

THE VAGINAL EXFOLIATIVE CYTOLOGY OF AWASSI EWES DURING POST-PARTURIENT PERIOD

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Summary

This trail was carried out on twelve Awassi ewes in the Animal Farm of College of Veterinary Medicine in June 2005 to study the cellular changes that occur in the vaginal epithelium after parturition, by using vaginal smear method. The post-parturient period was divided into three periods; 4th, 16th and 28th days after parturition. Each period presented different sizes of vaginal epithelial cells, some of them were predominant. Keratinization takes place in all periods. The 2nd period was characterized by cellular division, presence of vacuolated epithelial cells and WBCs mainly neutrophils cells. The conclusion, variations in the vaginal epithelium may relate to the physiological status and the hormonal activity of the animals.

الأنسلاخ الخلوي في مهبل النعاج العواسية خلال فترة بعد الولادة

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الخلاصة

أجريت هذه التجربة على اثنتا عشر من النعاج العواسية الموجودة في الحقل الحيواني لكلية الطب البيطري في حزيران 2005 لدراسة التغيرات الخلوية التي تحصل في ظهارة المهبل بعد الولادة باستخدام طريقة المسحات المهبلية. قسمت الفترة بعد الولادة إلى ثلاث فترات؛ فترة اليوم الرابع، فترة اليوم السادس عشر وفترة اليوم الثامن والعشرين بعد الولاده. أظهرت كل فترة من الفترات الثلاث خلايا ظهارية مختلفه الأحجام، بعضها سائد. أظهرت الخلايا الظهارية في جميع المراحل تقرباً واضحاً. تميزت الفتره الثانيه بكثرة الأنشطة الخلوية ووجود الفجوات في هيولي الخلايا الظهارية وكثرة الخلايا الدمويه البيضاء وخاصة العدلات. تستنتج الدراسة بأن التغيرات في الخلايا الظهارية للمهبل يعود إلى حاله الفسيولوجيه والنشاط الهرموني للحيوانات.

Introduction

Many researchers have been studied the variations which occur in the vaginal mucosa at different phases of estrus cycle by using vaginal smears (1, 2, 3, 4, 5 and 6). These variations occur under the effect of estrogen and progesterone hormones that secreted from the ovary (7, 8 and 9). (10) Classified the vaginal epithelial cells into three cell types according to the differences in their sizes without doing measurement of their diameter. While (6) classified the vaginal cells into three basic cell types with different cell diameters; as a superficial squamous cells (40-65um diameter) with light cytoplasm, intermediate squamous cells (20-40um diameter) and parabasal cells (12-15um diameter), both with blue cytoplasm. (11 and 12) reported that the vaginal epithelial cells thrown into four type's viz. parabasal, intermediate, superficial intermediate and superficial cells. Moreover, (13) found that there were three groups of vaginal cells viz. circular, squamous and leukocytic cells. The relative proportion of different types of vaginal epithelial cells can be used as a marker of the endocrine environment (9 and 14). (15) Stated that cell volume previously reduced to one-half by mitosis, then restored to its normal size. (6) Mentioned that under the influence of estrogen, the epithelial cells accumulate large amount of glycogen and undergo cell proliferation in the basal and parabasal layers. (16) Found that vacuoles have different diameter and may reach 0.5um. Due to the importance and effect of post parturient period on the reproduction, the present work was conducted to study the relation of the cellular changes with the ovarian rebound that not being studied before. Moreover the vaginal smear may be used clinically to evaluate the hormonal status, or / and the reproductive stages in the ewes, and also useful in the early detection of cervical cancer.

Materials and Methods

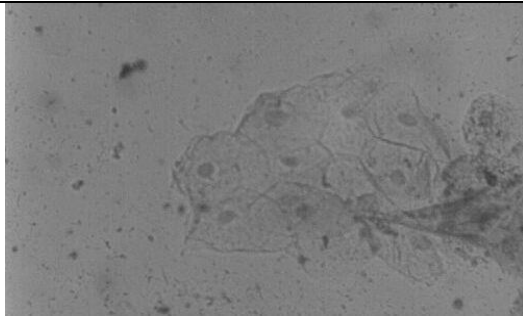
Twelve puerperium healthy adult Awassi ewes have been borrowed from the Animal Farm of College of Veterinary Medicine, Baghdad University, in June of 2005. The vaginal smears were taken according to (17). The period of this study was divided into three different stages (14). The first stage includes 4th day after parturition which characterized by the absence of ovarian activity. The second stage includes 16th day after parturition which characterized by resecretion of ovarian hormones while the third stage includes 28th day after parturition that represent complete ovarian rebound (14). The smears were stained by methylene blue (18). Vaginal mucus membrane smear were examined under light microscope. Cells measurements were done by using oculometer (19). Statistical analysis was calculated by LSD method (20).

