

Effect of Arginine and Selenium with Vitamin E on WBC and the level of hormones in Iraqi ewes discharged

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Abstract

This study was achieved in special farm in Basaer village which lies on the right side of the Euphrates in Heet district I Anbar, Heet lies 70 K.M west Ramadi during the period, From 17/4/2018 to 30/5/2018. 28 ewes aged 2-4 years of awassi with average weight 40-50 k.g. and a one lambing. All ewes were diagnosed by using the real-time Ultrasonography to make sure there is no pregnancy among the female before the experiment. All ewes were synchronized with intra vaginal sponges impregnated with 60 mg MAP for 12 days before sponges withdrawal and eCG 500 IU (Novormon made in Argentina). The pregnant ewes were divided randomly and equally (7 each group) into 4 groups. The ewes in the first group were received intra muscular injection of 160 Mμ of arginine 3 times daily. The second group were treated with 2ml/head a combination of selenium plus vitamin E (vitamin E 68mg/ml Selenium 1.5mg/ml). The third group was treated by injection of 160 Mμ of arginine and selenium with vitamin E 2 ml/head. The four groups were injected with normal saline which was considered as a control group. The ewes were inseminated by ram at the end of the hormonal program for five days. The samples of blood were taken from the Jugular vein at four day treatment. Also, in the 4, 10, 15, 18. The results of experiment showed no significant difference in WBC, Progesterone, estrogen, growth hormone, prolactin, early of pregnancy in the treatment with control group. The experiment showed significant difference in the Progesterone concentration within a single treatment in three treatment without control. Concluded from this study that use of arginine and selenium with vitamin E improves ewes health, increasing the progesterone to maintain pregnancy.

تأثير الأرجنين والسيلينيوم مع فيتامين E في قيمة WBC ومستوى الهرمونات في النعاج العراقية المسفدة
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الخلاصة

أجريت الدراسة في احد حقول القطاع الخاص ضمن قرية بصائر (Basaer) التابعة لقضاء هيت/الأنبار وتبعد 70 كم غرب الرمادي، للفترة من بين 17/4/2018 ولغاية 30/5/2018. على 28 نعجة بعمر تراوح بين 2-4 سنوات وذات وزن يتراوح بين 40-50 كغم، وذات ولادة واحدة على الأقل وتم فحص جميع النعاج باستخدام جهاز الموجات فورت الصوتية (السونار) للتأكد من خلوها من الحمل قبل بداية التجربة. تم توحيد الشبق في النعاج باستخدام الاسفنجيات المهبلية MAP 60 ملغم لمدة 12 يوم وعند الازالة حقن eCG 500 وحدة دولية بالعضل (تصنيع شركة Novormon الأرجنتينية). ثم سفدت النعاج من خلال اطلاق الكباش معها عند نهاية البرنامج الهرموني واستمر وجود الكباش مع الإناث لمدة خمسة أيام، ثم قسمت النعاج عشوائياً الى اربع مجاميع متساوية (7 نعاج في كل مجموعة) عوملت إناث المجموعة الاولى بحقن الأرجنين بالعضل بمقدار 160 مايكرو مول/كغم وزن الجسم ثلاث مرات يوميا وحقن من اليوم الخامس من التسفيد لغاية اليوم 20 من التسفيد (15 يوم بداية الحمل)، وعوملت نعاج المجموعة الثانية بحقن مستحلب السيلينيوم فيتامين E بمقدار 2 مل/رأس بالعضل (فيتامين E 68 ملغم/مل والسيلينيوم 1.5 ملغم/مل شركة Norbrook البريطانية)، وكانت الجرعة الاولى في اليوم 5 والثانية في اليوم 19 من التسفيد في بداية الحمل، اما المجموعة الثالثة حقنت الأرجنين 160 Mμ مع مستحلب السيلينيوم مع فيتامين E 2 مل/رأس عضليا، وحقنت نعاج المجموعة الرابعة بالمحلول الملحي الفسيولوجي واعتبرت مجموعة سيطرة. سحبت عينات الدم من الوريد الوداجي للإناث قبل المعاملة بيوم (اليوم 4 من التسفيد) وكذلك عند الأيام 10، 15، 18 من الحمل. لم تظهر نتائج الدراسة الحالية فروق معنوية بداية الحمل في قيمة WBC وكل من هرمون البروجيستيرون والاستروجين والبرولاكتين وهرمون النمو في بداية الحمل للاغنام. وظهرت النتائج فرق معنوي بين فترات السحب المختلفة ضمن المعاملة الواحدة في مجاميع المعاملات دون السيطرة في تركيز هرمون البروجيستيرون، ولم تظهر باقي الهرمونات فروق معنوية بين فترات السحب المختلفة ضمن المعاملة الواحدة. يمكن الاستنتاج من هذه الدراسة بان استخدام الأرجنين والسيلينيوم مع فيتامين E تؤدي الى تحسين صحة الام من خلال زيادة افراز هرمون البروجيستيرون للحفاظ على الحمل بعد التسفيد.

