The Effect of Vitamin A Supplement on Vaginal Epithelium During Pregnancy and Post Partum in Ewes

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Abstract

This trail was carried out on (12) Awassi ewes in the animal farm of College of Veterinary Medicine, in Baghdad University, during the period from May to November 2007, to study the cellular changes that occur in the vaginal epithelium during pregnancy and after birth. The animals were divided into two groups. The first group (treated group) gave (60.000) IU orally vitamin A once weekly. The second group left as control group. High mitotical figures have been observed in the vaginal smear of treated group. The size of the cells and the nuclei was significantly increased in the treated group, the affinity of the cytoplasm and nucleus for staining was elevated. No changes in keratinization and vacuolation intensity, whereas the presence of neutrophils in the treated group was obvious. These observations indicate the importance of vitamin A during pregnancy and pureperium to increase cell proliferation and cellular immunity.

تأثير إضافة فيتامين أ على الخلايا المهبلية المنسلخة للنعاج خلال الحمل وبعد الولادة

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الخلاصة

أجريت هذه التجربة على اثنتا عشر (12) من النعاج العواسية الموجودة في الحقل الحيواني لكلية الطب البيطري، جامعة بغداد للفترة من آيار إلى تشرين الثاني 2007لدراسة التغيرات الخلوية التي تحصل في ظهارة المهبل خلال الحمل وبعد الولادة. قسمت حيوانات التجربة إلى مجموعتين، المجموعة المعالجة والتي جرعت فموياً بـ(60.00) وحدة دولية من فيتامين أ مرة واحدة أسبوعياً والمجموعة الثانية والتي تركت كمجموعة سيطرة. لوحظ زيادة الانقسام الخلوي للمسحات المهبلية في المجموعة المعالجة. أزداد حجم الخلايا والأنوية بشكل معنوي في المجموعة المعالجة، وارتفعت آلفة الهيولي والأنوية للأصباغ. في حين لم يظهر أ ي اختلاف في وجود التقرن والفجوات. بينما لوحظ وجود واضح للخلايا العدلة في المجموعة المعالجة. أكد ت الدراسة الحالية أهمية فيتامين أ خلال الحمل وبعد الولادة لزيادة الانقسامات والمناعة الخلوية.

Introduction

Vitamin A considered as a fatty soluble vitamin which have a wide physiological uses (1). This vitamin has an essential role in cell growth and differentiation especially the epithelial cells (2). The hormonal-like action of vitamin A can keep the process of cellular differentiation (3). This vitamin have the ability to effect on the enzyme which inter in the synthesis of glycoprotein and glycosaminglycan as a component of cellular surface (4). Vitamin A affects cell morphogenesis through its effect on the gap junctional spaces (5). As it is known that the epithelium of the vagina is mostly stratified squamous epithelium, those epithelium like other reproductive tissues is

hormonal dependant, so that the height of the epithelium and the degree of keratinization vary with hormonal fluctuations during estrus cycle (6), the vaginal keratinization occur more with administration of estrogen (7). Vitamin A plays an important role in reproduction in the ewes which attributed to increasing ovarian activity, vaginal secretion, hormonal secretion and fertilization rate (8). Vitamin A is one of the critical vitamins in domestic animals, the decrease of its level causes reproductive imbalance that lead to decrease of reproductive efficiency (9). Fiori (10) found that the vaginal exfoliated cytology is correlated with the ovarian cycle.

A little knowledge is known about the cellular effect of vitamin A on the vaginal epithelium, so that, this study was conducted to evaluate the effect of vitamin A on the vaginal cytology during pregnancy and post parturition.

Materials and Methods

This study was conduct on (12) adult healthy Awassi ewes. These animals was divided into two groups, the first group was given (60.000) IU/animal orally once weekly during pregnancy and post partum. The second group left as a control group. These animals were kept in the farm animals of Veterinary Medicine College in Baghdad University during the period from May to November 2007. All animals were immunized against internal and external parasites and contagious diseases. The diet of these animals was ad libitum in two periods (after morning and after noon), the water and minerals was provided in the farm.

