The Neutrophilic Cellular Penetration

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Abstract

Vaginal smears have been taken from Awassi sheep in 2006. The smears were stained by methylene blue. The relation between the exfoliated vaginal cells and the neutrophils were examined under light microscope. Firstly, this study revealed that the neutorphils were unable to phagocytize the larger size cells directly, however they can attack and kill them according to the theory of "neutrophilic cellular penetration" registered firstly in this study. This theory containes five steps. The direct process of true phagocytosis of these large cells have been done by another larger phagocytic cell.

"Neutrophil" الأختراق الخلوي للخلية العدلة

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الخلاصة

أخذت مسحات مهبلية من نعاج عواسية عام 2006. صبغت المسحات بالمثيلين الأزرق. فحصت العلاقة بين الخلايا المهبلية المنسلخة والخلايا العدلة تحت المجهر الضوئي. أظهر ت الدراسة لأول مرة أن الخلايا العدلة غير قادرة على بلعمة الخلايا المهبلية المنسلخة الكبيرة الحجم مباشرةً ومع ذلك فأنها تستطيع أن تهاجمها وتقتلها بموجب نظرية الأختراق الخلوي للخلية العدلة التي تسجل لأول مرة في هذه الدراسة والتي تتضمن خمس خطوات. أن عملية البلعمة الحقيقية المباشرة للخلايا الكبيرة تتم بواسطة خلايا بلعمية أخرى كبيرة الحجم.

Introduction

Phagocytosis is the process by which bacteria, small particles and dead tissues are engulfed by neutorphils. Phagocytosis is more commonly used as a defense mechanism. Neutrophils contain two types of cytoplasmic granules, these granules contain the bacteriostatic and bacteriocidal agents. As a result of tissue injury, neutrophils migrate from the blood stream into the connective tissue in huge numbers followed by monocytes which thought to secrete a various enzymes and neutralized substances such as antibodies. Large cells and particles may engulfed by other larger cells, the macrophages which fuse together to form giant cells, that are capable of phagocytizing large particles, such as yeast cells, tubercle bacill, fungi and neutrophilic leukocytes in inflammation (1-5). Neutrophils advanced by an ameboid movement, usually with the nucleus in the rear and leave the blood in a random fashion (1,6). Many authors classified the endocytosis into two types. i.e., phagocytosis which deals with the solid particles, and the pinocytosis that deals with the fluid materials (2, 4 and 7). In case of more rapidly moving neutrophils, engulfment is facilitated by the passage of neutrophil over the target cell, histiocytes with less rapid locomotion come in contact with the

material to be engulfed by sending out pseudopodia which adhere to the debris and surround it either by drawing it toward the histiocyte or by expanding the pseudopodium (4,6), While, Dudek (8) demonstrated that neutrophils phagocytize dead cells by using antibody receptors, complement factors and bacterial polysaccharides to bind to the foreign material, the attachment of antibody to antigens may enhance phagocytosis (opsinazation) or precipitate complement activation, resulting in chemotaxis of neutrophils and even lysis of the invader (diagram 1). Neutrophils must bind to the foreign material to being phagocytosis. On the other hand Dudek (8) stated that the fluidity of the cell membrane was affected by many factors. i.e., elevated temperature and unsaturated fatty acids which increase the fluidity while elevated the level of cholesterol will decrease this fluidity. Under the need of many factors. The cell membrane opens channels for transporting hormones, proteins, receptors and ions exchange between the interior and exterior of the cell membrane (1, 5, 8 and 9).

Hussain and Danel (10) found that the mean percentage of neutrophils in the uterine fluid of the cow activity phagocytizing peaked at the 5^{th} day of postpartum and the phagocytic activity of leukocytes seems to play a crucial role in stopping the establishment of bacterial infection in the uterus immediately after calving and there after.

(11) and (12) also demonstrated that 16 days after parturition was characterized by the presence of numerous waves of neutrophils and concluded that these changes were induced by the hormonal changes of the animals. On the other hand (5) reported that plasma factors act on the bacteria to make them "tasty" to the phagocytes opsonization.

Little is known concerning the mechanism dealing with the phagocytosis of large cells. The present study was the first approach to the process of neutrophilic attacking to the exfoliated vaginal cells from the histological site of view.

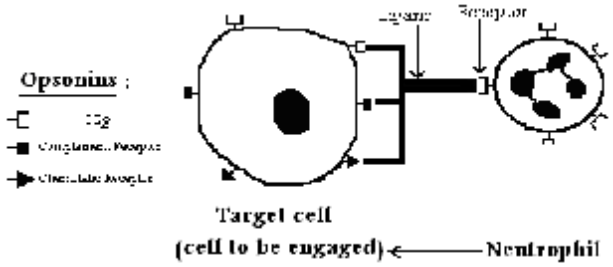

Diagram (1) Illustrates the process of attachment between neutrophilic receptors and the ligand formed from the receptors of the cell to be engaged

Materials and Methods

Fifteen healthy Awassi ewes have been borrowed from the animal farm of the college of veterinary medicine, Baghdad University in 2006. The vaginal smears were taken according to Heama (13). The smears were stained with methylene blue (14). The neutrophilic attack of the exfoliated vaginal cells were studied histologically under light microscope.