Results

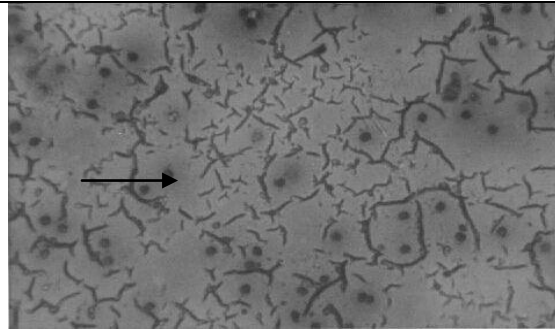
The present study revealed that there were polyhedral forms of vaginal epithelial cells (fig 1). The results found that the predominant size of the vaginal

epithelial cells in the first stage was the large which reach more than 37um. The second stage, the predominant size of the cells were less than 22um. While the third stage showed medium size range 22-37um (Table 1). The size of nuclei ranged from small (less than 5 um) to medium (5-7.5 um) and large (more than 7.5 um) (Table 2). There was a significant increase ($P<0.01$) in the large size nuclei in the three stages in comparison with small and medium size nuclei (Table 2). All stages showed a significant increase ($P<0.01$) of keratinized cells in comparison with nucleus of other cells. The cells had small pyknotic nuclei and have ample cytoplasm. Some of these cells were devoid of nuclei (fig 2). The 2nd stage showed prominent cellular division (fig 3, 4), and presence of vacuoles in their cytoplasm (fig5) which measured 2.5-3um, presence of a large number of WBCs were also noticed in the 2nd and 3rd stages (Table 3). The affinity for staining of the cytoplasm ranges from light to dark purplish or magenta (fig 6) and the number of dark cells were showed non significant increase from 1st stage toward the 3rd stage (Table 3).

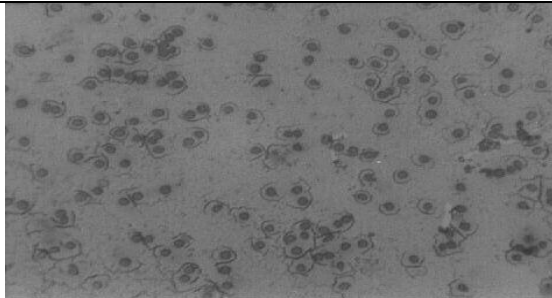
Figures (1-6) showed the vaginal epithelial cells changes during post-parturition periods in Awassi ewes (n=12).



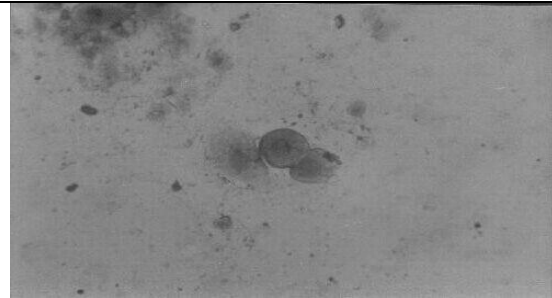
**Figure (1): Polyhedral vaginal epithelial cells. (2nd stage).
Methylene blue stain. 400X**



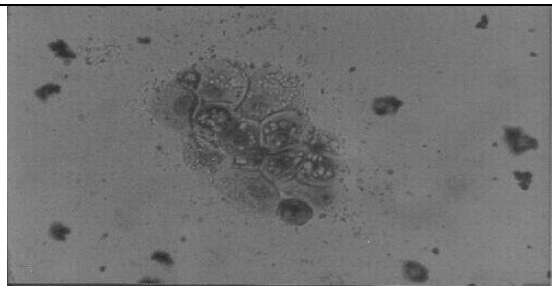
**Figure (2): Keratinization of vaginal cells (arrow). (1st stage).
Methylene blue stain. 400X**



