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Evaluating The Influence of Vitamin E and Selenium on The Uterine Health of Adult Female Goats: A Histological Study

Alaa Shamil Alalaf * **and Raghad Alnuaimy**

Department of Animal Production, College of Agriculture and forestry , University of Mosul, Mosul, Iraq.

Abstract

THE uterus is a remarkable organ. with an unmatched ability to grow and regenerate, undergoing continuous cyclic transformations. Vitamins E and selenium renowned for their antioxidant properties and reparative actions in microstructural rehabilitation and repair. Therefore, the current study was conducted to investigate the influence of vitamin E and selenium on the histomorphology of uterine tissue in adult female goats (*Capra hircus*). Ten healthy animals were used in this study. And divided into two groups. The first group was the control group fed regular diet (standard) and provided water ad libitum. The second group received the same food but with a combination of vitamin E and selenium given 2ml per day in water for 25 days. Uterine samples were collected and preserved in 10% NBF. Routine histological processing was performed and sections were stained with Harries hematoxylin and eosin stain, and Masson's trichrome stain, as well as additional slides were stained with Periodic Acid Schiff (PAS) and Alcian blue 2.5 pH. The observations revealed that vitamin E/selenium supplemented animals had healthier uterine microstructure, since it fixed the epithelial disintegration, uterine glands integrity, and uterine microvasculature, furthermore the supplemented animal had larger caruncles and thicker myometrial musculature, The histochemical findings showed an intense PAS reaction in the uterine epithelium basement membrane, uterine glands, wall of blood vessels. The alcian blue stain showed positive reaction in the endometrium ground substance. Encouragingly, incorporating vitamin E and selenium synergistic supplements holds promise for augmenting animal fertility and increasing conception rates.

Keywords: Animal, Histochemical, Goats, Uterus, Vitamin E/Selenium.

Introduction

Minerals and vitamins are important for animals' reproductive functions as they participate in various enzyme systems. The reproductive success of farm animals depends on having the right balance of essential minerals and vitamins, as they are crucial for cellular metabolism, and growth [1]. Imbalances or deficiencies in the minerals and vitamins can lead to inactive ovaries and repeat breeding in dairy animals [2].

Vitamin E is an important biological antioxidant is essential for optimal nutrition and a powerful free radical scavenger. The generation of free radicals can potentially lead to infertility [3]. malformed spermatozoa [4], and preimplantation embryos [5].

Studies have shown that the administration of vitamin E and selenium can enhance bovine fertility [6]. Other reports that demonstrated significant number of antioxidants in the diet of high-producing cows are necessary to maintain stable metabolic health and maximize milk production [7].

Additionally, the treatment of cows in late pregnancy with vitamin E and selenium has been shown to reduce the incidence of retained placenta [8]. While, selenium deficiencies have been found to suppress the immune response in different species of animals, this suppression of the immune system can indirectly contribute to reproductive inefficiencies [9]. Moreover, cattle experience a great response to selenium in many reproductive disorders, including retained placenta, abortion, stillbirth, irregular estrous cycles, early embryonic mortality, and cystic ovaries, and found that Supplementing selenium,

*Corresponding author: Alaa Shamil Alalaf, E-mail: alaa.shamil@uomosul.edu.iq , Tel.: +9647717894851
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periodically, and offering Se-mineral block are all possible treatments for selenium deficiency-related infertility [10].

Several studies have investigated the effects of vitamin E and selenium supplementation on reproductive performance and immune activity in various animal species, including cows [11], humans [12], pigs [11], Baladi sheep [12], and rats [13]. However,[10], also report that toxicity with vit E and selenium particularly cause, hair loss, muscle tremors, and lightheadedness, and in severe cases heart attack, and kidney failure or respiratory distress was recorded. There is a notable gap in the literature concerning the impact of vitamin E and selenium supplementation on adult female goats. Therefore, the objectives of this study are to investigate the influence of vitamin E and selenium on the microstructure histomorphology of uterine tissue in adult female goats (*Capra hircus*).

Material and Methods

Study design

The study was conducted using ten healthy adult female goats (*Capra hircus*) at 1.5 and 2 years age. obtained separately from reputable farms in Bashiqa-Mosul provinces-Iraq (36.45046°: 43.34977°, Lat: Long) The animals were divided into two groups. The first group served as the control group fed regular diet (standard) and provided water ad libitum. The second group received the same food as the control group but with the addition of a combination of vitamin E and selenium (Growvite®, Ireland) as a supplement given 2ml per liter of water (in 500L trough) once a day for 25 days each 1ml contain (100 mg of vitamin E and 200 mcg sodium selenite), according to the company instructions.