Results

Firstly, the present study revealed that neutrophil alone was unable to phagocytize larger size cell (target cell) by the ordinary process of endocytosis "internal killing", so that one or more neutrophils surround and attack such larger cell and try to destroy it firstly by killing its nucleus "external killing", then do penetrate the target cell it self. Both neutrophil and the target cell may die through this struggle according to the

"theory of neutrophilic cellular penetration" which is the most striking result of this study. We can divide this theory into five steps:

1- The step of migration: In response to the exfoliation of the vaginal mucosa, neutrophils rapidly migrated from the blood stream to the mucosa. The movement of the activated neutrophils occurred firstly as along stands, then at the site of exfoliation they spread randomly among and around the exfoliated vaginal cells (Fig. 1 and 2).

2- The step of engagement: Cells too large to be engulfed by one neutrophil were surrounded and attacked by more than one neutrophil (Fig. 3). The activated neutrophils then attached to the cell membrane of the target cells and tried to push this membrane in order to open a window in it. In response to this pressure, the cell membrane envaginated (Fig. 4, 5 and 6).

3- The step of "nuclear killing": The nucleus of the target cell was ruptured, it was common to see the nucleoplasm distributed throughout the cytoplasm. Neutorphil attacked the nucleus of the target cell before entering inside the cell itself (Fig. 7). Clear cells may be seen surrounded by one or more neutrophils. The clear cell was appear to be devoid of nucleus and other cytoplasmic contents (Fig. 8).

4- The step of penetration: As a result of the continuous pressure of neutrophil on the cell membrane of the target cell it responded to this pressure and opened a channel to pass through inside the cytoplasm. So that it was common to see engulfing neutrophil inside the cytoplasm of the attacked target cell (Fig. 9). No neutrophil have been seen over the target cell.

5- The step of "struggle for existence": Struggle between the penetrated neutrophil and the target cell occurred. Cells from both sides may die through this struggle (Fig. 10 and 11). Typical apoptosis was seen which have a condensed nuclei and vacuolated cytoplasm (Fig. 7 and 12) and fragmentation of the cytoplasm. Monocyte appeared in the site of exfoliation near neutrophils (Fig. 13). Other cells like lymphocytes, eosinophils, basophils and fibroblast were not seen in this process.

As a result of the struggle between the neutrophils and the exfoliated vaginal cells, small particles of dead tissues were seen. It is clear to see other neutrophils and macrophage to phagocitize these small particles (Fig. 14 and 15) and bacteria (Fig. 16).

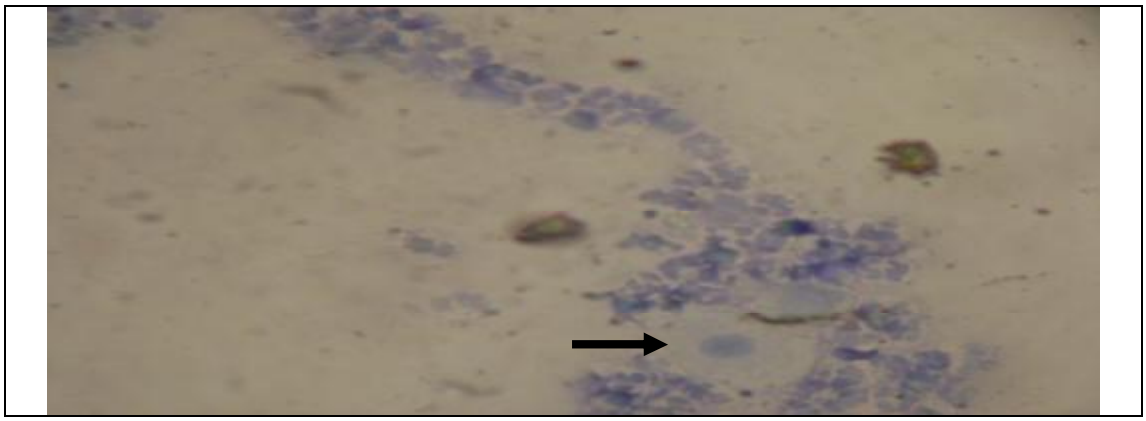

Fig. (1) Step of neutrophilic migration. The neutrophils migrate as strand (upper) and scattered randomly among the exfoliated vaginal cell (lower). Methylene blue stain. 400X

