# The Theory of Keratinization

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**Abstract:**

This study was carried out on eight of Awassi ewes in estrus during which keratinization can occur in the animal farm of college of veterinary medicine, Baghdad university in June 2006. In order to study the process of keratinization histologically, samples of vaginal smears were taken and stained by methylene blue. The present study recognized five stages of keratinization namely (stage of mitosis, stage of cellular transformation, stage of cellular adhesion, stage of endogenous bacterial invasion and stage of sloughing), during which the cells undergo a series of transformations in shape, size, colour and cornification. The study found also that the cells were transformed from small spherical basophilic shape to large polygonal acidophilic shape as the polygonal shape was suitable in hidden the interstitial spaces between the cells, when these cells try to building a compact sheet or packet which act as a defensive barrier to protect the internal environment against the external environment, finally the endogenous bacteria metabolize the glycogen into lactic acid which decrease the pH of the vagina and protect it from the pathogenic bacteria. Then these attacked cells start to exfoliated so that, we can regard the process of keratinization as a defensive mechanism in the vagina.

# نـظريـة الـتـقرن

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**الخلاصة:**

اجريت هذه الدراسة على ثمانية من النعاج العواسية في الحقل الحيواني لكلية الطب البيطري بجامعة بغداد في حزيران 2006. ولهذا الغرض تم جمع مسحات مهبلية من النعاج في مرحلة الشبق وهي الفترة التي يحدث بها التقرن. صبغت المسحات بالمثلين الأزرق لدراسة السلوك الخلوي خلال عملية التقرن في المهبل. بينت الدراسة أن عملية التقرن تقع في خمسة مراحل وهي (مرحلة الأنقسام الخيطي، مرحلة التحول الخلوي، مرحلة الالتصاق الخلوي، مرحلة غزو الجراثيم المتوطنة ومرحلة الانسلاخ) تعاني الخلايا خلالها من أنقسامات خيطية ومن تغيرات مورفولوجية في الشكل والحجم واللون والتقرن. بينت الدراسة كذلك أن الخلايا تتحول خلال عملية التقرن من خلايا كروية صغيرة قاعدية إلى خلايا مضلعة كبيرة حمضية وأن الشكل المضلع هو أنسب الأشكال لأخفاء المسافات البينية بين الخلايا عند قيامها بالأتصال لبناء قطع (sheets) أو مساحات متراصة (packets) وبالتالي عمل

حاجز دفاعي يبطن المهبل ويحفظ البيئة الداخلية ضد البيئة الخارجية. ثم تقوم الجراثيم المتوطنة في المهبل بتحويل الكلايكوجين المتواجد في الخلايا المهبلية إلى حامض اللاكتيك لعمل وسط حامضي داخل المهبل يمنع معيشة الجراثيم المرضية ثم تقوم الخلايا المستهدفة بالأنسلاخ لتحل محلها خلايا أخرى لذلك بامكاننا اعتبار التقرن وسيلة دفاعية للجسم.

## Introduction:

Keratinazation is the conversion of the granular cells to cornified cells. Under the stimulus of estrogen, the vaginal epithelium normally synthesizes and accumulates large quantity of glycogen, which deposit in the lumen of the vagina when the superficial vaginal cells continuously desquamated (1 , 2). Bacteria in the vagina metabolize glycogen and form lactic acid, which is responsible for the low pH of the vagina. The acidic vaginal environment provides a protective action against some pathogenic microorganisms. Both the metabolized glycogen and the exfoliated cells were transported to the uterus (3, 4 and 5). Also, under the influence of estrogen, the vaginal epithelium proliferates and becomes infliltrated with leukocytes (6, 7, 8 and 9). (10) and (11) found that the keratinization occurs in post parturient periods in Awassi ewes. In women, intense keratinization with the cells changing into keratin plates, does not occur (12). On the other hand (10) stated that the affinity of cytoplasm for staining in the process of keratinization may be attributed to the accumulation of cytoplasmic glycogen, while (13) explained that the degenerative process of keratinization begins with a change to reddish-violet. Besides, (14) reported that the keratin arise from the combination of keratohyalin granules and the tonofibrils.

Recent investigations indicate that keratinocytes produce immunogenic molecules and probably active in the immune process (7). Evidence also shows that these cells are capable of producing several interleukins, colony-stimulating factors, interferons, tumor-necrosis factors as well as platelets and fibroblast-stimulating growth factor (12 and 15).

