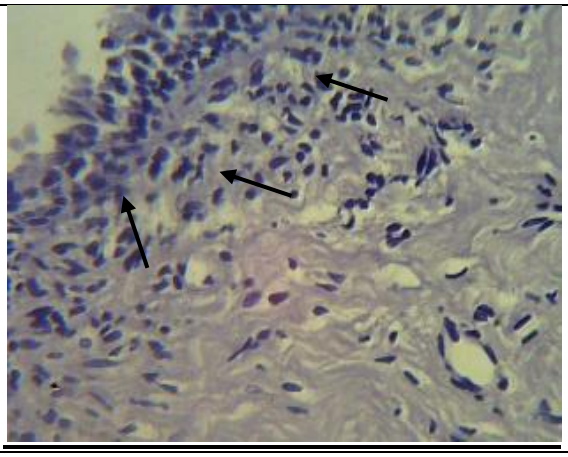

Fig. 7: Arrows pointed to upward migration of the defensive cells. H & E stain. 1000X.

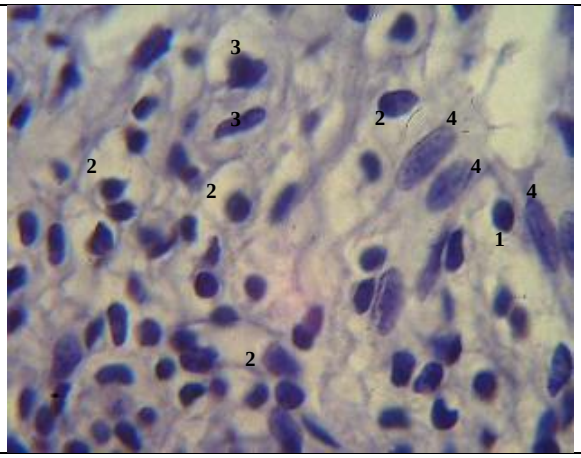

Fig. 8: Lymphocyte (1), plasma cells (2), macrophage (3) and muscle cell fiber (4). H & E stain. 1000X.

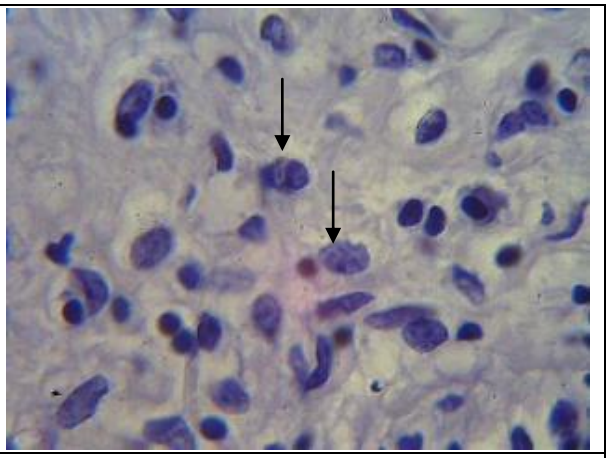

Fig. 9: Arrows points to the mast cell at the periphery of blood vessel. H & E stain. 1000X.

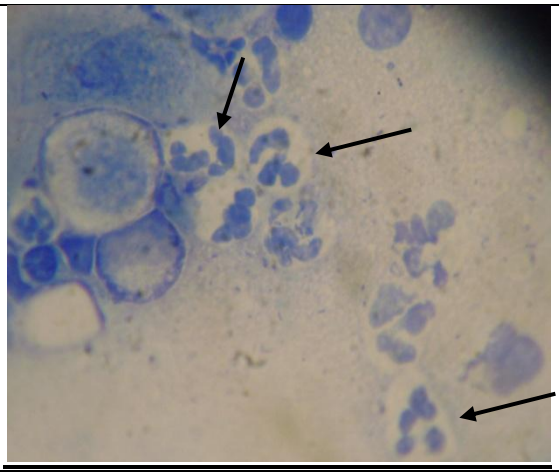

Fig. 10: Neutrophilic infiltration (arrows). M B stain. 1000X.