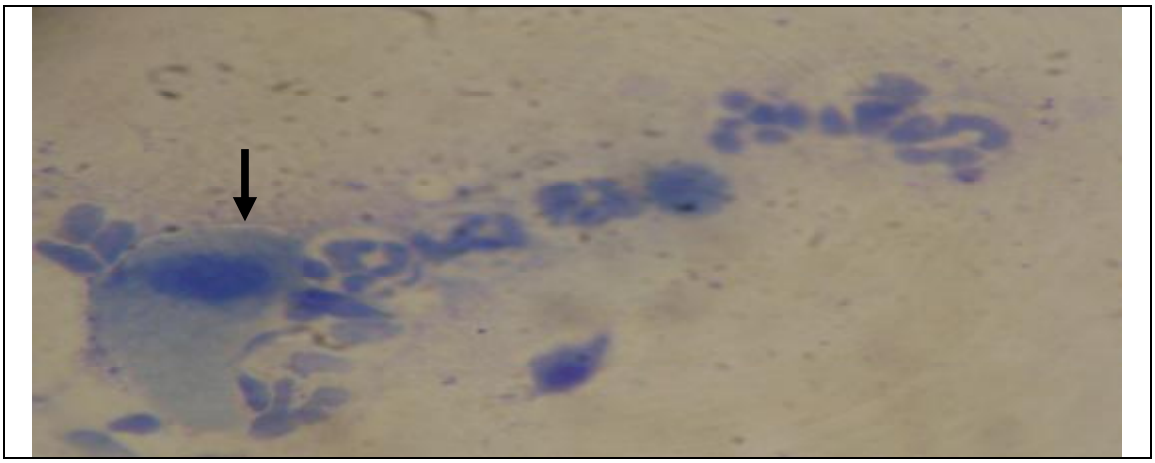

Fig. (2) Step of neutrophilic migration. The neutrophils oriented as strand and then scattered randomly around the exfoliated vaginal cell (arrow). Methylene blue stain. 1000X

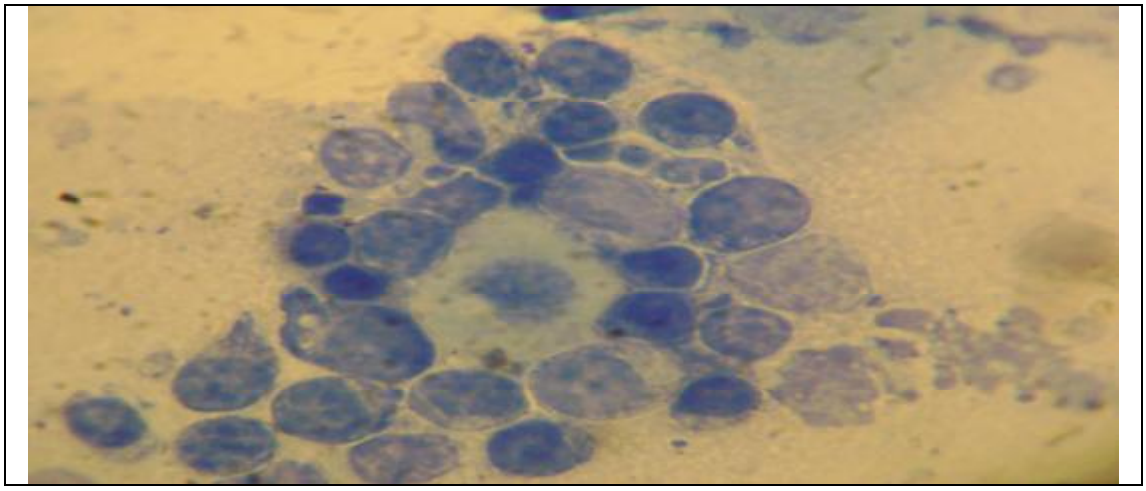

Fig. (3) step of engagement. Numerous neutrophils encircle and engage vaginal exfoliated cell. Methylene blue stain. 1000X

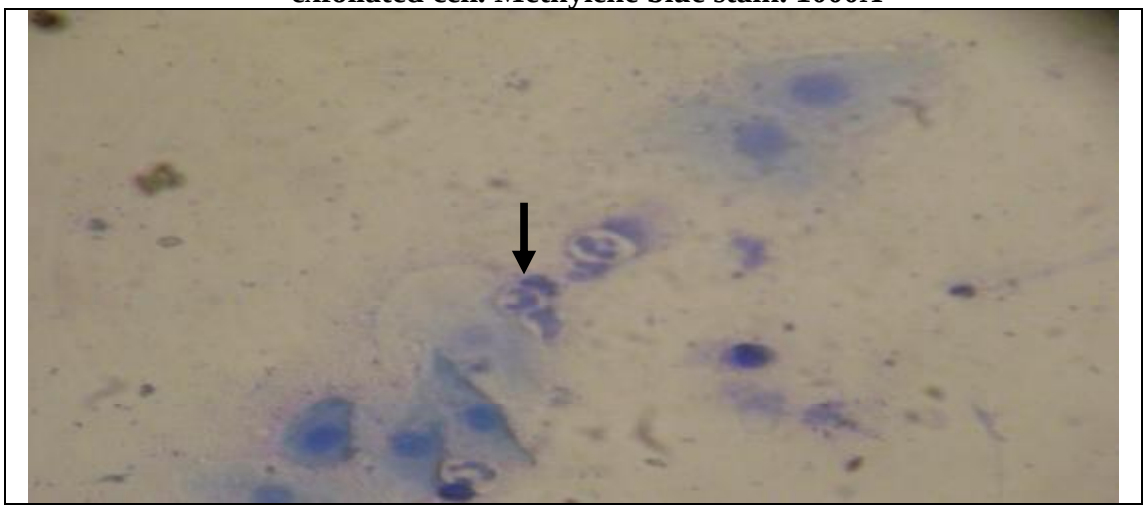

Fig. (4) Step of engagement showing that the neutrophil pushes the cell membrane of the exfoliated vaginal cell (arrow). Methylene blue stain. 400X

