

Metachromasia in the exfoliated vaginal cells of Awassi ewes

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Abstract:

Smears from exfoliated vaginal cells of eight Awassi ewes were obtained from the animal farm of Veterinary College, Baghdad University during 2007. The smears were stained with Methylene blue and examined under light microscope. The present results clearly indicated that, through its upward migration, the vaginal epithelial cells undergo shifting in colour of both cytoplasm and nucleus, metachromasia. This may related to the different amount of glycogen loaded in the cells and the different amount of keratohylin which occupied the cell gradually and the pH of these cells after invasion with the endogens lactobacillus normally found in the vagina.

التغيرات اللونية لخلايا المهبل المنسلخة لنعاج العواسي

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الخلاصة:

أخذت مسحات مهبلية من ثمانية نعاج عواسية من الحقل الحيواني لكلية الطب البيطري، جامعة بغداد في 2007. صبغت المسحات بصبغة المثلين الأزرق ثم فحصت تحت المجهر الضوئي. بينت الدراسة الحالية أن هيولي ونواة الخلية يتعرضان إلى تغيرات لونية خلال هجرة خلايا الظهارة المهبلية من الطبقات القاعدية إلى الطبقات السطحية حيث يتحول اللون من الأزرق إلى الأحمر ثم الأرجواني وبشكل تدريجي وتعتقد الدراسة أن سبب ذلك يرجع إلى تجمع كميات مختلفة من الكلايوجين في الخلايا تبعاً للمراحل الوظيفية المختلفة وكذلك تبعاً لتراكم كميات مختلفة من الكيراتين الذي يغزو الخلايا تدريجياً إضافة إلى ذلك فإن عامل الأس الهيدروجيني يعتبر عاملاً آخر يؤدي إلى تغير لون الصبغات عند تعاملها مع الأس الهيدروجيني الحامضي بفعل البكتريا المتوطنة التي تقوم بتحويل الكلايوجين إلى حامض اللاكتيك.

Introduction:

The normal shifting of the colour from blue to red or purple when tissue components react with certain basic dyes is called metachromasia. Cells and tissue structures that have high concentration of ionized sulphate and phosphate groups, such as the ground substance of cartilage,

the heparin containing granules of the mast cells, and the rough endoplasmic reticulum of plasma cells, will exhibit metachromasia, (1, 2 & 3), stated that the mast cell granules were metachromatic because of the high content of acidic radicals in the glycosaminoglycans.

Metachromasia is a property of certain molecules that changes the

colour of some basic aniline dyes (ex. Toluidine blue) (1). Any metachromatic molecules take on a different colour (purple-red) different from that of the applied dye (blue) (4). The heterochromatin is metabolically condensed inactive, on the other hand, the euchromatin is depressed or extended active chromatin (5). So the light microscopist then can gain information about the relative activity of the cell by noting the appearance of the chromatin (4). Components that carry a net negative charge include:

The phosphate groups of nucleic acid which found in the nuclei, the sulphate groups of glycosaminoglycans which present in the ground substance and cells, carboxyl groups of proteins which is richly present in the most component of the cells (1).

Many researchers noted different characteristics of vaginal epithelium to diagnose the estrus cycle phases, pregnancy and post parturition. These different characteristics were related to the hormonal effects and ovarian activity (6-12). Less is

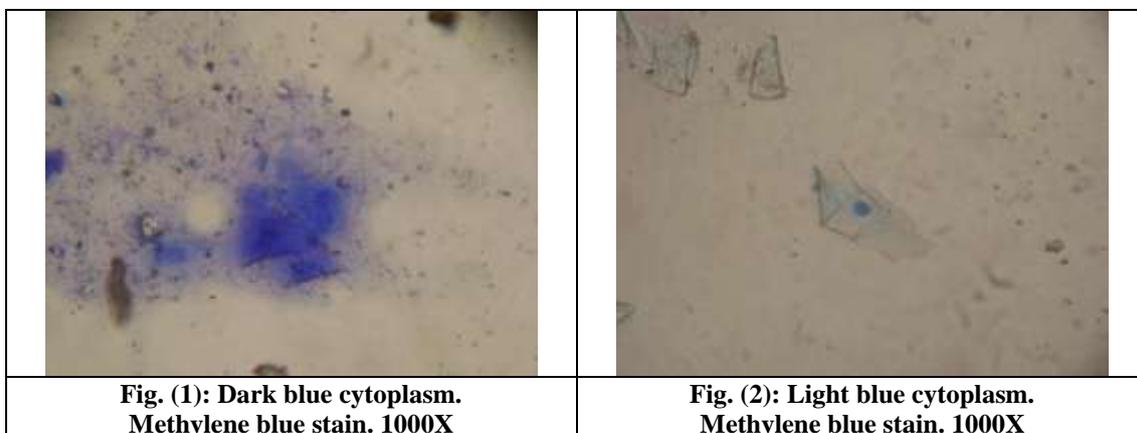
known about metachromasia so that this study try to throw more light on this phenomenon.