الكلمات المفتاحية: أرجنين، سيلينيوم و الفيتامين هـ ، بداية الحمل ، الهرمونات ، كريات الدم البيض

Keywords :- : Arginine, Selenium with vitamin E, Early pregnancy, Hormones, WBC.

Introduction

The Iraq in particular livestock sector suffers from a constant deficit in providing the required needs,. There is a need to it. develop this sector to meet the challenges of increasing the demand for The breeders of sheep and goats suffer from some problems that decrease reproductive of animals. , Zoologists have focused on the past decades and are still focusing on the roles played by some important nutrients. of these nutrients, there were some essential elements of them Amino acids, especially L-arginine, because the body is usually produced in sufficient quantities. However, supplements may be needed at Special conditions such as malnutrition during fetal development and development (1). So many researchers in their studies have been interested in increasing production by increasing the number of animals by raising the reproductive efficiency of the proportion of fertility and the proportion of twins through the use of some including the methods use hormones (2) and some of them pointed to the application of certain nutrition oral sup such as vitamins and minerals, including vitamin E and selenium (3) and amino acids, such as arginine (4). Both nutrition, immunology, endocrinology and reproductive studies suggest that the lack of selenium and vitamin E leads to decreased fertility, placental retention, miscarriage and poor fetuses (5). Arginine has an important role in regulating growth and metabolism. arginine plays a role in stimulating the growth and development of the placenta (6), Prolactin and Growth hormone (7). The aim of study to showed the effect of arginine or selenium with vitamin E combination or both of them on the early pregnancy on WBC. and level of hormones that include progesterone, estrogen, prolactin and growth hormone at the early pregnancy.

Materials and methods

This study was achieved in special farm in Basaer village which lies on the right side of the Euphrates in Heet / Anbar, Heet lies 70 K.M west Ramadi during the period, From 17/4/2018 to 30/5/2018 .Twenty-eight non-pregnant local ewes were selected with good condition and at least one birth with an average age of 2-4 years and an average weight of 40-50 kg. were included in the present study. The ewes were

born from one to two months and completely isolated from the rams. Ultrasonography to ensure that they were used non pregnant before the start of the experiment. Ewes were synchronized with vaginal sponges 60 mg MAP eCG of 500 IU (Novormon made in Argentina). seven rams were selected for insemination. The rams were kept for five days, then the ewes were divided into four equal groups (7 ewes in each group). Each group was then placed in a 16 m² area with 4 x 4 fenced. The animals were fed twice daily at the morning and evening and at 500 g concentrate per head, which represents the barley, the corn, the bran, the wheat bran, using the fodder in each fold, the coarse feed, which represents the straw, the green and the dry, the duration of the experiment. Mineral licks block were used.

The ewes were randomly assigned to four equal groups (7 ewes / group), were divided into groups. During the study, the first group I (T1) was injected with arginine by 160 µMol / kg body weight and 3 times daily from 5 day to 20 days (15 day) of pregnancy. The second group (T2) injected a selenium vitamin E mixture (made in Company Norbrook United Kingdom) with 2 ml of head / day 5 and day 19 of the early pregnancy. The third group (T3) was injected with the combination of selenium vitamin E 2 ml with arginine 160 µMol. (T4) was injected with the normal saline solution (control group).