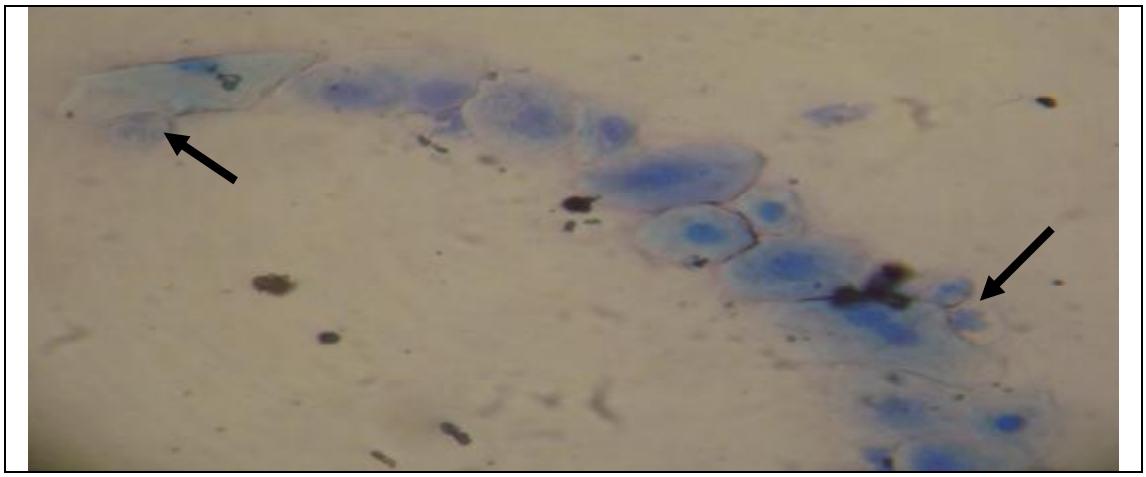

Fig. (5) Step of engagement showing that the neutrophil pushes the cell membrane of the exfoliated vaginal cell (arrows). Methylene blue stain. 400X

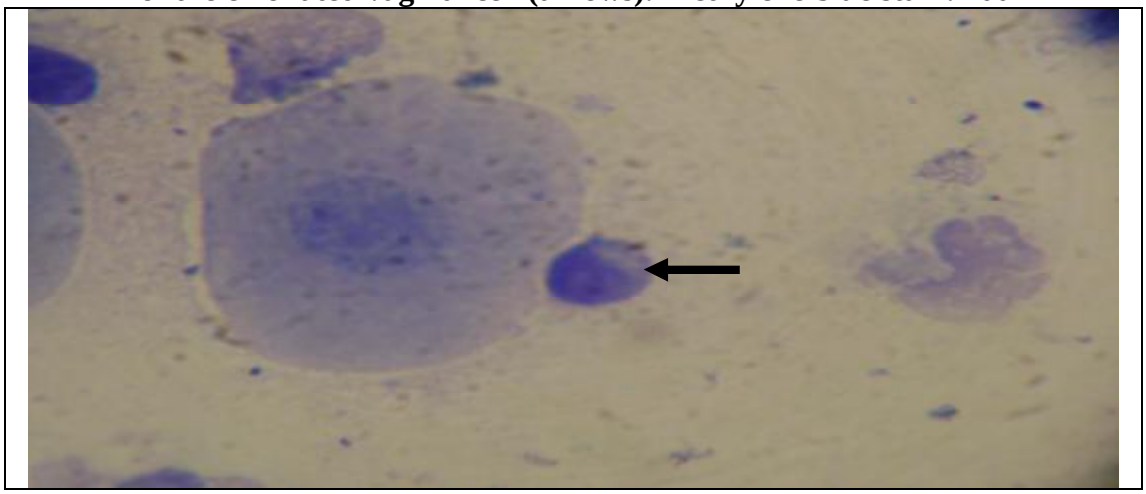

Fig. (6) Step of engagement showing that the neutrophil (arrow) pushes the cell membrane of the exfoliated vaginal cell. Methylene blue stain. 1000X

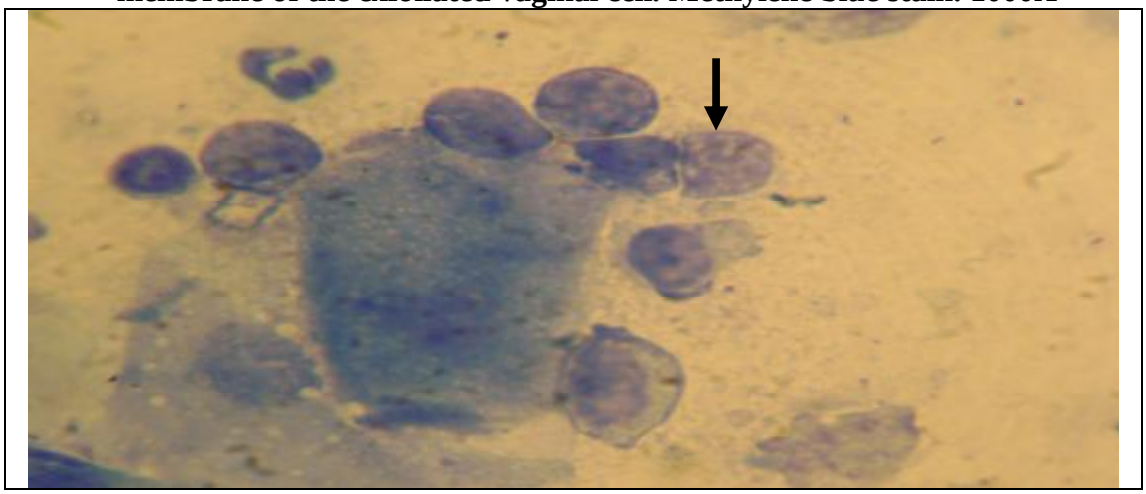

Fig. (7) Step of nuclear killing showing that the nucleus of the exfoliated vaginal cell was burst and the nucleoplasm was distributed throughout the cytoplasm. Vacuolated neutrophil (arrow). Methylene blue stain. 1000X