The study was scheduled from July to November 2023 and performed in the same farm fields, the animals slaughtered and the samples taken followed guidelines set by the American Veterinary Medical Association (AVMA) [16]. Uterine samples were collected and examined for gross integrity, and small specimens were taken from the uterine horn, uterine body, and cervix. These specimens preserved in 10% neutral buffered formalin for histological processing.

Histological analysis

Afterward, the collected samples were prepared for histological processing, dehydrated using ascending series of alcohol concentrations (70%, 80%, 90%, and 100%). For the next step the sample were cleared with xylene for 20 minutes and infiltrated with melted paraffin at 58ºC. Subsequently, paraffin blocks were prepared. Thin sections of 5 micrometers were trimmed using a rotary microtome (BR Biochem, BRM-1120, India) and mounted on glass slides.

To visualize the tissue components, the sections were stained with Harries hematoxylin and eosin stain, as well as Masson's trichrome stain (ThermoFisher chemicals, USA), following the protocol of [17]. The stained sections examined under light microscope (Olympus, Japan), for routine histological assessments.

Histomorphometric analysis

For histomorphometric analysis of the uterus, a 3.0 USB Microscope camera (HDC DF2, 2.9x2.9 µm ,China) with ImageJ software by (NIH Image, USA) was used. The measured parameters included the thickness of the endometrium (functional and proliferative layers), myometrium thickness, perimetrium thickness, diameter of uterine glands, and diameter uterine arteries [18].

Furthermore, additional sections stained with Periodic Acid Schiff (PAS) and Alcian blue stain (2.5 pH) to detect the presence of glycoproteins and acidic mucopolysaccharides, following the protocol described by [17].

Statistical analysis

The data generated from the histomorphometric study were analyzed to calculate the Mean ± standard error. Additionally, an independent t-test analysis was performed to evaluate the differences between the two groups. This analysis was conducted using (IBM SPSS v. 25,UK) software with a significance level set at $P \leq 0.05$. [19].

Results

Uterine horns

In both groups, the uterine horn was composed of three distinct layers; the endometrium, myometrium, and perimetrium. The endometrial layer, also considered as the mucosal and submucosal layer, comprises of two distinct zones. The first zone was the functional zone, which was the superficial layer that shed during estrus. The second zone was the basal zone, which was the thin, deep generative layer that remains after shedding of the functional layer.

The myometrium was composed of smooth muscle fibers that arranged into two layers. The inner layer had a circular arrangement and was relatively thick, while the outer layer had a longitudinal arrangement. The perimetrium, on the other hand, consisted of loose connective tissue that was lined by the peritoneal mesothelium (Fig. 1).

The histological observations of the uterine horns in the control and vit E/selenium groups showed obvious significant differences. Starting with the epithelium, the majority of epithelium was sloughed off and the remaining was mostly pseudostratified columnar epithelium in the control animals while shedding was less extended and partially disintegrated in the supplemented group (Fig. 1). The

functional layer of the endometrium was filled with a high number of large well-developed, coiled and branched uterine glands lined with simple cuboidal and pseudostratified columnar epithelium in the supplemented group, whereas the functional layer in the control group was lake or had a little number of small uterine glands (Fig.2). Moreover, the blood vessels were present in a high density in the supplemented group compared to the control group.

The functional layer showed a large uterine caruncle in the supplemented group. The basal layer was filled with a numerous small, branched uterine glands in both supplemented and control groups endometrium of the left and right uterine horns (Fig. 3).

The myometrium muscular layers were thick in the supplemented group. The inner layer was thicker than the outer, whereas it was thinner in the control group, and both muscular layers (inner and outer) had same thickness (Figs. 4, 5).

The perimetrium was slightly thicker in the supplemented animal group compared to the control, and it was attached to the uterine ligament. Masson's trichrome findings revealed that connective tissue distribution was unequal throughout the endometrium and in the supplemented animals and the density of connective tissue was higher in the basal layer than the functional layer compared to control groups. The connective tissue was clear around the large blood vessels between the myometrial layers, particularly in the supplemented group compared to the control animals. The density of connective tissue was moderate in the perimetrium in both animal groups (Fig. 5, Table 1).

The histochemical findings revealed an intense positive reaction of PAS stain at the uterine epithelium basement membrane, uterine glands, wall of blood vessels, and the perimetrium serosal layer. The intensity increased noticeably in the supplemented group's uterine horns. The alcian blue stain was positive in the endometrium ground substance, and the intensity became moderate with the control group (Fig. 6, 7).

The positive PAS staining reflects the glycogen intercellular granules and the neutral glycoprotein throughout uterine horns' structures, while the alcian blue stain reflects the acidic glycoprotein content with the endometrium ground substance.