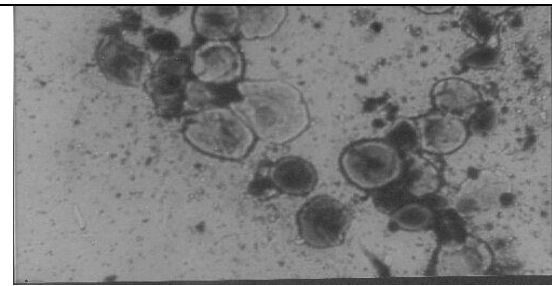
**Figure (3): Cellular division of vaginal epithelium. (2nd stage).
Methylene blue stain. 400X**



**Figure (4): Cellular division of vaginal epithelial cell. (2nd stage).
Methylene blue stain. 400X**



**Figure (5): Vacuolation in the cytoplasm of vaginal epithelium. (3rd stage).
Methylene blue stain. 400X**



**Figure (6): Vaginal epithelial cells with different sizes showing different affinities for staining. (3rd stage).
Methylene blue stain. 400X**

Table 1: Size of vaginal epithelial cells during post parturient periods in Awassi ewes (n=12).

Post parturient period	1 st stage 4 th day	2 nd stage 16 th day	3 rd stage 28 th day
Cell size (milimicrone)			
Small size (less than 22um)	27.75±1.40 a	50.00±2.40 a	28.25±1.20 a
Medium size (37-22um)	22.25±0.90 a	31.50±1.70 a	43.75±2.30 a
Large size (more than 37um)	50.00±0.90 a	18.50±1.20 a	28.00±1.40 a

The numbers represent the mean ± the standard error, the small letter represent significant differences at P<0.01.

Table 2: Size of nuclei during post parturient periods in Awassi ewes (n=12).

Post parturient period	1 st stage 4 th day	2 nd stage 16 th day	3 rd stage 28 th day
Nucleus size (micrometer)			
Small size (less than 5um)	10.0±0.8 b	5.0±0.3 ab	2.0±0.1 a
Medium size (7.5-5um)	5.0±0.2 ab	6.0±0.3 ab	8.0±0.4 ab
Large size (more than 7.5um)	34.5±1.2 c	40.5±2.3 c	38±3.3 c
Keratinized (without nucleus)	50.5±0.7 d	48.5±1.9 d	52.0±1.6 d

The numbers represent the mean ± the standard error, the small letter represent significant differences at P<0.01.

Table 3: Shows presence of WBCs (neutrophil) and cytoplasmic color during post parturient periods in Awassi ewes (n=12).

Post parturient period	1 st stage 4 th day	2 nd stage 16 th day	3 rd stage 28 th day
WBCs (neutrophil)	—	+++	+
Number of dark cells	32±1.3 a	34±1.5 a	36±2.2 a

The numbers represent the mean ± the standard error, the small letter represent significant differences at P<0.01.

Discussion

To describe the exfoliative cytology of the vaginal epithelium, the present study depends on the measurement of the cellular, nuclear diameter and the affinity for staining. The predominancy of vaginal epithelial cells was differ from stage to another. This may be due to the changes in physiological and hormonal status of the animal during the post parturient period. This is in agreement with (6, 9, 21, 22 and 23). The vaginal epithelial cells were classified according to their location in the vaginal mucosa as parabasal, intermediate, superficial intermediate and superficial cells (3, 10, 11, 12 and 13). In the present work the common polyhedral forms of the vaginal epithelial cells were resulted from their juxtaposition in cellular layers or masses. This is coincided with (15). There were some differences in the values of the vaginal cell diameters that recorded by (6) in comparison with this study due to species variation. The affinity of the cellular cytoplasm for staining may depend on the accumulation of cytoplasmic glycogen due to increases in metabolic activities which give the purple or magenta color (6, 24 and 25).

The variation in the size of the vaginal epithelial cells noticed in the second stage indicates that there were cellular divisions. This is in accordance with (6 and 15). Present results revealed the presence of keratinization occur in the cells suffering from apoptosis (9). While, the presence of vacuoles in the cytoplasm during the 2nd stage may reach 2.5-3um in diameter. This may be due to glycogen consumption during metabolic activity. This is similar to the conclusion of (16) who found that the vacuoles excrete secretary product of different density. Moreover, (14) stated that the vacuolation are typical in the ovarian activity. The presence of neutrophila was well observed in the 2nd stage. This is in accordance to (14 and 26) who mentioned that neutrophilia was followed by a recovery period. The conclusion of the present study showed that the

exfoliative cytology of Awassi vaginal ewes was affected by the hormonal status of the animals during the post parturient periods.