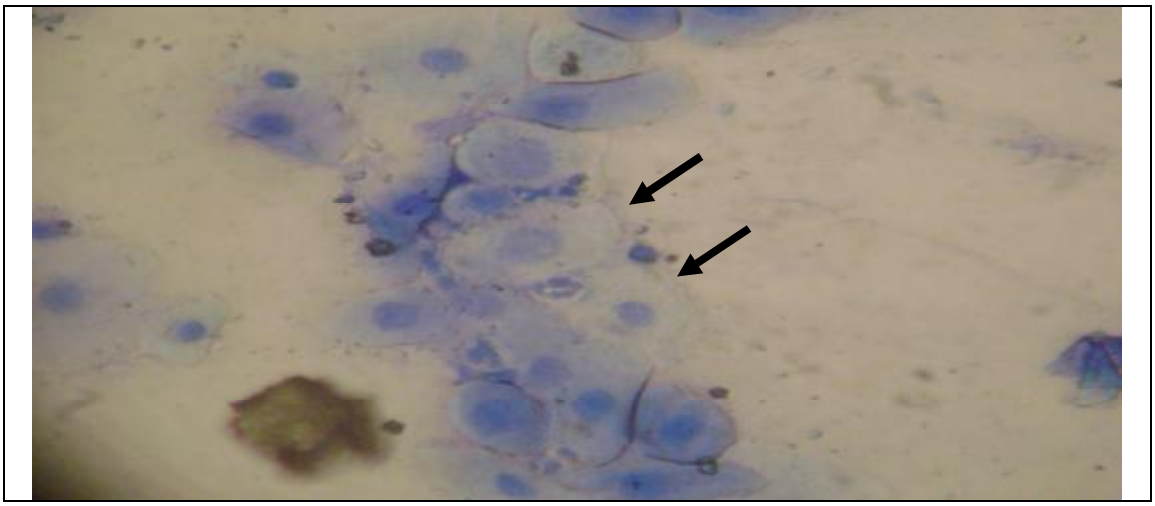

Fig. (8) Showing two clear cells (arrows). Methylene blue stain. 400X

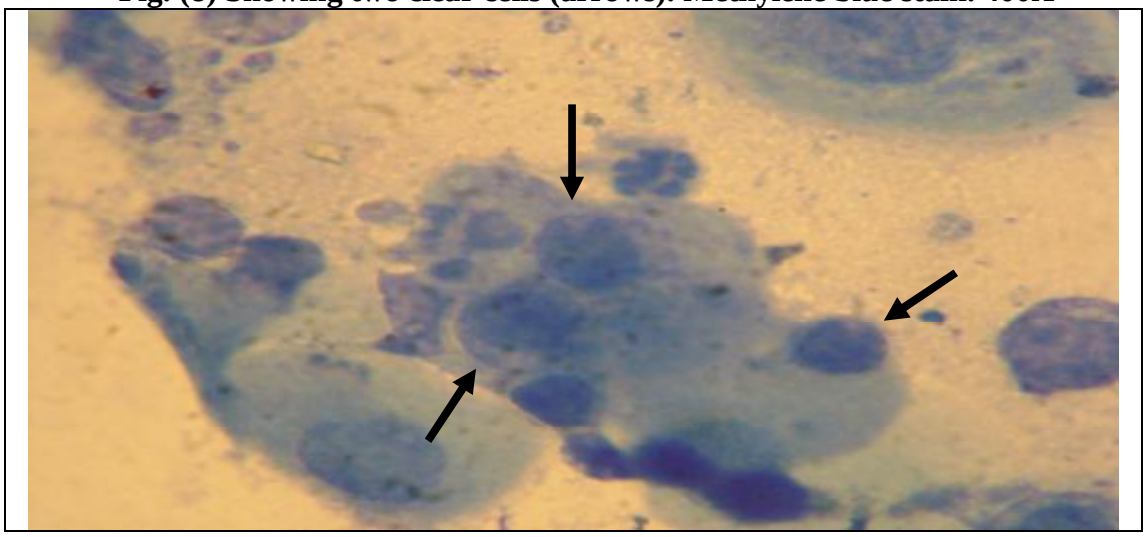

Fig. (9) Step of neutrophilic penetration. Many neutrophils appear penetrating the exfoliated vaginal cell (arrows). Methylene blue stain. 1000X