The uterine body

The histology of the uterus was similar to the uterine horns. In the supplemented group, the endometrial functional layer showed large, coiled, and branched uterine glands, compared to the control group which was smaller in size. Furthermore, the basal zone showed many small, branched, and simple uterine glands in both animal groups. The uterine blood vessels were numerous and large in the supplemented group compared to control animals. and large parts of the endometrium were disintegrated in both groups. The myometrium composed of inner circular and outer longitudinal layers of muscular fibers interspersed with large uterine arteries. The myometrium and the perimetrium were thicker in supplemented animals.

The histochemical analysis revealed an intensely positive reaction to PAS staining at uterine glands, and wall of blood vessels. The intensity of PAS staining increased notably in supplemented alcian blue staining was positive at endometrial ground substance in the supplemented group of animals, compared with its moderate intensity in the control group.

Cervix

Observations of the cervix in supplemented goats revealed that the cervix comprised of three layers. endocervix layer was lined by stratified columnar epithelium with wide mucosal folds. Turned to non-keratinized epithelium near the vagina. The lamina propria submucosa contained a thin layer of loose connective tissue. This layer was denser composed of collagen and smooth muscle fibers supplemented animals compared to the control group. With uneven connective tissue distribution between endocervix and ectocervix as well as, around the large blood vessels, with low density and thin muscular layers. PAS stain positivity was observed in the cervical glands, and blood vessel walls (Fig. 8).

The histomorphometerical analysis showed significant differences in the thickness of endometrium, myometrium and glandular diameter between control and supplemented animals with obvious increase in the thickness of uterine and glandular epithelium in the supplemented group at significant level of $P \leq 0.05$(Table 1).

Discussion

The current study explains the influence of vitamin E and selenium on the uterine microstructure histomorphology in adult female goats. Vitamin E/selenium supplemented animals showed healthier uterine microstructure, since it was fixed the epithelial disintegration, integrity of uterine glands, and uterine microvasculature, furthermore the supplemented animal had larger caruncles and thicker myometrial musculature , [20] mentioned that vitamin E /Selenium had an ameliorated antioxidant effect in the uterine tissue , hence it improved the blood vessels health and uterine glands, less sloughed epithelium , and thicker myometrium . selenium exhibits a protective role in the study of [21] as he mentioned that animals treated with selenium showed increased uterine glands with less epithelial degeneration, reduced hemorrhage and fixed blood vessels, with marked increased

myometrial thickness, and suggested that supplementation of selenium for 5 days may increase the probability to repair histological microstructure against cellular damage.

Observations of the cervix revealed that the endocervix, was lined by stratified columnar epithelium featuring wide mucosal folds. This epithelium transitioned into a non-keratinized form near the vagina. Within the lamina propria submucosa, a thin layer of loose connective tissue was present, which appeared denser in supplemented animals compared to the control group. Masson's trichrome stain highlighted an unequal distribution of connective tissue between the endocervix and ectocervix, and around large blood vessels, where there was a low density and thin muscular layers. [20] also mentioned that the antioxidant effect of vitamin E/selenium has shown a great impact on tissue proliferation, as it helps with in the maintenance of endometrial epithelium sloughing and acts as a protective agent, according to [22] , it has been confirmed that higher levels of vitamin E in the blood are associated with a lower risk of cervical cancer. [23], also mentioned that selenium plays an important role in the formation of thyroid hormones, which in turn has a significant impact on the repair of uterus and cervix tissue after the estrus cycle, including the regeneration of blood vessels and repair of the endometrium, as well as enhancing embryo implantation. Their deficiency is associated with reduced fertility and disturbances in the cyclic performance. Furthermore, according to the study performed [24], vitamin E and selenium have been found to have positive effects on the health of the uterus and cervix, increasing the pregnancy rate and calving conception, and reducing the risk of retained fetal membranes.

The histochemical findings showed a strong positive reaction to the PAS stain in the uterine epithelium basement membrane, uterine glands, wall of blood vessels, and the perimetrium serosal layer. The intensity of the staining was noticeably increased in the uterus of the supplemented group. The alcian blue stain was positive in the endometrium ground substance, and the intensity was moderate in the control animal group. [25], mentioned similar observations and reported that the intensity of PAS staining increases during the estrous period in cattle, particularly in the cervical epithelium and cervical glands, indicating neutral mucin secretion and glycogen formation during this period. The acidic mucin (Alcian blue, pH 2.5) was greater in the basal areas compared to the apical areas of the cervix.

The distribution of mucin in the uterus was explained by [26], they found that neutral mucin was present in the uterine cervical glands and the basement membrane of the uterine epithelium, while acidic mucin showed lower intensity and was present around the adjacent tissues. Vitamin E and selenium enhance the production of neutral and acidic mucin, which in turn enhances the function of the uterus and regulates its cyclic activity. [25,26], found that the secretion of mucin is uncontrolled and influenced by the activity of estrogen and progesterone hormones.