The cytological examination of cells which collected from the vagina provides information of clinical importance (12, 16, 17, 18 and 19). The vaginal smear is also useful in early detection of cervical cancer (5). Less is known about the cellular behaviour of the vaginal epithelium during the process of keratinization. The present study designed to throw further light on this aspect and deals at first time with the process of keratinization as a new theory.

## Materials and Methods:

In order to study the keratinization in the vaginal smears, eight healthy adult Awassi ewes have been borrowed from the animal farm of college of veterinary medicine, Baghdad University in June 2006. Forty vaginal smears were taken during estrus, by using sterile swabs that rolled three times inside the vagina, this was done according to (20). Three hundred cells were examine per slide by using light

microscope. The smears were stained by methylene blue (21).

**Results:**

The present work found that the process of keratinization in the vagina of Awassi ewes was thrown into five stages, namely:

**1- Stage of Mitosis:**

Spherical to slightly oval basophilic cells arranged in pairs or tetrads were recognized. The nuclei of these cells were large and basophilic and more closely packed. Binucleated cells were common (Fig. 1).

**2-Stage of Cellular Transform-ation:**

Morphological changes were easily recognized in the cells. As these cells start to divide, their boundaries showing thickening and cornification at the periphery and cellular bridges (Fig. 2 and 3), then the cornification occupies all the cell (Fig. 4). The keratin were readily visible with light microscope. It tend to be chromophobic and cytologicaly homogeneous when treated with Hematoxylin and Eosin stain.

The cells were changed from spherical or slightly oval basophilic (Fig. 5), to polygonal acidophilic (Fig. 6) according to the differences in the affinity of secretory granules for staining. The polygonal cells showed folding at their periphery (Fig. 7). The nuclei changed from large dark blue in the spherical cells to small purple or even pyknotic in the polygonal cells (Fig. 7). The limits of the nuclei disappear then the nucleoplasm was spread throughout the cytoplasm (Fig. 8). Disappearance of the nuclei was common (Fig. 9), some of the spaces that they occupied can be seen. Extruded nuclei was also seen, vacuolation of the cytoplasm was evident (Fig. 10) so that keratinocytes finally were devoid of nuclei and organells, and had a thickened cell membrane and had a cytoplasm filled with keratin.

**3- Stage of Cellular Adhesion:**

The flattened polygonal cells were start to accumulate and fuse to each other leaving no intercellular spaces forming broad sheets or packets which line the vagina (Fig. 11). The process of keratinization terminates when the cells become as a mature keratinized cells, eventually to be sloughed off at the surface of the epithelium.

**4- Stage of Endogenous Bacterial Invasion:**

Accumulation of endogenous bacillus bacteria was found distributed over the keratinized sheets of the cells. They were clearly discernible (Fig. 12).

**5- Stage of Sloughing:**

Later on, the fusion between superficial polygonal cells were detached and these cells were sloughed off.

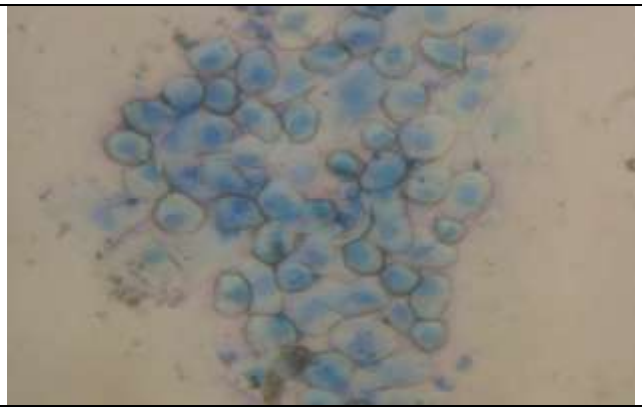

**Fig. 1: Stages of mitosis.**
**Methylene blue stain. 400X**

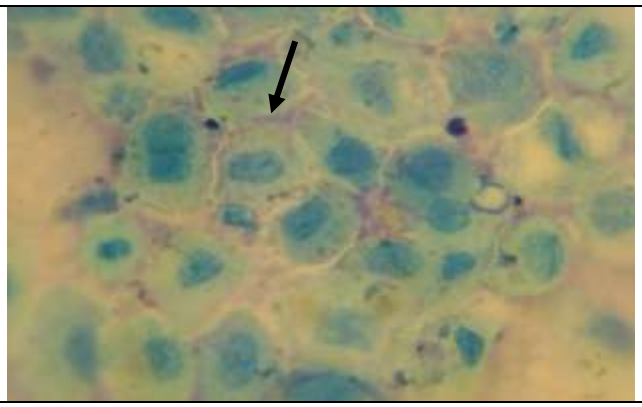

**Fig. 2: Stage of Cellular Transformation. Notice the thickening of cellular limits (arrow).**
**Methylene blue stain. 1000X**