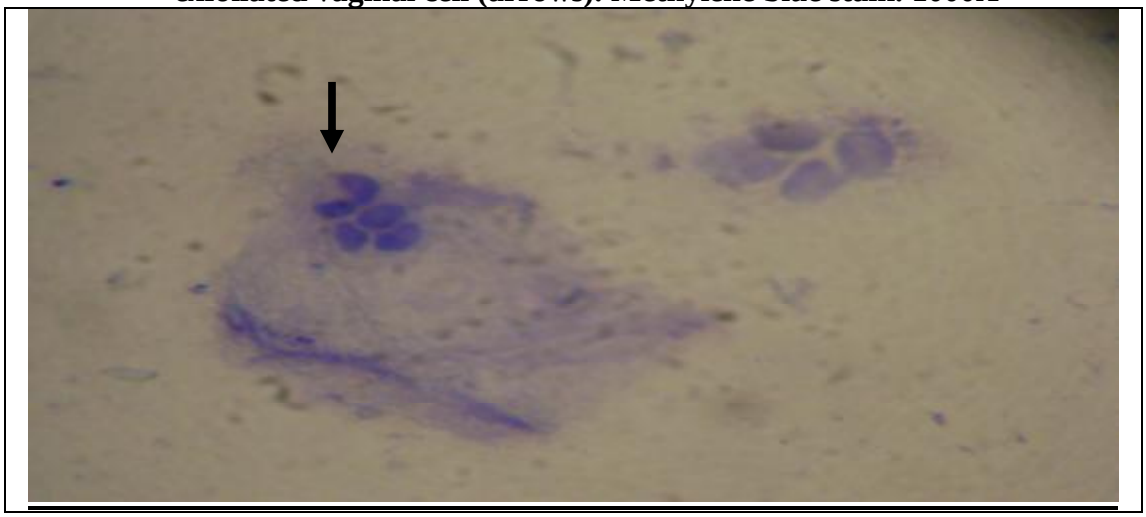

Fig. (10) Step of Struggle for existence. Both cells were die, the chromatin strands of neutrophil were detached. The exfoliated vaginal cell was raptured and the nucleus was disappeared (arrow). Methylen blue stain. 1000X

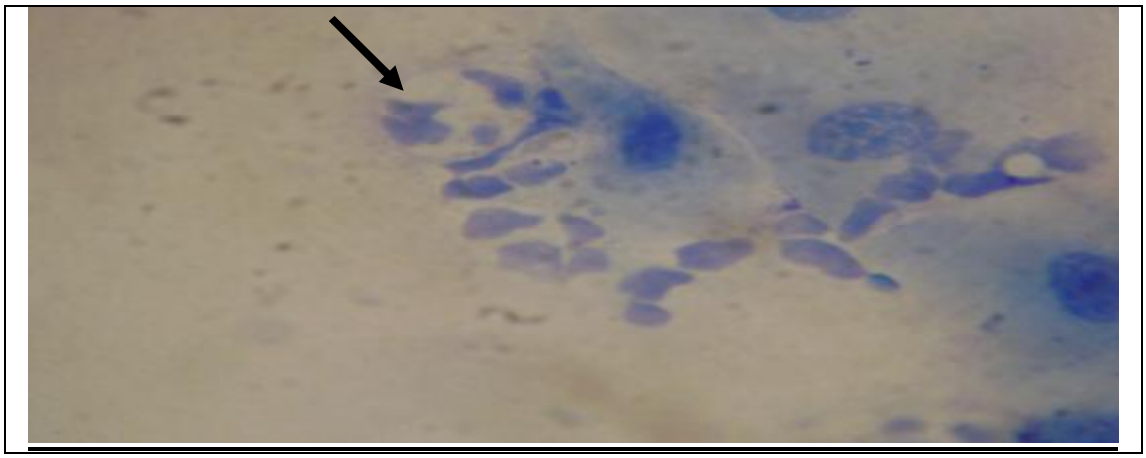

Fig. (11) Step of Struggle for existence. Both cells were die, the chromatin strands of neutrophil were detached. Methylen blue stain. 1000X

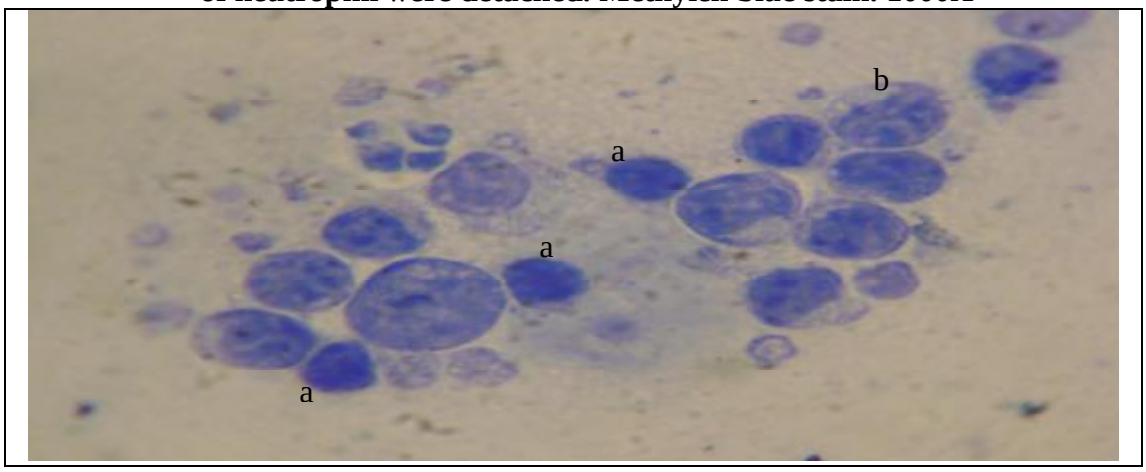

Fig. (12) Step of Struggle for existence. a- Typical apoptosis with condensed nuclei. b- vacuolation of neutrophil. Methylene blue stain. 1000X