Conclusions

The uterus exhibits unique proliferative and regenerative capabilities, undergoing continuous changes. Vitamin E and selenium, are well-known for their antioxidant properties and reparative actions, play important roles in microstructural recovery and repair. Supplementation with these nutrients enhances the maintenance of endometrial epithelium and, renewal of blood vessels, and facilitates the regeneration of uterine glands. Incorporating vitamin E and selenium supplements holds promise for augmenting animal fertility and increasing conception rates. particularly, their effects manifest at the cellular level within the female reproductive system.

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Conflicts of interest

None

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TABLE 1. the microscopic measurements of uterine layers and uterine glands

Parameters	Control group Mean ± SEM	Supplemented group Mean ± SEM
Uterine epithelium thickness (µm)	13.4 ±1.06	16.5 ± 0.72
Endometrial thickness (µm)	756.2 ± 0.14	871.2 ± 0.22*
Myometrial thickness (µm)	698.3 ± 0.48	810.5 ± 1.01*
Perimetrial thickness (µm)	50.6 ± 0.67	53.7 ± 0.71
Glandular epithelium thickness(µm)	15.9 ± 0.23	16.1± 0.61
Glandular diameter (µm)	48.3 ± 1.22	54.3 ± 0.41*

* : Indicate statistical significant differences in each parameter within both groups ,at significant level of $P \leq 0.05$

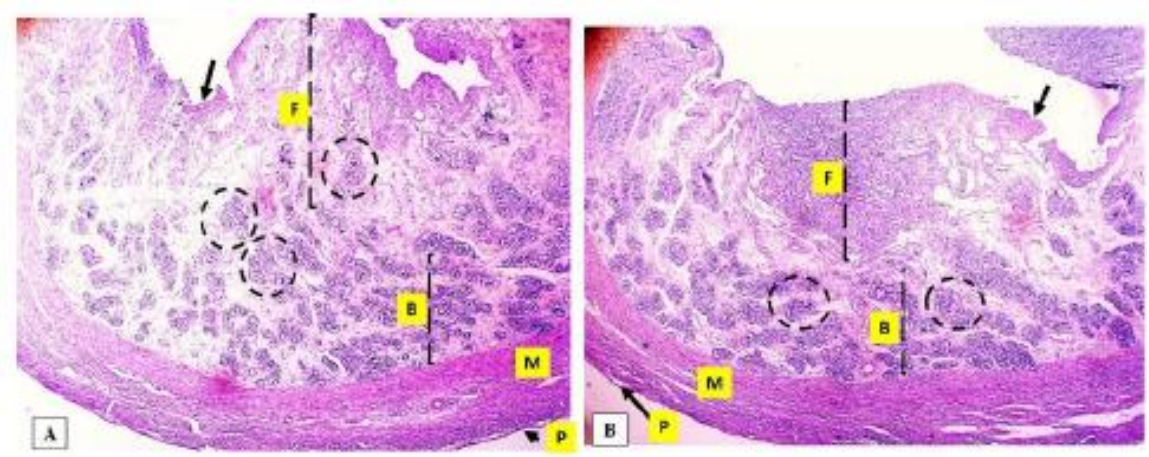

Fig. 1. Microphotograph shows the uterine layers, in Vit E/selenium supplemented group (A), and control group (B), The figures refer to endometrial functional layer (F), endometrial basal layer (B), myometrium (M) , perimetrium (P) ,the endometrial epithelium (arrows) , the uterine glands (dashed circles) , (H&E , 40X).