The vaginal smears were taken from all animals once weekly according to the methods of Neama (11). The staining was done by using methylene blue stain. The examination was done under light microscope and the measurement of the cells was done by using the ocular micrometer according to Coles (12). The data were calculated by using ANOVA test, the LSD was determine the statistical differences between the mean of the groups according to Al-Mohammed *et al.* (13).

Results

The present study revealed that the exfoliated vaginal cells have different shapes, sizes and cytoplasmic affinity for staining. The predominant cells were the identical neighbouring spherical cells with large spherical bluish nuclei, and of the size (22.5 µm) that indicated the state of mitosis, the remainder of the cells were the oval or even irregular polygonal cells (Fig. 1).

Table (1) showed that the small exfoliated vaginal cells (mitotic cells) were significantly increased ($P<0.01$) in the treated group comparison with other cells or even with the non-treated group.

The cytoplasmic affinity for staining varies with the different functional status, some cells have grayish magenta cytoplasm, others have dark magenta or even light greenish blue cytoplasm, some cells have homogenous cytoplasm, others have granular cytoplasm depending on the activity of the cell (Fig. 2).

The light cytoplasm was significantly increased ($P<0.01$) in the treated group more than the dark cytoplasm in the non treated group (Table 2).

The nuclei of the exfoliated vaginal cells may be central or marginal or even absent. The nuclei may be spherical or oval, magenta or bluish sky stain. Nucleoli may or may not be present in the nuclei depending on the activity of the cells in keratin synthesis (Fig. 3, 4 and 5).

Table (3) showed that there was significant increase ($P<0.01$) of the small-sized nuclei in the treated group in comparison with the non treated one. On the other hand the affinity of nucleus for staining in (Table 4) showed that the light nucleus was significantly increased ($P<0.01$) than the dark one and even the non treated group.

The keratinized cells were devoid of nuclei and have a thickened cell membrane because of the deposition of proteinous compound and have a keratin-filled cytoplasm (Fig. 5).

The treated group showed less keratinization than the non treated group during pregnancy months and post parturition (Table 5).

The presence of vacuolated cells and the folded cells were more numerous in the treated group than the non treated group (Fig. 6 and 7), (Table 6 and 7).

Some of the exfoliated vaginal cells have a water-clear cytoplasm without nuclei and surrounded by phagocytized neutrophils. This may be due to the role of phagocytosis done by the neutorphils to the organells of the exfoliated cells (Fig. 8).

(Table 8) showed elevation of neurophils in the treated group in comparison with the non treated group.

Table (1) The effect of vitamin A supplement on the exfoliated vaginal cell size during different pregnancy months and post parturition

Cell size / Reproductive stage	Non treated group			Treated group		
	Large (50-75)µm	Medium (30-50)µm	Small (12-25)µm	Large (50-75)µm	Medium (30-50)µm	Small (12-25)µm
Month 1	43 bf	37.5 di	19.5 kp	16.25 mp	38 ci	45.75 be
Month 2	42 bf	32 el	26 io	27.75 gm	34.5 ei	37.75 di
Month 3	34 ej	32 el	34 ej	13 np	35 ei	52 be
Month 4	27 in	38.5 ci	34.5 ei	10.5 p	27.5 gm	69 a
Month 5	18.5 lp	51.5 bd	30 fm	20 jp	45 be	35 ei
Post parturient	18.2 lp	41.4 ch	40.4 ch	11.9 p	36.7 ei	51.4 bd
Non pregnant	25.5 io	41.58 cg	32.92 ek	16.5 mp	27.33 hm	56.17 ab

- The numbers represented the percentage% of observation.
- The small letters represented significant differences at (P<0.01).

Table (2) The effect of vitamin A supplement on cytoplasm's affinity for staining during different pregnancy months and post parturition

Cytoplasm affinity for staining / Reproductive stages	Non treated group		Treated group	
	Dark	Light	Dark	Light
Month 1	25.125 lo	74.875 ad	39 hm	61 ch
Month 2	22.5 lo	77.5 ad	37.75 in	62.25 bg
Month 3	11 o	89 a	43 gn	57 ci
Month 4	16 no	84 ab	69 af	31 jo
Month 5	21 mo	79 ac	52.5 ej	47.5 fk
Post parturient	28.8 ko	71.2 ae	44.4 gl	55.6 di
Non pregnant	50.58 ek	49.42 ek	57 ci	43 gm

- The numbers represented the percentage% of observation.
- The small letters represented significant differences at (P<0.01).