**Fig. 3: Stage of Cellular Transformation. Notice the thickening of cellular limits and intercellular bridges.**
**Methylene blue stain. 400X**

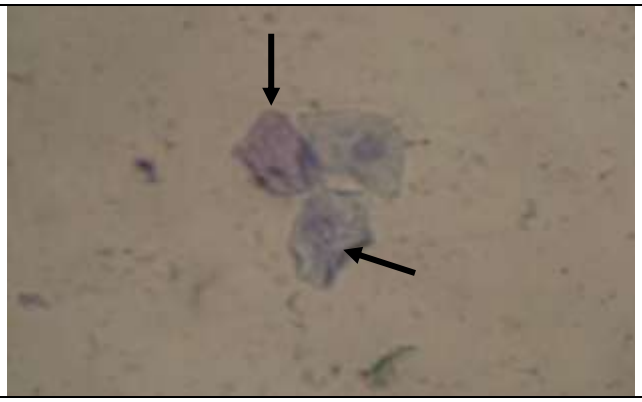

**Fig. 4: Stage of Cellular Transformation. The keratin occupies all the cells (arrows).**
**Methylene blue stain. 1000X**

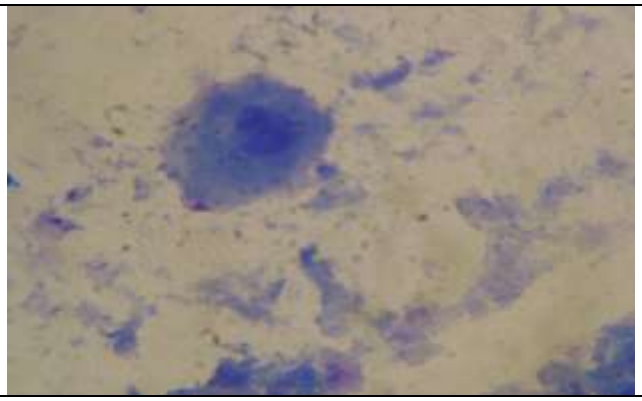

**Fig. 5: Stage of Cellular Transformation. Oval basophilic cell.**
**Methylene blue stain. 1000X**

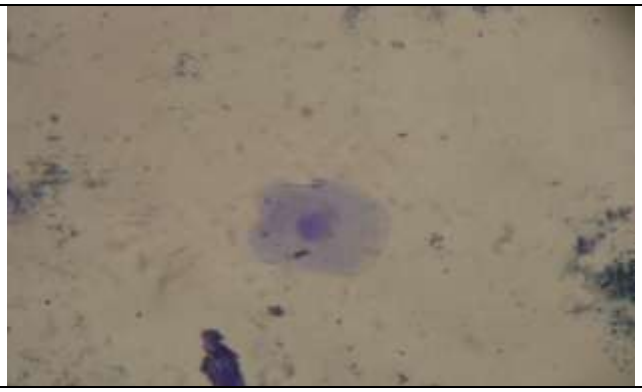

**Fig. 6: Stage of Cellular Transformation. Slightly acidophilic cell.**
**Methylene blue stain. 1000X**

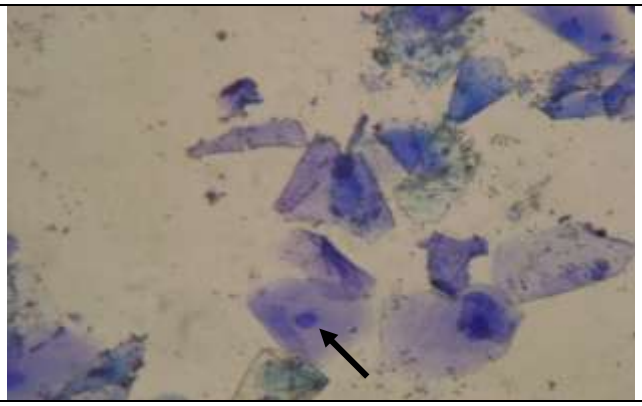

**Fig. 7: Stage of Cellular Transformation. Polygonal folded cells. Pyknotic nucleus (arrow).**
**Methylene blue stain. 1000X**