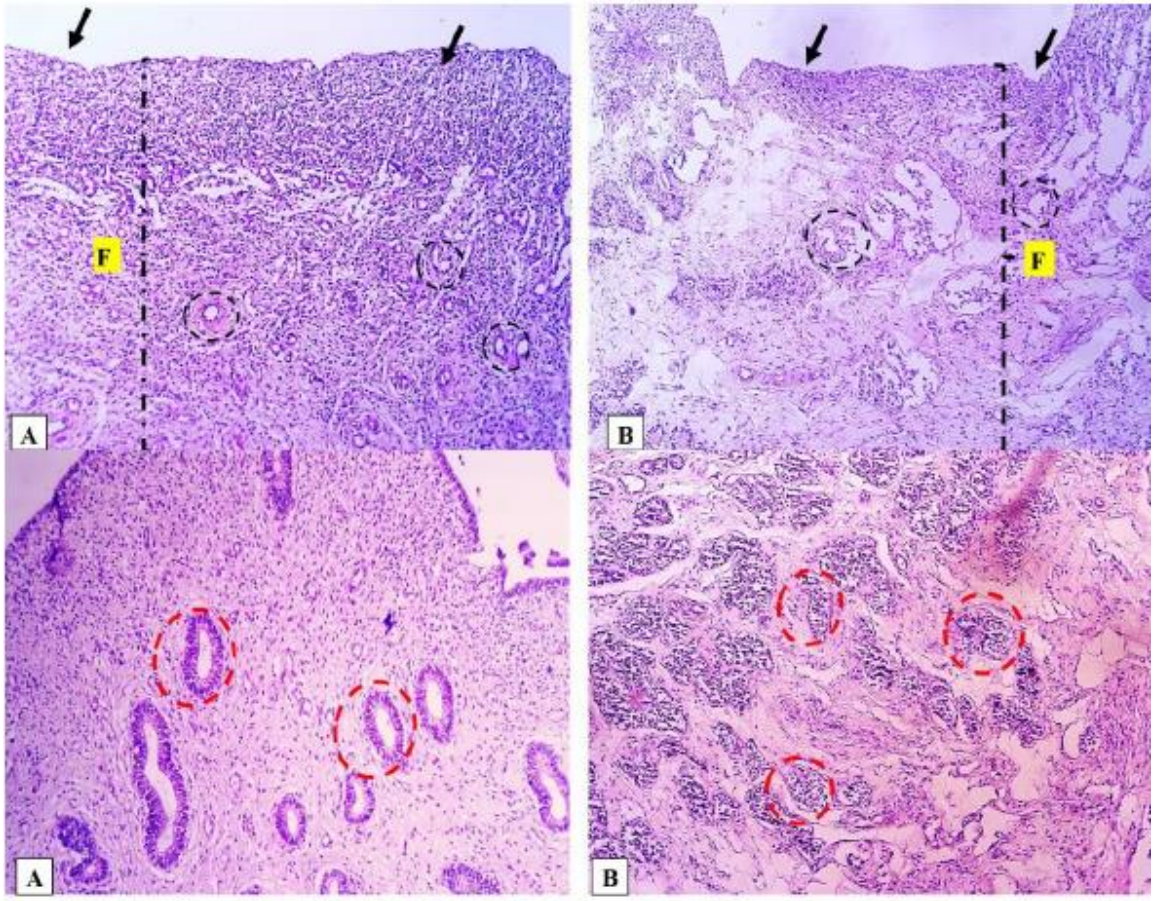

Fig. 2. Microphotograph shows the endometrium (functional zone) at the upper set of figures and basal zone at the lower set of figures in, Vit E/selenium supplemented group (A), and control group (B)., The figures depict to the endometrial epithelium (arrows), the fictional zone (F), the uterine glands (dashed circles), (H&E, 100X).

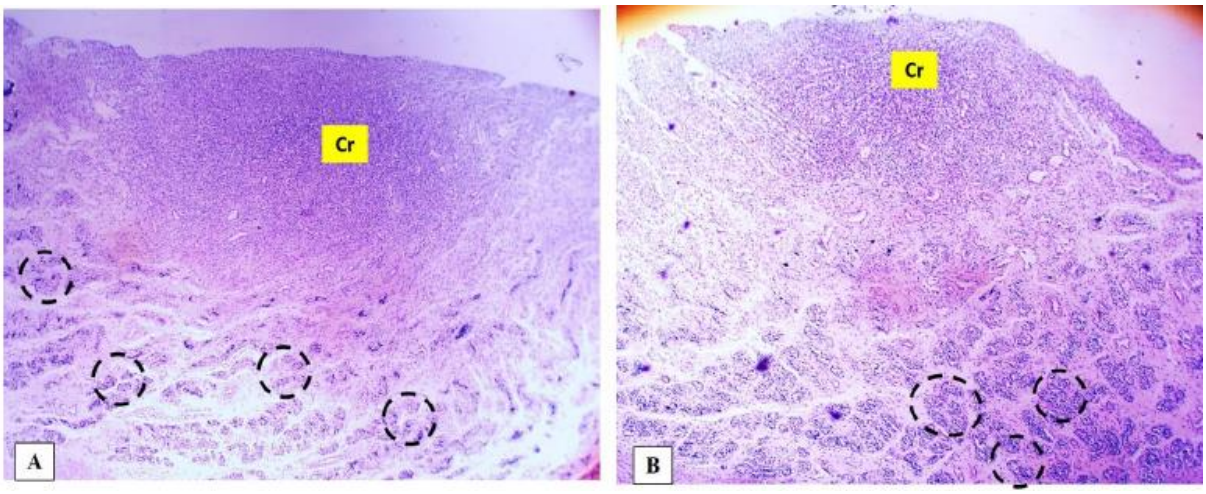

Fig 3. Microphotograph shows the caruncles (Cr) within endometrial functional zone, in Vit E/selenium supplemented group (A), and control group (B), the uterine glands (dashed circles) filled the basal endometrial layer , (H&E , 40X).