Table (3) The effect of vitamin A supplement on nuclear size during different pregnancy months and post parturition

Nucleus size / Reproductive stage	Non treated group				Treated group			
	Large (<12.5)µm	Medium (7-12.5) µm	Small (>7)µm	Without	Large (<12.5)µm	Medium (7-12.5) µm	Small (>7)µm	Without
Month 1	43.25 ag	4 ux	29.875 fn	22.875 jr	28.25 hp	27.25 hp	9.5 qx	35 ck
Month 2	56 a	6 sx	20 ls	18 mu	38.25 bi	23.75 ir	10 qx	28 hp
Month 3	42 ah	6 sx	28 hp	24 iq	28 hp	45.5 ae	5 tx	21.5 ks
Month 4	49.5 ac	11.5 qx	15.5 nw	23.5 iv	44.5 af	37 bj	17 mv	1.5 wx
Month 5	57 a	17 mv	3 vx	23 jr	41 bh	9 rx	0 x	50 ab
Post parturient	45.5 ae	34.4 dl	9.1 qx	11 qx	15.7 nw	47.2 ad	19.5 lt	17.6 mv
Non pregnant	41.17 bh	29.33 go	14 px	15.5 nw	31.92 en	33.5 dl	14.42 ox	20.16 ks

- The numbers represented the percentage% of observation.
- The small letters represented significant differences at (P<0.01).

Table (4) The effect of vitamin A supplement on nucleus affinity for staining during different pregnancy months and post parturition

Nucleus affinity for staining / Reproductive stages	Non treated group		Treated group	
	dark	light	dark	light
Month 1	66.32 be	33.68 hk	50.5 eh	49.5 eh
Month 2	71 ac	29 jl	46.75 fi	53.25 dg
Month 3	73 ac	27 jl	15 l	85 a
Month 4	52.5 dg	47.5 fi	60 bf	40 gk
Month 5	32 ik	68 bd	43.5 fj	56.5 cg
Post parturient	70.5 ac	29.5 jl	25.8 kl	74.2 ab
Non pregnant	49.7 eh	50.3 eh	47.75 fi	52.25 dg

- The numbers represented the percentage% of observation.
- The small letters represented significant differences at (P<0.01).

Table (5) The effect of vitamin A supplement on the keratinization during different pregnancy months and post parturition

The groups / Reproductive stages	Non treated group	Treated group
Month 1	31.125 a	37 a
Month 2	26 a	47 a
Month 3	43 a	23 a
Month 4	31.75 a	7 a
Month 5	20..5 a	26.5 a
Post parturient	15.2 a	20.4 a
Non pregnant	45.5 a	43.1 a

- The numbers represented the percentage% of observation.
- The small letters represented no significant differences.

Table (6) The effect of vitamin A supplement on the vacuolation during different pregnancy months and post parturition

The groups / Reproductive stages	Non treated group	Treated group
Month 1	1.25 a	4 a
Month 2	0 a	4 a
Month 3	0 a	2.5 a
Month 4	2.5 a	5 a
Month 5	5 a	6 a
Post parturient	12.1 a	0 a
Non pregnant	1.3 a	6.92 a

- The numbers represented the percentage% of observation.
- The small letters represented no significant differences.

Table (7) The effect of vitamin A supplement on the folding during different pregnancy months and post parturition

The groups / Reproductive stages	Non treated group	Treated group
Month 1	2.125 a	5 a
Month 2	4 a	8.25 a
Month 3	6 a	6.5 a
Month 4	5.25 a	1 a
Month 5	4.5 a	0 a
Post parturient	3 a	5.6 a
Non pregnant	2.5 a	9.58 a

- The numbers represented the percentage% of observation.
- The small letters represented no significant differences.