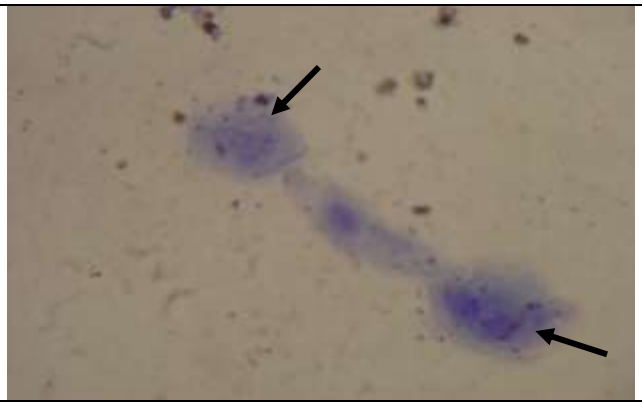

**Fig. 8: Stage of Cellular Transformation. Spread nucleoplasm (arrows).**
**Methylene blue stain. 1000X**

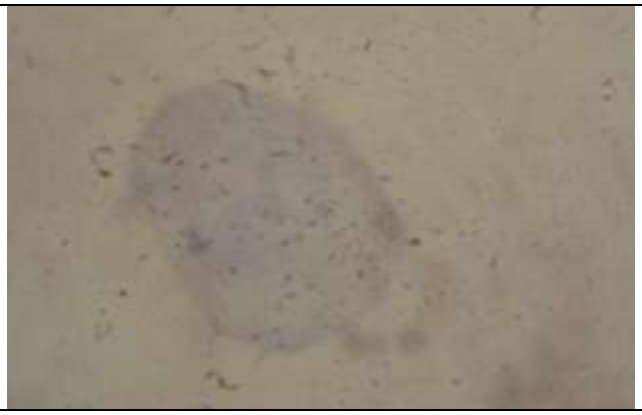

**Fig. 9: Stage of Cellular Transformation. Disappearance of the nucleus.**
**Methylene blue stain. 1000X**

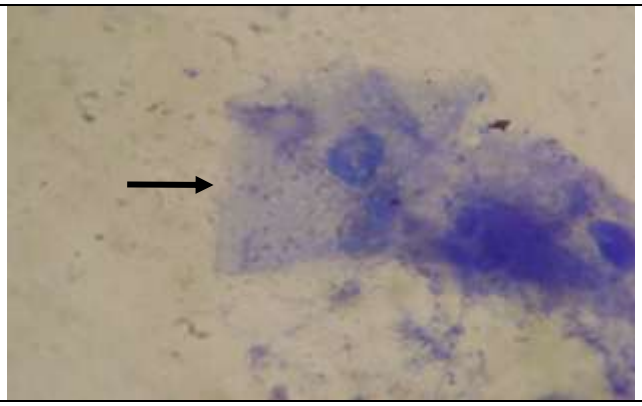

**Fig. 10: Stage of Cellular Transformation. Vacuolated cytoplasm (arrow). Methylene blue stain. 1000X**

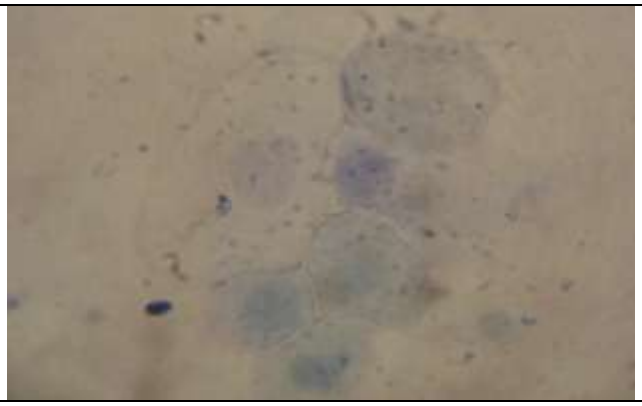

**Fig. 11: Stage of Cellular Fusion. Methylene blue stain. 1000X**

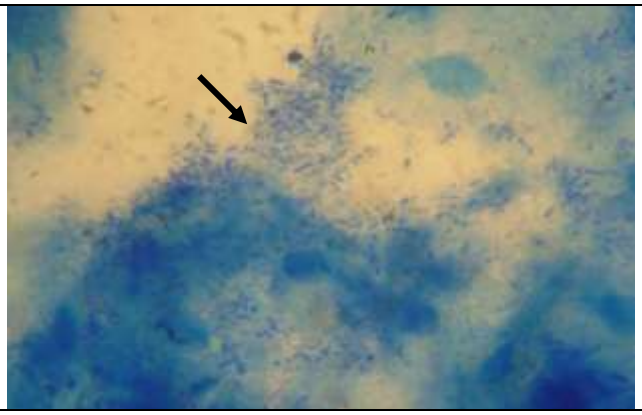

**Fig. 12: Stage of Endogenous Bacterial Invasion. Methylene blue stain. 1000X**

## Discussion:

The present study revealed that the basophilic cells of this layer have less cytoplasm than the cells in the layer above, consequently their nuclei were more closely spaced (3, 4 and 22). The basal layer was mitotically activated to compensate the dead keratinized cells of the outermost superficial layer which were fairly resistant to environment irritants. The increase in the number of proliferating cells will result in greater epithelial thickness and more rapid renewal of the epithelium to

increase its defensive immunity (4). The cytoplasm of the immature keratinocyte appears basophilic in histological sections because of the very large number of free ribosomes, most of which were engaged in the synthesis of intermediate filaments, the tonofilaments (1, 6 and 22). As the cells enter and were moved through the upper layers, the synthesis of tonofilaments continues, and the filaments become grouped into bundles sufficiently thick to be visualized with the light microscope. These bundles were called tonofibrils. The keratohyalin granules were also synthesized by free ribosomes during their formation. A substance contained in the keratohyalin granules combines with the tonofibrils converting them in to keratin (5 and 22). The cytoplasm becomes eosinophilic as the cells move upward due to the staining reaction of the tonofibrils that fill more and more of the cytoplasm (15).

The cell membrane has dynamic structure being constantly renewed at different rates. This is similar to the findings of (13). The intense flatness of the polygonal cells may explain the folding at the periphery of these cells.

The change in the size of the cells may be due to the increase in number and size of the organells which was necessary for the formation of the protein, keratin and also due to the increase in glycogen accumulation. This is in consequence with (22 and 23). The shifting in colour from blue to red or even purple, the metachromasia, was due to the reaction between certain basic dyes (methylene blue) and the tissue components. Cells and tissues that have high concentration with rough endoplasmic reticulum will exhibit metachromasia (22).