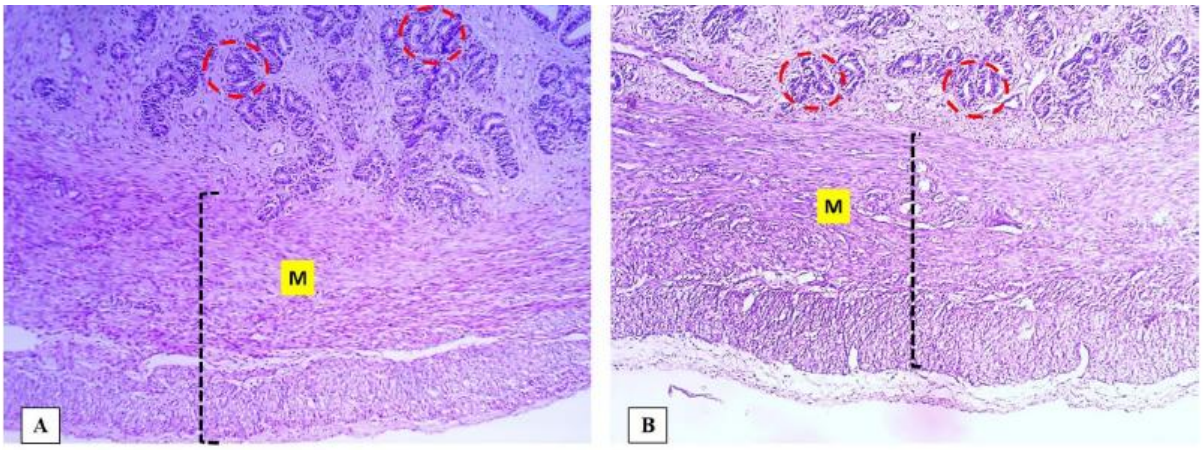

Fig 4. Microphotograph shows the myometrium (M), in Vit E/selenium supplemented group (A), and control group (B), the figures refer to the uterine glands (dashed circles), (H&E , 40X).

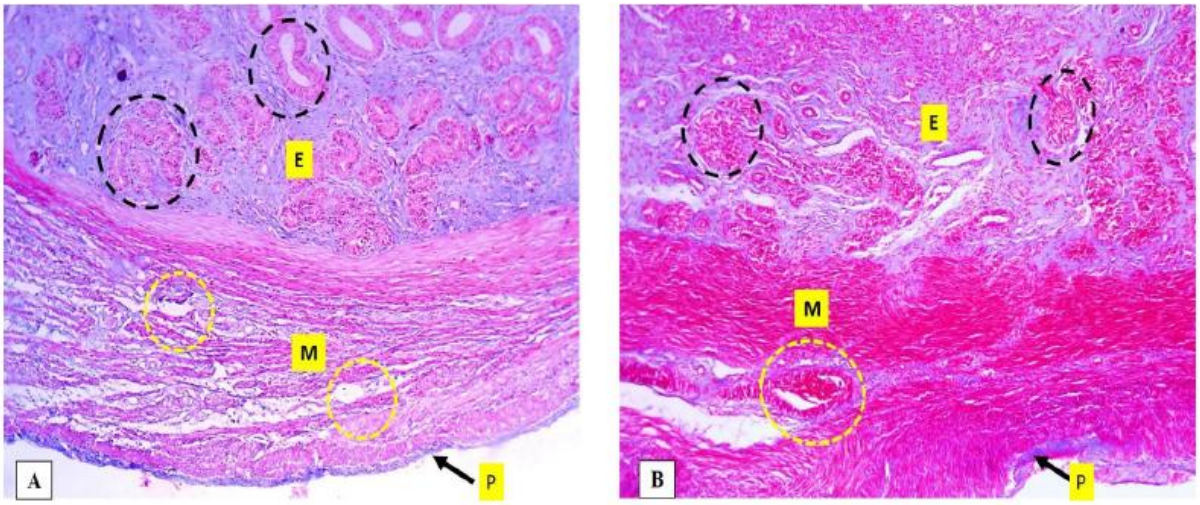

Fig. 5. Microphotograph shows the endometrial basal layer (E), myometrium (M) and the perimetrium (P), in Vit E/selenium supplemented group (A), and control group (B), The figures refer to the uterine glands (dashed circles) uterine vessels within myometrium (yellow circles), (MTS , 40X).