Table (8) The effect of vitamin A supplement on the white blood cells during different pregnancy months and post parturition

The groups / Reproductive stages	Non treated group	Treated group
Month 1	-	+++
Month 2	++	+
Month 3	-	-
Month 4	-	-
Month 5	-	+
Post parturient	-	-
Non pregnant	-	++

- The marks represented the presence of neutrophils.

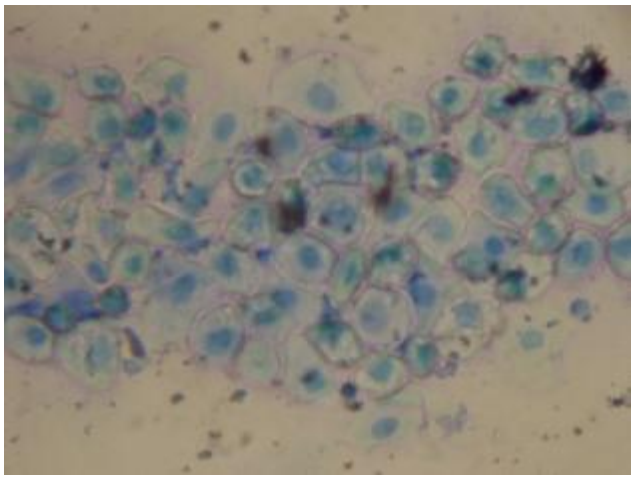

Fig. (1) Exfoliated vaginal epithelial cells with different sizes.
Methylene blue stain 400X.

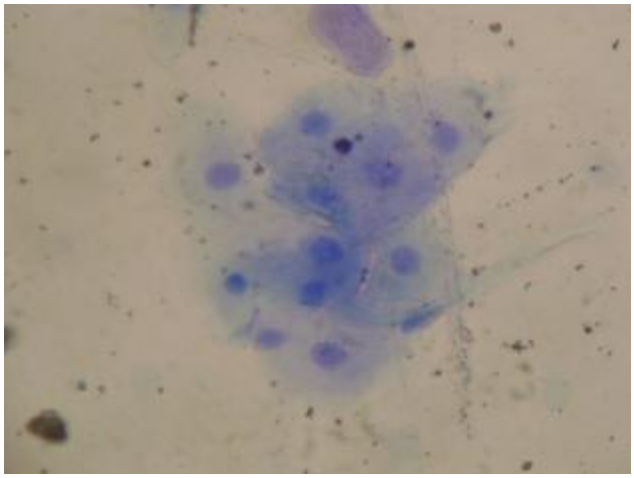

Fig. (2) Exfoliated vaginal epithelial cells with different cytoplasmic affinity for staining.
Methylene blue stain 400X.

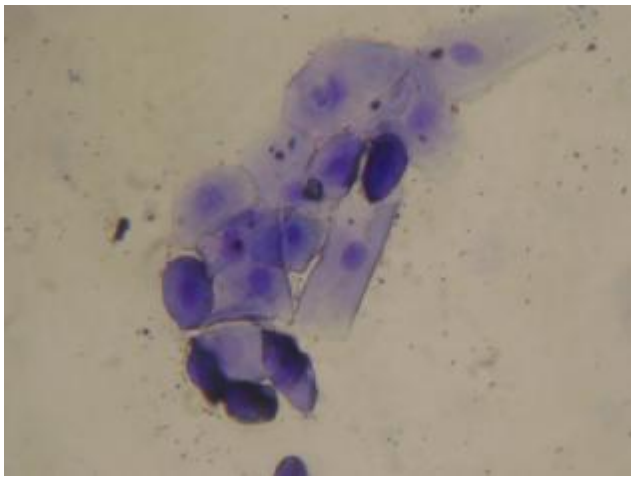

Fig. (3) Exfoliated vaginal epithelial cells with different nuclear sizes.
Methylene blue stain 1000X.