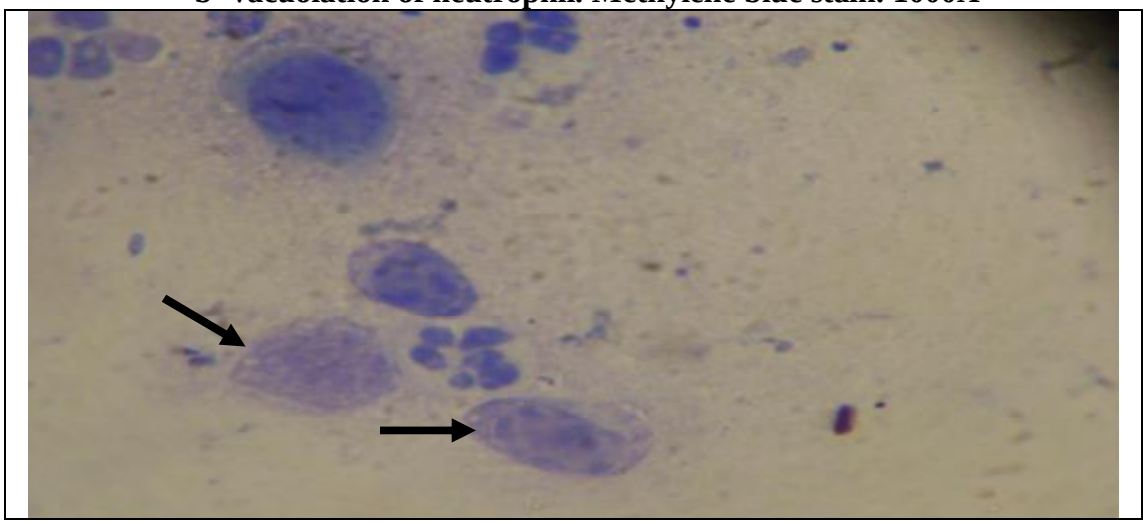

Fig. (13) Showing the presence of monocyte (arrows). Methylene blue stain. 1000X

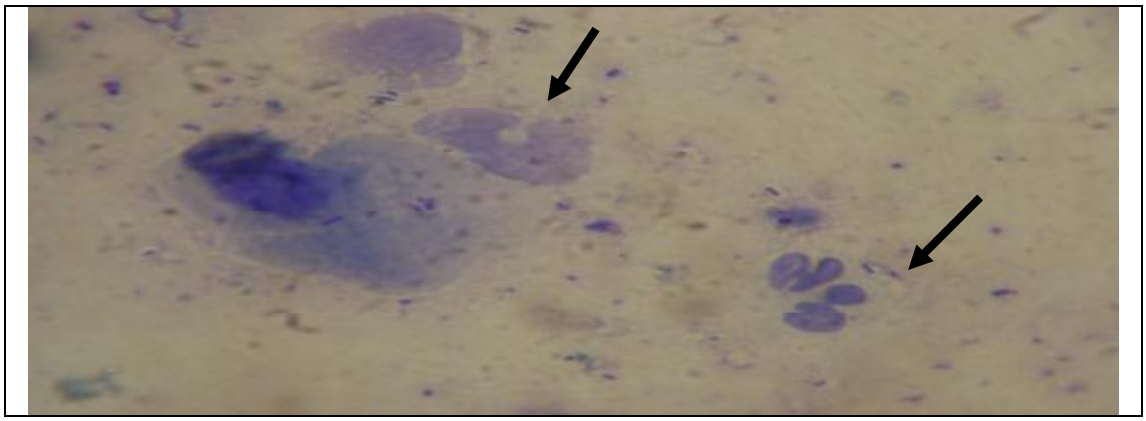

Fig. (14) note that neutrophil and macrophage in the state of endocytosis (arrows). Methylene blue stain. 1000X

Fig. (15) Step of phagocytosis showing small particles inside the neutrophil (arrow). Methylene blue stain. 1000X