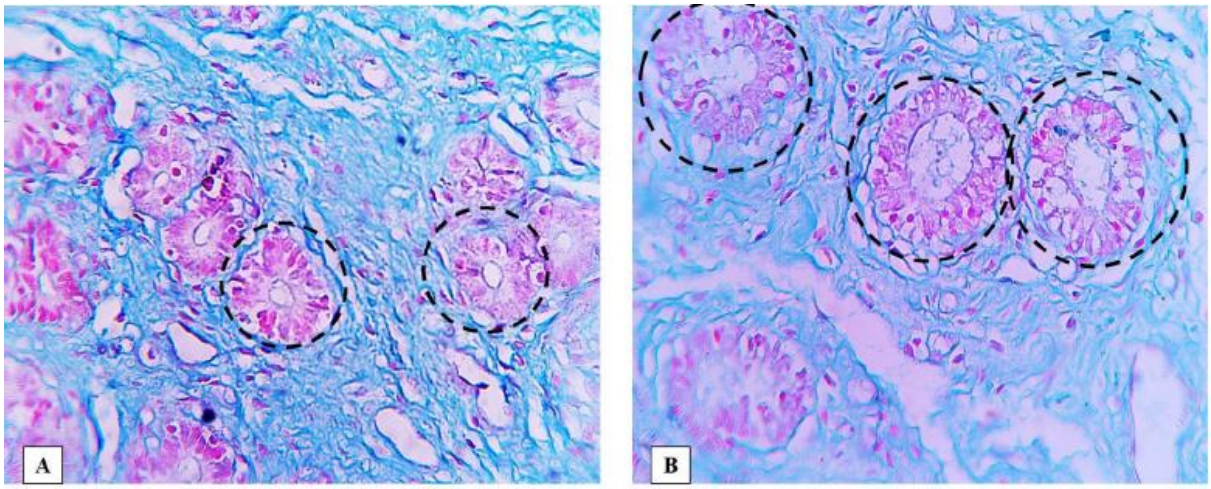

Fig 6. Microphotograph shows the Endometrium , in Vit E/selenium supplemented group (A), and control group (B), The figures refer to the uterine glands (dashed circles) , (PAS/AB , 400X).

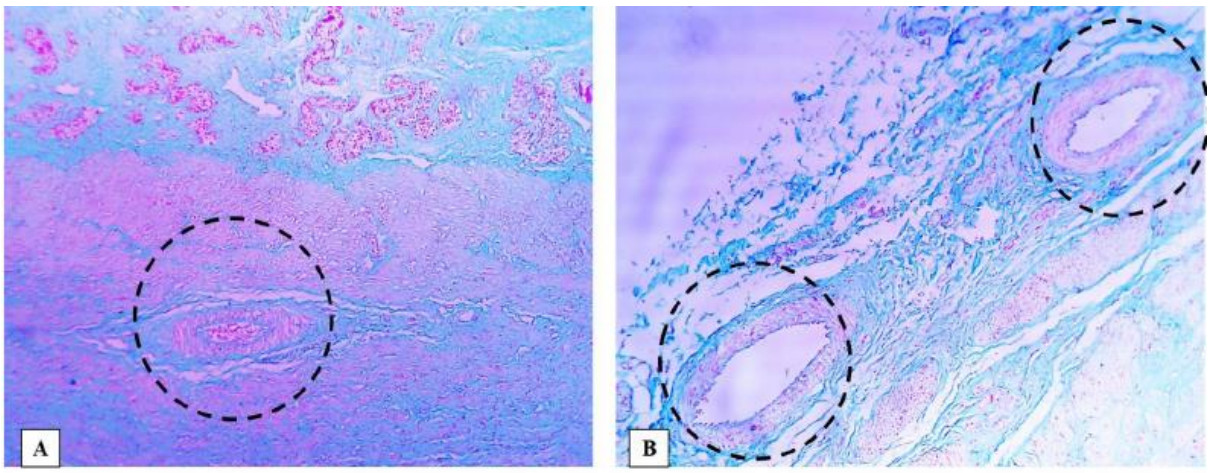

Fig. 7. Microphotograph shows the uterine large blood vessels (dashed circles), in Vit E/selenium supplemented group (A), and control group (B), The figures refer to the uterine glands (PAS/AB , 100X).