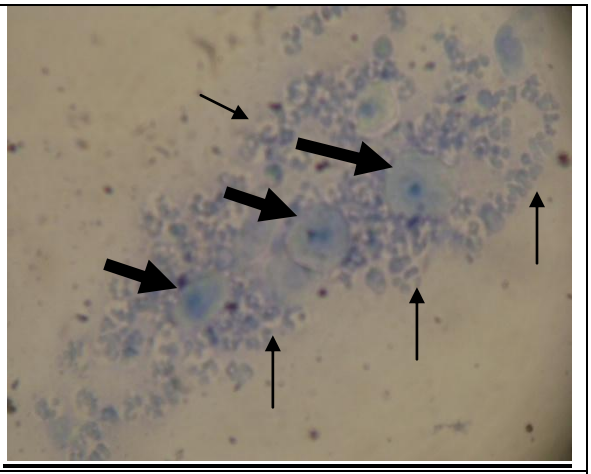

Fig. 11: Neutrophilic infiltration (small arrows) surrounding the exfoliated vaginal cells (large arrows). M B stain. 1000X.

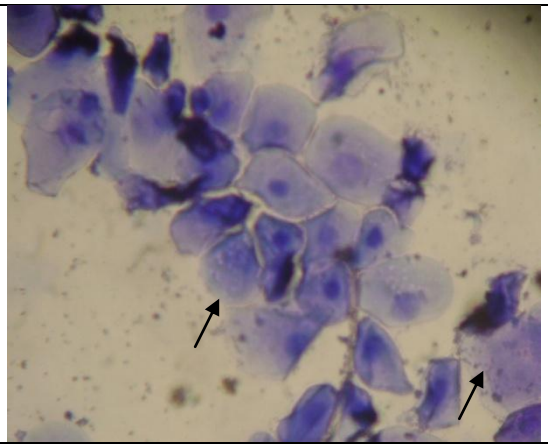

Fig. 12: Arrows pointed to the presence of Vacuolation in vaginal smears. M B stain. 400X.

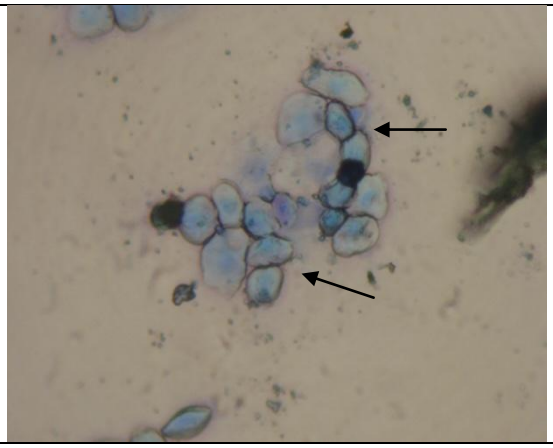

Fig. 13: Keratinization in the vaginal smears showing thickening at the plasma membrane (arrows). M B stain. 400X.

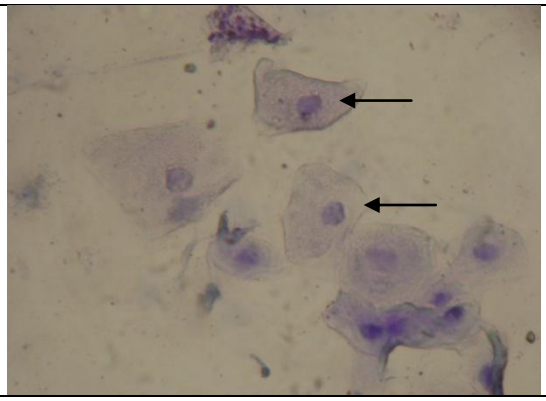

Fig. 14: Keratinization and pyknotic nuclei (arrows) in the vaginal smears. M B stain. 400X.

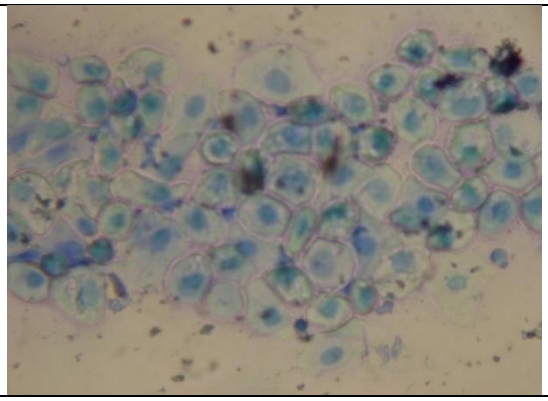

Fig. 15: Sheet formation in vaginal smears. M B stain. 400X.