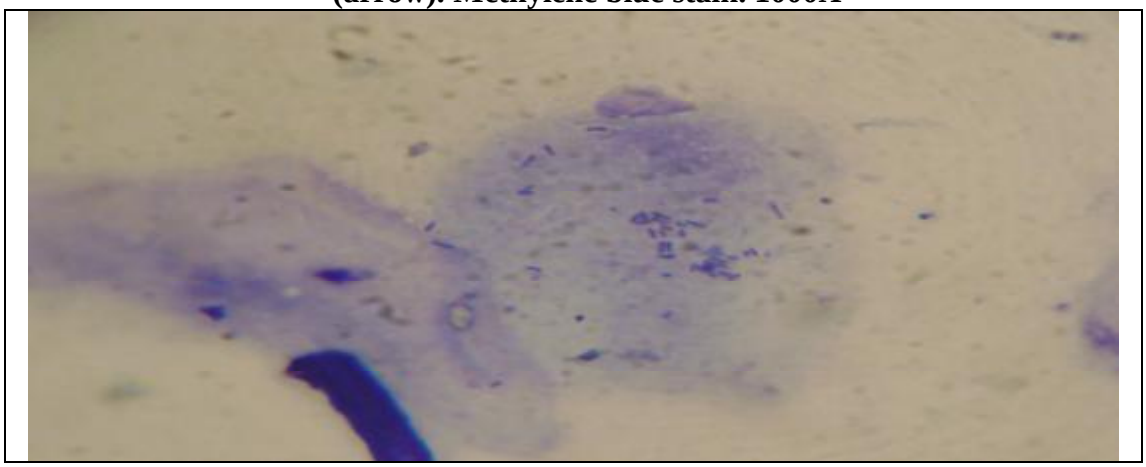

Fig. (16) Showing exfoliated vaginal cell attacked by the bacteria. Methylene blue stain. 1000X

Discussion

The study of phagocytosis has a great attention in recent years due to its relation to inflammation, treatment of diseases, wound healing and tumor researches (5, 8, 15, 16, 17, 18). The present result was in variance in part with other workers varies (1, 3, and 6) whom stated that neutrophils move randomly toward the site of infection. It has been known for a long time that the major function of neutrophils is phagocytosis of foreign materials as a general (1, 6, 19, 20, 21). The current study declared that neutrophils can only phagocytize bacteria and small particles, this is similar to the finding of Judith, etal.,(22). It is easy for neutrophil to engulf and phagocytized such small particles by the

way of endocytosis. On the other hand it is impossible for neutrophil to engulf cells equal to or larger than the its size, because the size of neutrophil was smaller enough to cover the need of engulfing large cell by forming invagination of the cell membrane by the process of endocytosis or to encircle such large cell by short pseudopodia, in contrast to the larger size phagocytizing macrophage which has longer slender processes enablins it to phagocitize larger size cells (23). The macrophages themselves frequently fuse together to form the larger giant cell which are capable of phagocytizing any larger particles or cells. This is in variance in part with others (1, 3, 24 and 25). In stead of phagocytosis, the present result revealed that the neutrophil firstly kill the large organism or large cells to dividing it into small particles and to insure that the organisms not grow and divide inside it. This result was similar in part to the finding of many researches (3, 8, 26 and 27) whom found that the neutrophil must kill the organisms by some means such as oxidative burst before the beginning of phagocytizing process or by using lysozyme which can destroy the glycosides found in the cell membrane of the organism. This is in contrast to the finding of researches (5, 6, 25) when they reported that neutrophils engulf then kill the particles in a process generally similar to those occurring in macrophages. Neutrophils used the effective smaller lysosomes granules to attack the nucleus of target cell through the cell membrane firstly before making a channel by the pressure on the cell membrane to pass through inside the cytoplasm, in a manner similar to the penetration of acrosome of the sperm to the ovum. This is in accordance to the reports of other worker (4, 5 and 8). Dudek(8) regarding that the cell membrane consists of phospholipids leaflets which can dilate laterally under the effect of many factors such as temperature, fatty acids and cholesterol. Moreover the present result add the effect of "mechanical pressure" done by neutrophils on the cell membrane, to those factors, this is reinforced our result in regarding the activated neutrophil only to penetrate the target cell and not to pass over it, as mentioned by (4,6). Both cells, neutrophil and exfoliated vaginal cell possesses a defensive factors like enzymes so that one of them or both may die during the step of struggle. Apoptosis showing that its cytoplasm is undergoing a process of fragmentation in blebs that preserve their plasma membrane. These blebs are phagocytized by macrophage without eliciting an inflammatory reaction. Then macrophage may migrate also in response to chemotactic stimuli to start engulfment of the tissue debris of the two. This is similar to the finding of others (1, 3, 25, 28, 29).

The present results were in accordance with the findings of (11, 12 and 30) whom referred to the presence of neutrophils in 16^{th} and 28^{th} days during post parturient periods in Awassi ewes to increase the immunity of the epithelium by phagocytozing any bacteria and small particles. The absence of other blood and connective tissue cells were because the early stages of inflammation involved only neutrophils, the first line of defense, other cells like macrophage, basophil, eosinophil and fibroblast comes later. Phagocytosis is important for the elaboration of a specific immune response rather for directly destroying the pathogens (much more later) there is no previous histological approach to the process of neutrophilic penetration of the large cells as a theory, moreover, the "theory of neutrophilic pentration" was not mentioned before. We concluded that:

1- The exfoliation of the vaginal epithelium was regarded as an early stage of inflammation or natural tissue injury.
2- When a cell loses its attachment to other cells like in exfoliation, the body will regard it as a devitalized cell and try to get rid of it by phagocytosis or any other process.
3- Neutrophils kill the large cell firstly before attacking it according to the neutrophilic penetration theory. The role of phagocytosis will come later by macrophage.

4- Cell membrane respond to the pressing and open a window for injecting neutrophilic lysosome or passing through the cell membrane to the cytoplasm of neutrophil.

We recommend that the phagocytosis must classified into:

1- Direct phagocytosis which deals with small solid materials.

2- Indirect phagocytosis which deals with large particles and large cells.

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