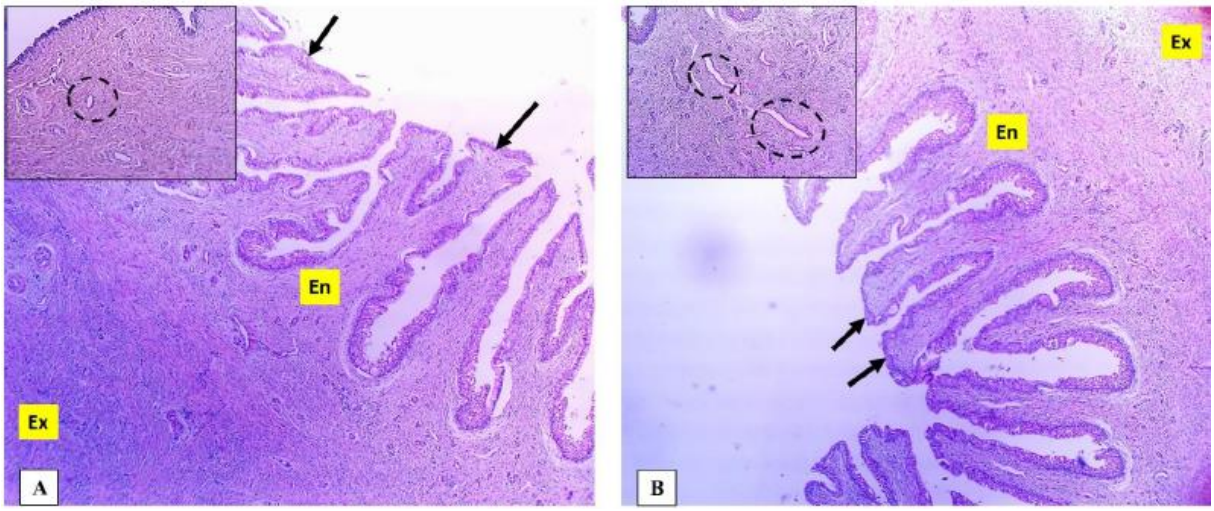

Fig. 8. Microphotograph shows the cervical layers and cervical folds, in Vit E/selenium supplemented group (A), and control group (B), The figures refer to the endocervix (En), the ectocervix (Ex), and the cervical epithelium (arrows). The magnified figure show the blood vessel within cervical lamina propria (dashed circles) (H&E, 40X magnified figure in 100X).

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تقدير تأثير فيتامين E والسلينيوم في صحة الرحم لأناث المعز البالغة (دراسة نسيجية)

الاء شامل العلاف ورغد النعيمي

قسم الإنتاج الحيواني، كلية الزراعة والغابات، جامعة الموصل، الموصل، العراق.

الملخص

يتميز الرحم بقدرة لا مثيل لها على النمو والتجدد، ويخضع لتحولات دورية مستمرة. اذ يشتهر فيتامين هـ والسيلينيوم بخصائصها المضادة للأكسدة وقدرته الترميمية في اصلاح البنية المجهرية للانسجة المختلفة. لذلك، أجريت الدراسة الحالية للتحقق من تأثير فيتامين هـ والسيلينيوم على التركيب المجهري لأنسجة الرحم في إناث الماعز (*Capra hircus*). استخدمت عشرة حيوانات بالغة سليمة في هذه الدراسة، وقسمت إلى مجموعتين. كانت المجموعة الأولى هي المجموعة الضابطة التي تم تغذيتها بنظام غذائي (قياسي) مع توفير الماء حسب الحاجة. تلقت المجموعة الثانية نفس الغذاء ولكن مع مزيج من فيتامين هـ والسيلينيوم اعطي بمقدار ٢ مل في الماء يوميا لمدة ٢٥ يوماً. جمعت عينات الرحم وحفظت في محلول فورمالين١٠٪. ثم إجرايت معاملات التقطيع النسيجي الروتينية عليها، صبغت المقاطع بصبغة الهيماتوكسيلين والأيوسين وبملون ماسون ثلاثي الصبغ، وصبغت شرائح إضافية بحمض شيف الدوري (PAS) وصبغة الاليشيان الزرقاء pH٢,٥ . اظهرت النتائج أن الرحم في الحيوانات المدعمة بفيتامين هـ/سيلينيوم ذا بنية مجهرية أكثر صحة، حيث تم إصلاح وترميم الظهارة والغدد الرحمية والأوعية الدموية الصغيرة في الرحم، علاوة على ذلك كان لدى الحيوانات المكملة بفيتامين هـ/سيلينيوم لحيمات رحمية أكبر حجماً وغلالة عضلية أكثر سمكاً، وأظهرت النتائج النسيجية الكيميائية تفاعلاً شديد مع صبغة PAS في الغشاء القاعدي لظهارة الرحم والغدد الرحمية وجدار الأوعية الدموية. فيما كانت صبغة الألشيان االزرقاء إيجابية في المادة الأرضية لبطانة الرحم. ويمكن الاشارة الى ان استخدام مكملات فيتامين هـ والسيلينيوم كمركب تأزري يعد امراً واعدً في زيادة خصوبة الحيوانات وزيادة معدلات الحمل. اذ تظهر آثارها بصورة مباشرة على مستوى البنية المجهرية.

الكلمات الدالة: الحيوانات، دراسة كيميائية نسيجية، المعز ،الرحم، فيتامين E/ والسلينيوم.