References:

- 1-Sanger VL, Engle PH and Bell DS (1958) Age changes in the number of antral follicles in the lambs and ability of oocytes to mature. *Ani Breed Abst* 56: 814.
- 2-Ducker MJ and Boyed JS (1974) An evaluation of the vaginal smears technique for detecting of occurrence of ovulation in the ewe. *J Rep and Fert* 41: 249-251.
- 3-Zourgui A, Neophytou G and Elize K (1976) Results of vaginal cytology studies and examination of the arborization phenomenon of the vaginal mucus for the determination of estrus cycle activity in sheep. *Arch Exp Veterinarmed* 30(6): 889-896.
- 4-Al-Wahab RMH and Bryant MJ (1977) Reproductive sexes ewe lambs mated at and induced puberty. *Ani Prod* 24:129.
- 5-Al-Ameri MHM (1999) Comparison of some reproductive performance feature between the Awassi and Tetra Assaf and Tetra Turkey sheep. Master Thesis - Agriculture College - Baghdad University.
- 6-Dudek RW (2004) *High Yield Histology*. 3rd ed. Philadelphia: Lippincott Williams and Wilkins p:222-223.
- 7-McDonald LE (1980) *Veterinary Endocrinology and Reproduction*. 32nd ed. Philadelphia USA: Lea and Febiger.
- 8-Peterson SL (1986) Age-related changed in plasma estrogen concentration, behavioral responsiveness to oestrogen, and reproductive success in female Gray-Tailed Voles, *Microtus canicaudus*. *J of Rep and Fert* 78: 57-64.
- 9-Lamond DR and Lang DR (2005) Investigation of the vaginal smear (Allen-Doisy) assay of oestrogen in ovariectomized ewes. *Aust J of Agri Res* 16(2): 201-210.
- 10-Khedeyer ARN (1980) The study of circulatory and reproductive capacity of Awassi ewe lamb and determination of their age at puberty by means of vaginal smearing. Master Thesis – Veterinary College – Baghdad University.
- 11-Bacha WJ and Wood LM (1990) *Color Atlas of Veterinary Histology*. Philadelphia USA: Lea and Febiger.
- 12-Yamada MLA and Kozicki LE (1998) Gestation diagnostic in *Capra hircus*, through histological and cytological analysis of the vaginal epithelium. *Brazilian J of Vet Res and Ani Sci* 35(6).

- 13-Neama HF (2000) Evaluation of reproductive performance of Awassi ewe lambs by using some hormonal programs. Master Thesis – Agriculture College – Baghdad University.
- 14-Arthur GH, Noakes DE, Pearson H and Parkinson TJ (1996) Veterinary Reproduction and Obstetrics. 7th ed. WB Saunders Company.
- 15-Junqueira LC, Carneiro J and Kelley RO (1998) Basic Histology. 9th ed. Lange Medical Book McGraw-Hill.
- 16-Lesson CR, Lesson TS and Paparo AA (1985) Textbook of Histology. 5th ed. Lg Aky Shoin Sandirs.
- 17-Willard MD, Tvedten H and Turnwad GH (1989) Small Animal Clinical Diagnosis by Laboratory Methods. WB Saunders Company.
- 18-Coles EN (1986) Veterinary Clinical Pathology. 4th ed. Philadelphia USA: WB Saunders Company.
- 19-Al-Mukhtar KA, Al-Allaf SM and Al-Attar AA (1982) Microscopic Preparations. 1st ed. Iraq: Ministry of Higher Education and Scientific Researches pp: 276-277.
- 20- Al-Mohammed NT, Al-Rawi KM, Younis MA and Al-Marrany WK (1986) Principles of Statistics. Mousl: Book House for Printing and Publishing.
- 21-Trautman A and Fiebiger J (1957) Fundamental of the Histology of Domestic Animal. New York: Comstock Publishing Associated.
- 22-Copenhaver WN, Vang RT and Bynge MV (1971) Bailye's Textbook of Histology. 16th ed. Williams Company.
- 23-Dellmann HE (1993) Textbook of Veterinary Histology. 4th ed. Philadelphia USA: Lea and Febiger.
- 24-Breazile JE (1971) Textbook of Veterinary Physiology. Philadelphia USA: Lea and Febiger.
- 25-Derobertis EDP and Derobertis EMS (1981) Cell and Molecular Biology. 7th ed. Philadelphia: Canders College pp: 250-270.
- 26-Al-Najar HAS (1998) The effect of dam age and the lambing programs in the puberty age of the Awassi female lambs. Master Thesis - Agriculture College– Baghdad University.