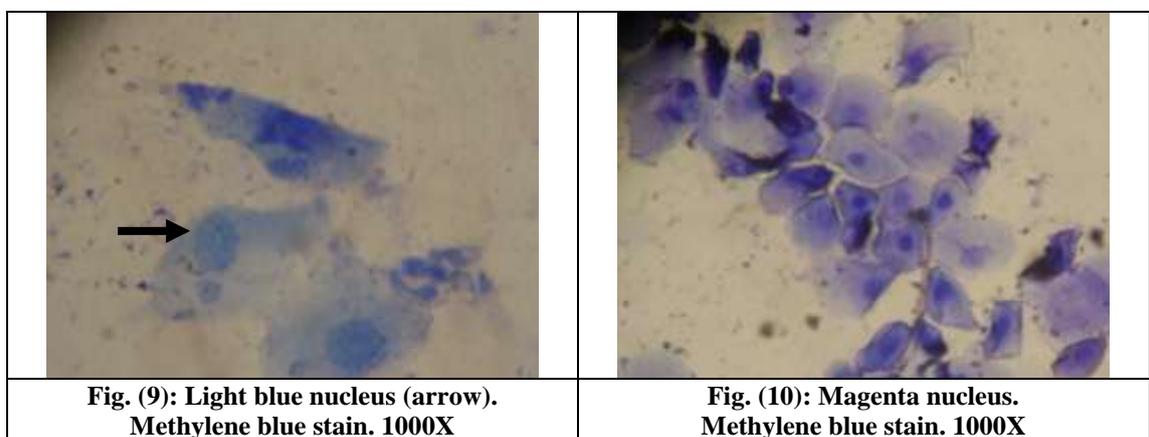
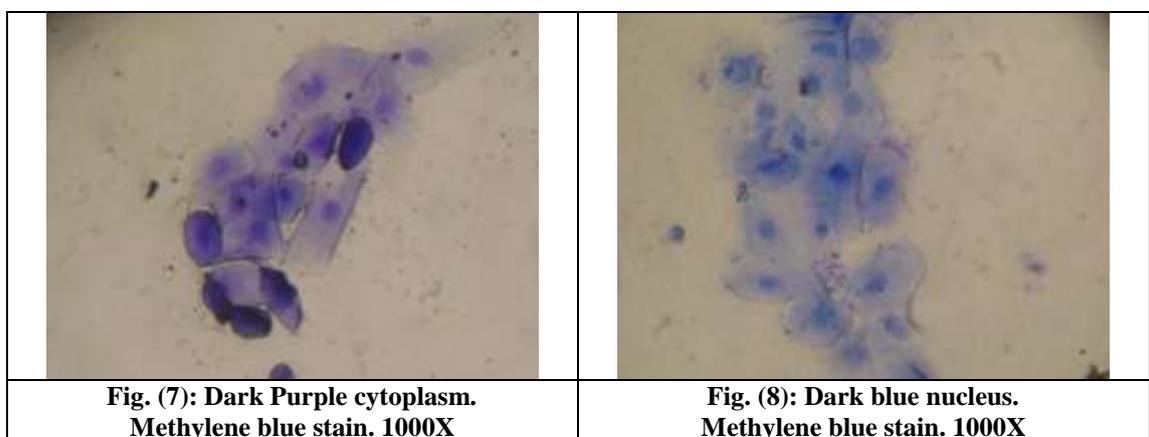
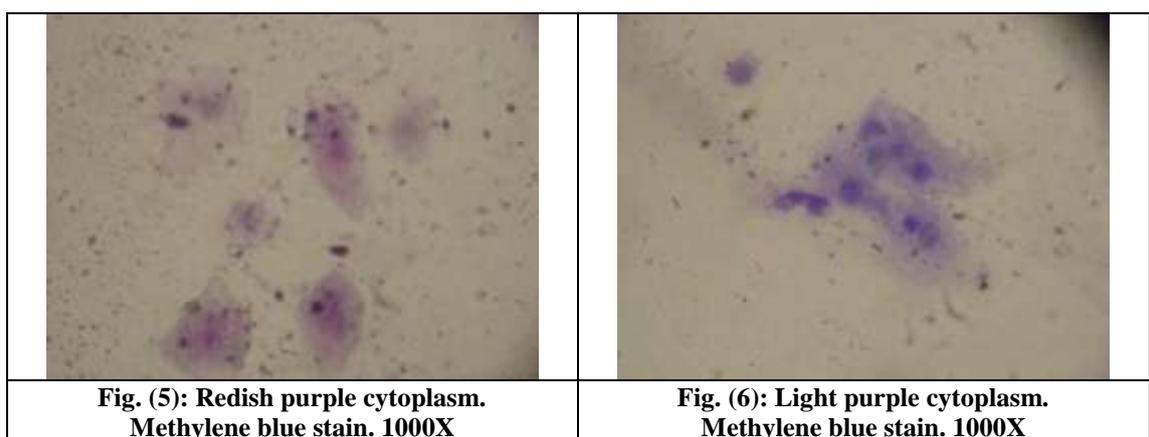
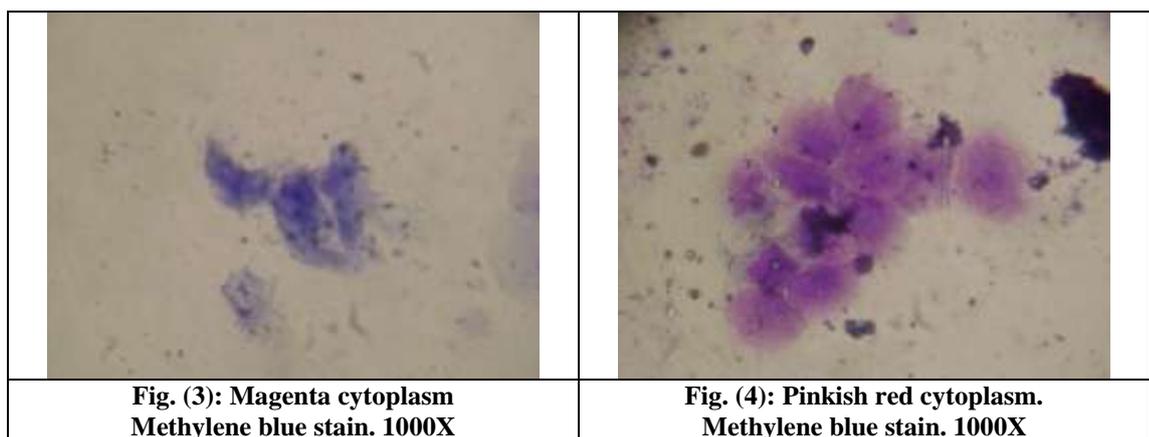
Materials and Methods:

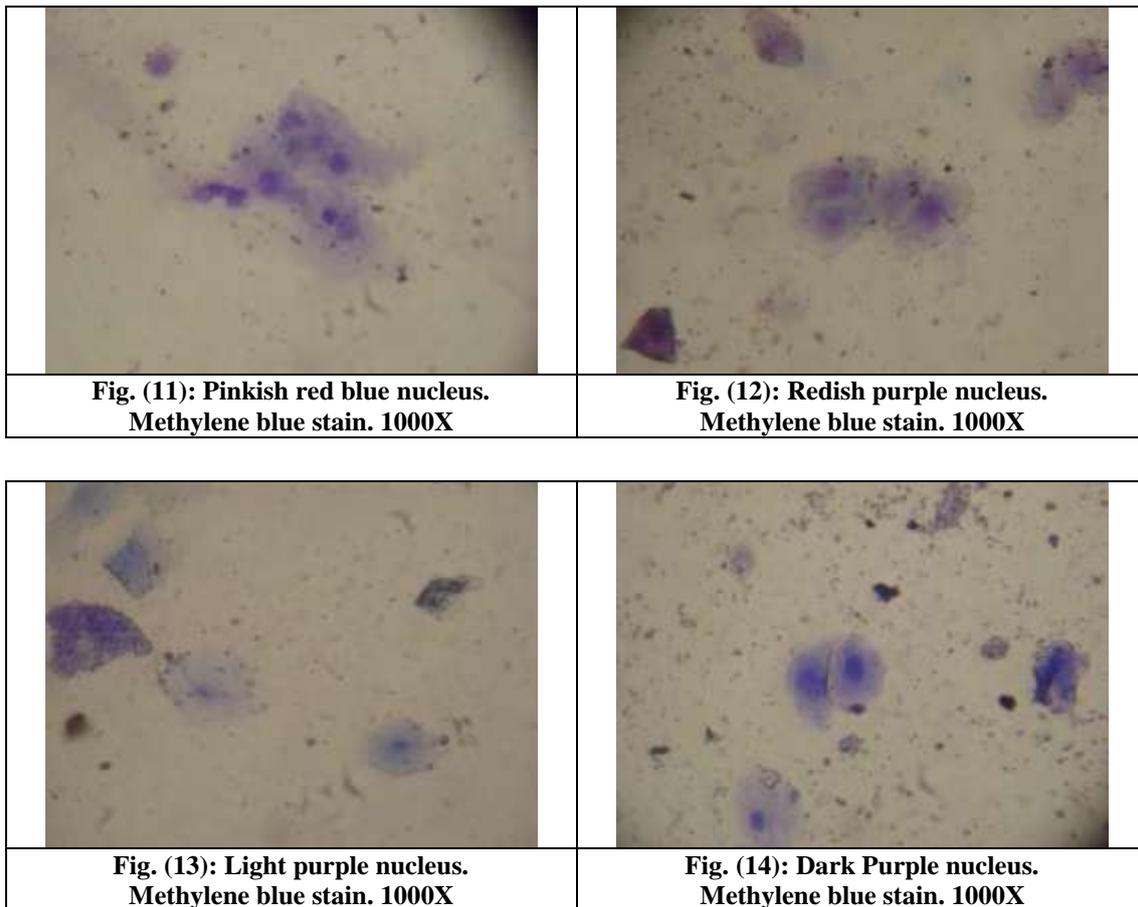
This study was carried out in the animal house of the College of Veterinary Medicine, Baghdad University during 2007, using eight adult healthy Awassi sheep. Vaginal smears were prepared and stained by using methylene blue, the slides were examined under light microscope according to Meander method which mentioned by (13). Ocular oculometer was used for measuring the diameters of the vaginal epithelial cells (14).