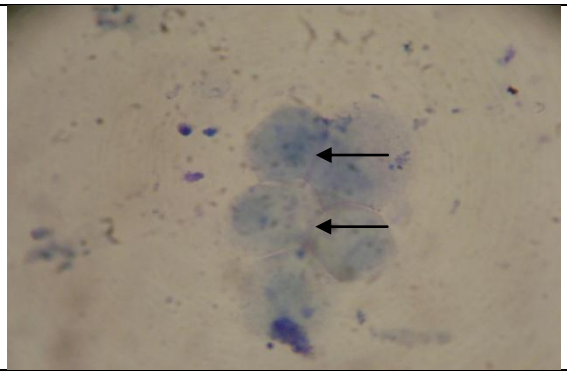

Fig. 16: Presence of cellular junctional complexes in vaginal smears (arrows). M B stain. 1000X.

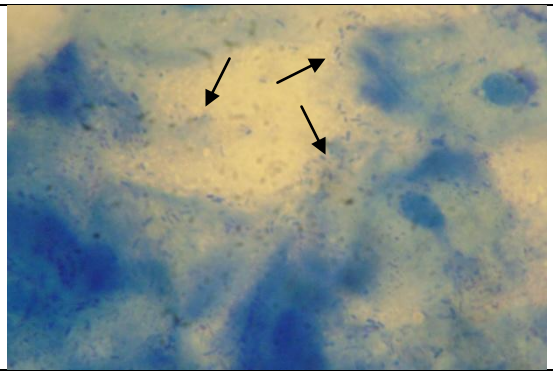

Fig. 17: Presence of bacteria in vaginal smears (arrows). M B stain. 1000X.

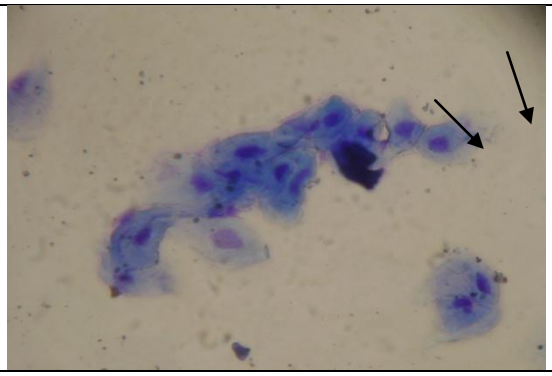

Fig. 18: Metachromasia of vaginal smears showing different cellular colours (arrows). M B stain. 400X.

Discussions

The external position of the vagina at the entrance of female genital system leading the lumen of the vagina to be opened to the external environment, besides, the vagina receives the male copulatory organ that give rise to introduction of different pathogens, moreover the lack of many defensive constituents from the wall of the vagina elicited many compensatory defensive mechanisms to occur (27). The mucosal folds of the vagina were increased and becomes markedly expanded and folded to provide a suitable way for increase immune response by increasing the relative number of leukocytic and defensive cell infiltration. This study firstly referred to the relation between these deep