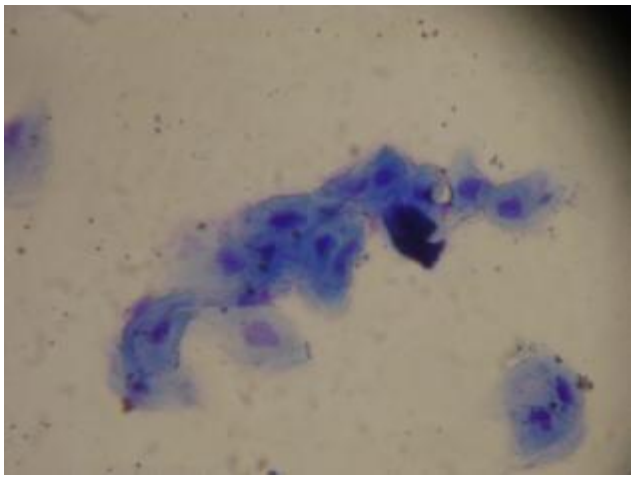

Fig. (4) Exfoliated vaginal epithelial cells with different nuclear affinity for staining. Methylene blue stain 1000X.

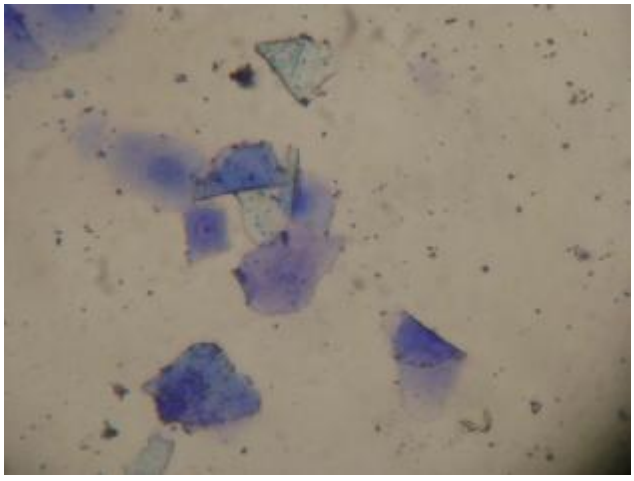

Fig. (5) Keratinization of the exfoliated vaginal epithelial cells. Methylene blue stain 1000X.

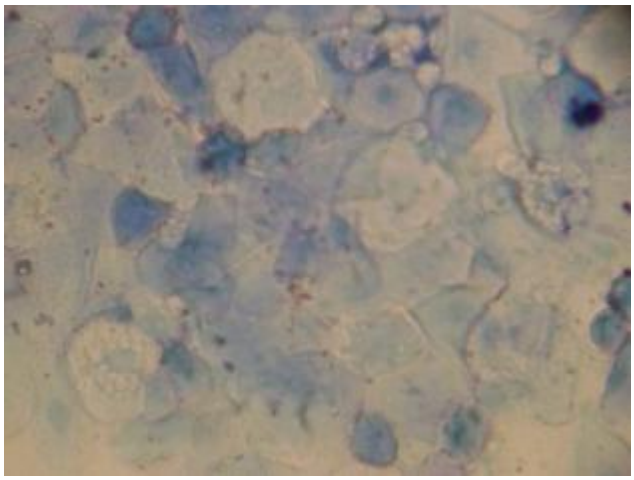

Fig. (6) vacoulation of the exfoliated vaginal epithelial cells. Methylene blue stain 1000X.

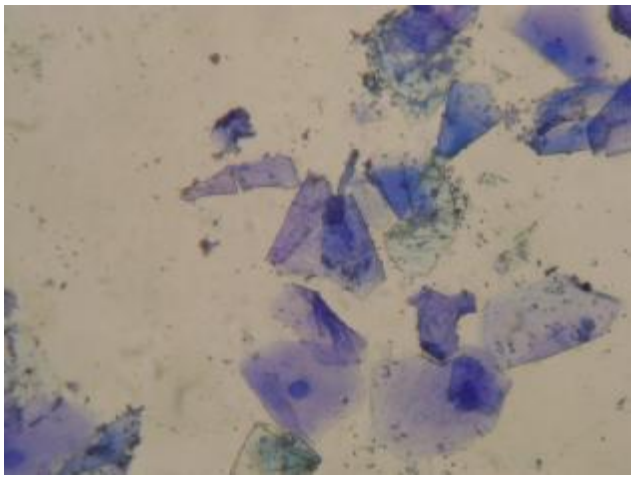

Fig. (7) Folding of the exfoliated vaginal epithelial cells. Methylene blue stain 1000X.