The shape of the cells in the basal layer changes from spherical to polygonal as they move to the superficial layer. This is because the polygonal shape was the preferable shape which can hides the intercellular spaces when the squamous polygonal cells fuse to form broad sheets (6). These junctions maximize the intercellular communications and reduce permeability to microbes, fluid … etc. so that 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 (15 and 24) who stated that keratinization activate immunization. On the other hand, this result was in variance in part with (5 and 10) who explained that the polyhedral shape of the cell was resulted from their juxtapositioned in cellular layers or masses as the present study referred to the presence of many isolated polygonal cell.

Any abnormal changes in the vagina will result in the entry of microbes so that periodic natural desquamation of the vaginal epithelium results in sloughing off any invading pathogens (12). In this

we agree with the latter author how regards the keratinization process as natural flushing action which assist in elimination of the infectious antigens. This mechanism is necessary for the vaginal epithelium as the wall of the vagina was devoid of glands and the mucus found in the vaginal lumen comes from the glands of the uterine cervix (3, 4 and 25).

Many authors reported that the disintegration of cell organells and the degeneration of the epithelial cells were apparently related to the presence of hydrolytic enzymes abundant in these cells, these lysosomal enzymes destroy the nuclei of the cell, mitochondria and other organells, the process of producing keratin and horny epithelium (1, 5 and 22).

(7) mentioned many types of cellular communications between the alive cells. When the cells die, these communications will stop. In this we agreed with the author as the desquamation process was probably due to the weakened junctional complexes between the surface cells. Cells that will desquamate, accumulate acid phosphatase which is thought to participate in the exfoliation of these keratinized cells (2 and 12). In primates, glycogen increases with the administration of estrogen. After death, it quickly reverts to soluble glucose (3). Glycogen stains reddish purple in the superficial polygonal cells (23). It is an important store of chemical energy in vaginal epithelium (7, 15 and 26). The endogenous bacillus normally metabolized the glycogen and give rise to lactic acid which decrease the pH to acidic and prevent the pathogenic b
acteria from alive in the vagina (6 and 7).

In conclusions, this study recognize at first time five stages of the process of keratinization. This findings were in variance in part with the authors who found that the superficial polygonal cells were continuously exfoliated as the current study revealed that the polygonal type try firstly to fuse to each other forming a broad sheets or packets and by the time these polygonal cells detaches the junctional complexes and then exfoliated.

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