mucosal folds and the increased defensive mechanisms. During embryonic development, glands were formed of epithelial invagination and cellular specialization to synthesize and secrete specific glandular product. The absence of such vaginal glands may indicates a failure during gland development. Moreover, the recent study did not referred to the presence of mucosal goblet cells or lymphatic nodules. This is in variance with the statement of (13) who referred to the presence of these both constituents in the cow. besides, this study was not in agreement with the statement of (28) who reported that the vaginal epithelium undergoes epithelial proliferation and projection into the underlying stroma because the epithelium commonly showing increase in thickness and not proliferative cellular activity. The muscularis mucosae forms the boundary between the mucosa and submucosa. The present study firstly revealed that the absence of the muscularis mucosae from the wall of the ewes vagina may facilitate the upward migration of the different defensive cells from the lamina propria toward the epithelium. There has been much controversy about the presence of the basement membrane in the vagina. Numerous authors neglected the reference to the basement membrane (13 and 29). Other authors did not refer clearly to the presence of this membrane (14) who stated that some epithelia rest on a basement membrane, while (30) reported that all epithelial tissue rest on a basement membrane. (31) confirms that the epithelium rests directly on a lamina propria. Our hypothesis declared that in areas devoid of cellular infiltration, the basement membrane appeared very clear, whereas in other areas where it is hidden by heavy cellular infiltration this membrane disappears. This phenomena misleading the above authors. (32) was the only who referred to the presence of this membrane in the vagina of the human. Our result firstly confirms the presence of such basement membrane in the vagina of the ewe. This membrane plays an important roles in cellular growth, proliferation, differentiation and pathway (15). The increase in vascularity of lamina propria lead to increase cells related to immune defense mechanism. The clearly standing out capillaries demonstrating the abundant blood supply of the underling tissue of the vagina. These capillaries provide metabolites, vitamins, growth factors, phagocytized cells and antibodies to the devoid-glands vaginal tissue (33).

Our recent study disclosed the great infiltration of neutrophils in the vaginal lumen as first line of defense. This finding in accordance with the findings of (5, 6 and 34) who stated that the presence of neutrophils in ewes increases the immunity of the epithelium by phagocitizing any bacteria and small particles. The present study was in variance with (28) who referred to the presence of leukocytes among the epithelial cell. The activated macrophages migrate in response to chemotatic stimuli and engulf and kill bacteria by process generally similar to those occurring in neutrophils (4 and 22). The recent study is similar to the statement of (12, 35 and 36) who referred that lymphocytes are found only in the stroma and not in the epithelium of ewes. Moreover, plasma cells secret large quantity of antibodies into the general circulation (16). Mast

cells are also found in areas rich in connective tissue and they are present beneath epithelial surface (16). Mast cells functions in inflammatory response, innate immunity and tissue repair. They found around the blood vessels. They are oval or round cells with small central nuclei. In spite of its important role in immune defense, our results did not clearly recognized the dendritic cells because it is difficult to identified in routinely stained sections, it appeared only in immunostained sections (14). The vacuolation of the cells may be due to glycogen consumption during metabolic activity. This is similar to the conclusion of (37) who found that the vacuoles excrete secretory product of different density. Moreover, (38) stated that the vacuolation are typical in the ovarian activity. The pale empty appearance of the vaginal epithelium cells is due, in part, to the fact that the cells accumulate glycogen during their migration from the basal layers toward the superficial layers and in the preparation of routine Hematoxylin and Eosin section, the glycogen is lost as it is water-soluble substance. When the vaginal cells desquamate, bacteria metabolize glycogen to lactic acid, causing a relatively low pH within the vaginal lumen which helps provide protection against pathogenic microorganisms. This is in accordance with (14). We can regard the process of keratinization as a defensive mechanism in the vagina. The function of keratinization was the production of keratin and the creation of an extracellular barrier. The importance of such mechanism was to protect the vaginal epithelium from invasion of microorganism to underlying tissue and finally raise the immunity of that epithelium. This finding was in accordance with the finding of (21 and 39) who stated that keratinization activate immunization.

The current study firstly declared that the sheet formation and the intercellular junctional complexes has a role in raising the immune defense, by serving to close the spaces between the vaginal linning cells to prevent the flow of materials between the cells and the underling tissues. This agrees partly with the finding of (40) during the study of keratinization in the vagina of ewe. This study referred to the importance of the endogenous bacteria which metabolize the glycogen into lactic acid in decreasing the pH of the vagina and protect it from the pathogenic bacteria. This is in accordance with the finding of (39, 40 and 41). Moreover, the herein study has been found that the cytoplasm of the vaginal epithelial cells with its nuclei undergo metachromasia, i. e. the tissue colour shifts from blue to red or purple. Cells and tissues have a high concentrations of rough endoplasmic reticulum will exhibit metachromasia which is important in forming the protein, keratin during the process of keratinizatin through its upward migration in the vagina. This is similar in part to the result of (42) who stated that the metachromasia affected by the hormonal and ovarian activity.

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