Results:

The present result revealed that the colour of both the cytoplasm and the nuclei of the exfoliated vaginal epithelial cells were gradually shifted, metachromatic, from the basic to reddish or purple colour (Fig. 1-14) during the upward migration of the vaginal epithelial cells from the basal layer to the superficial layer.







Discussion:

Many researchers mentioned that there were differences in the affinity for staining of the cytoplasm and nucleus in different physiological status (15-20), but they do not explain the causes of this phenomenon. Under the influence of estrogen, the epithelium cells accumulate large amounts of glycogen and undergo cell proliferation. The increase in glycogen accumulation toward the superficial layer and the increase in the amount of keratin which occupy the cytoplasm as well as the acidic pH of the vaginal lumen due to the presence of endogenous lactobacillus microorganism which metabolize the glycogen yielding lactic acid to decrease the pH of the vaginal lumen and finally to deters the invasion of

the pathogenic microorganism and fungi. All the above fore going factors result in different chemical composition of the different parts of the cells (1 & 5). According to the tissue components and the physiological status of the exfoliated vaginal cells they react differently with the different above status and finally undergo metachromas. The staining with basophilic blue stain revealed metachromatic staining along the collagen fibers and morphological changes (21). Cells and tissue structures that have high concentrations of ionized sulphate and phosphate groups, such as the heparin-containing granules and rough endoplasmic reticulum will exhibit metachromasia. Basic dyes

react with anionic components of the cells and tissues (1).

The ability of the anionic groups to react with the basic dyes is called basophilia. The staining with basic dyes may deal exactly with the pH of the cell. At high (about 10) all three above groups are ionized, at a slightly acid to neutral pH (5-7), sulphate and phosphate group, are ionized while at low pH (below 4) which is mostly present in the vaginal lumen, only sulphate groups remains ionized. When bacteria react with the glycogen at the superficial layer it decreases the pH to low pH. The exfoliated cells contain glycogen which is metabolized by commensal lactobacilli to lactic acid which forms an acidic (pH=3) environment to deters the invasion of bacterial pathogens and fungi (5). It was found that the pH fall from 7-8 to 6 at the end of follicular growth, low concentration of sulphated mucopolysaccharide are also evident in estrus (22). The cells with large pale nuclei containing little condensed chromatin are metabolically very active cells (4). The ribosomes serve like cytoplasm that have affinity for staining and containing RNA and protein (23). The rates of protein and glycoprotein synthesis in the uterus and cervix were higher in ewes with estrogen (24). (25) found that eosinophilic polychromatin fragmenting plasma cells account for the majority in healthy genital tract tissues during proestrus and estrus in the sheep. So the metachromatic stain is produced in certain elements colour different from that of the stain itself (26), the

new methylene blue stain a metachromatic stain used for staining of blood, vaginal smears and tissue samples for cytological examinations particularly for demonstration of reticulocytes.