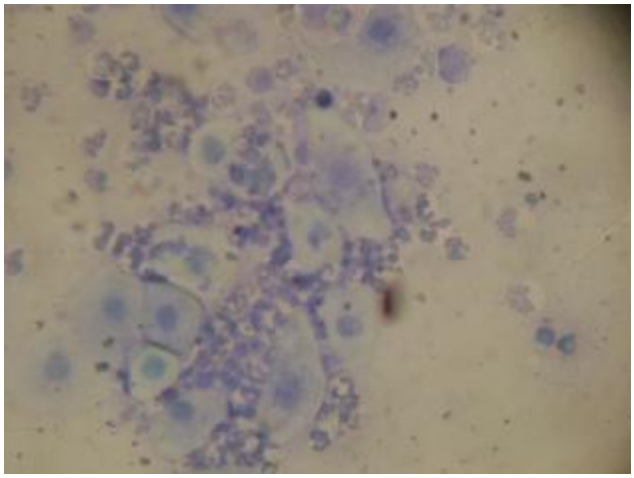

Fig. (8) Presence of neutrophiles in the exfoliated vaginal epithelial cells. Methylene blue stain 1000X.

Discussion

In fully mature vaginal mucosa, 5 types of cells were easily identified: cells of the basal layer (basal cells), cells above the basal layer (parabasal cells), cells of the intermediate layer, precornified cells and cornified cells, based on the numbers of cell types that appear in the vaginal smears (14). Many researchers have been studied variations which occur in the vaginal epithelium at pregnancy using vaginal smears (11, 15). They classified the vaginal epithelial cells into three types without measuring their diameters, while others (16, 17, 18) classify the vaginal cells into three basic cell types according to their diameters. These variations occur under the influence of estrogen and progesterone that secreted from the ovary (19) and the valuable informations can be obtained from the hormonal status of these hormones (14).

Vitamin A could be bound to transfer proteins in the circulation, which is necessary for cell development throughout the life (20). Ganong (21) found that vitamin A plays an important role in proliferation of the immune cells to protect the epithelial cells. While others (22, 23) stated that the principle role of vitamin A in decreasing the infection depend on the theory which stated that the vitamin A has two roles, the offensive role by protect the internal epithelium and the defensive role through protecting the immune system by activating of B and T cells.

Chew (24) explained that the reason of abortion caused by the deficiency of vitamin A in late gestation period was due to the decrease in the uterus production of the principle proteins necessary for embryo nutrition like uteroferrin which is glycoprotein

produced from the internal layer of uterus and responsed for transportation of iron from the dam to the embryo through placenta. It is well known that vitamin A is an essential nutrient for normal cellular function including reproduction and development (25).

The variation in the size of vaginal exfoliated epithelial cells and nuclei cells noticed in the treated group indicates that there were a state of cell divisions. Besides, the affinity of the cytoplasm and nucleus for staining may be depending on accumulation of cytoplasmic glycogen due to the increases in metabolic activities. This is similar to the finding of (17, 18).

In view of our finding, there were no significant differences in keratinization between treated and non treated groups. This agreed with the suggestion that the vitamin A apparently inhibits keratin formation, and the cells forming keratin exhibit partial atrophy (26). Moreover Takasugi (27) found that the high dose of vitamin A inhibits cornification of the vaginal epithelium.

The presence of vacuoles might be occurred due to glycogen consumption during ovarian activity (28), meanwhile Lesson *et al.* (29) claimed that the vacuoles excrete secretory product of different density.

The presence of neutrophils was well observed in the recent study. This is in accordance to other worker (17, 30) who mentioned that neutophil was followed by a recovery period, due to increase of immunity (18, 31). Lawson *et al.* (32) mentioned that vitamin A play an important role in stimulation of the gene responsible of reproduction and differentiation of neutrophils through RXR and RAR receptors. The inflammatory cells invading cervix toward late gestation provide a potational source of collagenous and neutral protection activity (33).

The conclusion of the present study showed that vitamin A protects the epithelial health by increasing the proliferation of cell mitosis and increasing the neutophil infiltration.

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