It has been known for along time that metachromasia involves only the cytoplasm of the cells. The present study at first time noticed that the metachromasia involves also the nucleus which may be due to the phosphate groups which affected by the hormonal and ovarian activity.

References:

- 1-Ross MH, Romrell LJ & Kaye GI (1995): Histology. 4th ed. Williams and Wilkins, Sydney, PP: 374-375.
- 2-Junqueira LC, Carneiro J & Kelley, R.O. (2003): Basic Histology. 10th ed. A Lange Medical Book. PP: 28-104.
- 3-Junquera LC & Carneiro J (2009): Basic Histology Text Atlas. 11th ed. www.mk:@msitstore:h:basic%20histology. 23/5/2009.
- 4-Copenhaver WN, Vang RT & Byng MV (1971): Baileys Textbook of Histology. 16th ed. Williams Company. PP: 62-182.
- 5-Dudek RW (2004): High Yield Histology. 3rd ed. Philadelphia: Lippincott Williams and Wilkis, PP: 222-223.
- 6-Al-Wahab RMH & Bryant MJ (1971): Reproductive success in ewe lambs mated at an induced puberty. Anim. Prod, 24, 25 (1-3): 129.

- 7-Richardson C (1972). Diagnosis of pregnancy in the ewe by vaginal biopsy. *Br Vet J*, 128: 316-330.
- 8-Ducker MJ & Boyd JS (1974). An evaluation of the vaginal smear technique for detection the occurrence of ovulation in the ewe. *J Reprod Fert*, 41: 249-254.
- 9-Bartol FF, Wiley AA & Goodlett DR (1988). Ovine uterine morphogenesis: histochemical aspects of endometrial development in the fetus and neonate. *J. Anim Sci*, 66:1303-1313.
- 10-Yamadai MLA & Kozicki LE (1998):Gestation diagnostic in *Capra hircus*, through histological and cytological analysis of the vaginal epithelium. *Brazilian J Vet Res Ani Sci*, 35 (6): <http://www.scielo.br>.
- 11-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.
- 12-Neama HF (2000): Evaluation of reproductive performance of Awassi ewe lambs by using some hormonal programs. Master Thesis – Agriculture College – Baghdad University.
- 13-Coles EN (1986): *Veterinary Clinical Pathology*. 4th ed.
- 14-Geswami P & Wani GM (2009): Cervical alterations during Pregnancy in Small Ruminants. <http://www.articlesbase.com/science-articles/cervical-alterations-during-pregnancy-in-small-ruminants-442936.html>, 07.03.2009.
- 15-Luna, G (1968): *Manual of Histological Staining Methods of the Armed Forced Institute of Pathology*. 3rd ed. Mc Graw Hill Book Company New York.
- 16-Hussin AM (2006): The vaginal exfoliative cytology of Awassi ewes during post-parturient periods. *Iraqi Journal Veterinary Medicine*, 30 (2): 130-137.
- 17-Zaid NW (2006): Effect of Prostaglandins F_{2α} on the Evaluation of Vaginal Epithelial Cells of Iraqi Ewes during Puerperium. *Iraqi Journal Veterinary Medicine*, 30 (2): 138-144.
- 18-Hussin AM (2009):The theory of neutrophilic cellular penetration. *Al-Anbar J Vet Sci*, 2 (1):58-68.
- 19-Hussin AM (2010a): The theory of keratinization. Unpublished paper.
- 20-Zaid NW (2010):The effect of vitamin A supplement on the vaginal epithelium during pregnancy months and post parturition. Unpublished paper.
- 21-Hussin AM (2010b): The histological effects of vitamin A supplement on the vaginal epithelial

cells during estrus and luteal phases in Awassi sheep. Unpublished paper.

22-Hughes RL & Rodger JC (2009). Studies on the vaginal mucus of the marsupial *Trichosurus vulpecula*. Australian J Zoology, 19 (1): 19-33.

23-Lesson CR, Lesson TS & Paparo AA (1985). Textbook of Histology. 5th ed. Lg Aky Shoin Saunders.

24-Tang BY & Adams NR (1981). Oestrogen receptors and metabolic activity in the genital tract after ovariectomy of ewes with permanent infertility caused by exposure to phyto-oestrogens. J Endocrinology, 89: 365-370.

25-Leucocyte-like cells of the vagina and uterus, and their modification during the normal estrous cycle and by progestin and estrogen treatment. J Anatomical Record, 155 (4): 591-601.

26-Internet (2010). metachromatic stain. Free Online Medical Dictionary, Thesaurus and Encyclopedia.htm, Free dictionary, www.thefreedictionary.com.