Blood samples (6 ml) were collected from the jugular vein of females as the blood divided into two parts I 1 ml placed in a tube containing anticoagulant to measure WBC. The second part of the blood was 5 mL, placed in a tube and left for two hours at room temperature, then centrifuged (3000 cycles / min) for 20 minutes. The serum was separated from the rest of the ingredients and kept at -20 ° C until the hormonal analysis included Progesterone, estrogen, prolactin hormone, growth hormone, Blood was collected from the female experiment before the initiation of injection of Arginine and Selenium vitamin E at the early of pregnancy day 4 of the benefit as well as on days 10, 15, 18. Using kits of analysis hormone by system Cobas e411 (Roche made in Germany) Statistical analysis type Duncan test by SAS program 9.1 (8)

Results and Discussion

Effect of arginine 160 μ mol, selenium and vitamin E 2 ml on White Blood Cells (Table -1)

The results of the present study showed that white blood cells were not affected when injecting arginine or selenium with vitamin E at early of pregnancy, and that there were no significant differences between different treatments at early pregnancy in white blood cells. The study were agreed with Sterndale et al., (9) who noticed that there were no significant differences between selenium plus vitamin E and control group in WBC. The results of the study were also agreed with Oh et al., (10), where there was no significant difference between the Arginine group and the control one on WBC value at the early pregnancy (11, 12). The results showed no significant differences in different collect periods within treatment. WBC was within the normal level of ewes.

Effect of arginine 160 μ mol, selenium and vitamin E 2 ml on the concentration of hormones at early of pregnancy

1- Concentration of the Progesterone hormone (Table -2)

The results of the present study showed no significant differences between the three experimental groups and the control group at the level of progesterone in the serum. The results of the study were agreed with Luther et al., (13) who noticed that there were no significant differences between the treatment of arginine and control in the concentration of progesterone at the early pregnancy (11, 14). But the results of the study showed a significant difference between the different withdrawal periods within the single treatment in the totals of treatment without control. This indicates that dietary supplements of arginine and selenium with vitamin E act to protect the corpus letum and progesterone from oxidation and regulation programmed death of the corpus letum and prevent hyperplasia of the corpus letum result in an increase in the concentration of progesterone, especially as increase was accompanied with the progress of pregnancy days in ewes. The results of the study agreed with Yildiz et al., (15) whom observed that injecting vitamin E at the early pregnancy leads to increased concentration of progesterone (16). Kamada (17) observed when giving selenium the

progesterone increased its concentration ($P \leq 0.05$) at late pregnancy. Yunta et al., (18) noticed that giving arginine during pregnancy significantly affects progesterone (19). The results of the study differed with that observed by Saevre et al., (20) that injection of arginine did not affect the concentration of progesterone for ewes on day 9 and 10 of pregnancy.

2-Concentration of the estrogen (Table- 3)

The results of the present study showed no significant differences in estrogen concentration between the three experimental groups and control group. As for the different periods of withdrawal, the results of the study did not showed any significant within the same treatment. Stability of estrogen concentration may be due to elevated progesterone during pregnancy which inhibits the action of sex hormones by negative feedback mechanism. The results of the study agreed with Al-Dabbas et al., (21) that there was no significant difference in the estrogen level between the arginine group and the control group. Li and Wu (22) reported that estrogen concentration was not affected by arginine supplementation and no significant differences with control group (11). The results of the current study disagreed with Zeitoun et al., (19) that giving low and high doses of arginine to pregnant ewes at the early pregnancy led to a decrease in the level of estrogen at the early pregnancy, Soleimanzadeh et al., (16) observed a rise in estrogen level when injecting selenium with vitamin E late pregnancy.

3- Concentration of prolactin (Table- 4)

The results of the present study showed no significant differences in the concentration of prolactin between the three experimental groups and control group. The results did not show any significant difference in the different withdrawal periods within the same treatment. This indicates that the dietary supplements used in the current study did not affect the prolactin at the early pregnancy. The results were similar with that of Yunta et al., (18) that giving arginine during pregnancy on the day 41 - 146 which might be not stimulate the secretion of prolactin. Bass et al (23) confirmed that injecting arginine into mature, non-pregnant ewes significantly increased the concentration of prolactin.

Table – 1: the effect of different treatments different days on WBC ($\times 10^6$ /ml)

Days	treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 558 ± 7330 a	A 968 ± 6740 a	A 720 ± 6440 a	A *430 ± 7730 a	N.S.**
Ten day	A 792 ± 5510 a	A 647 ± 6970 a	A 751 ± 5820 a	A 1238 ± 8320 a	N.S.
Fifteenth day	A 626 ± 6160 a	A 963 ± 7110 a	A 952 ± 7890 a	A 968 ± 8170 a	N.S.
Eighteen Day	A 486 ± 6594 a	A 608 ± 7380 a	A 575 ± 7590 a	A 857 ± 8130 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means ± SE.

** N.S = Mean No significant difference ($P \leq 0.05$).

Day	treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 0.271 ± 0.913 a	B 0.154 ± 0.632 a	B 0.124 ± 0.527 a	B *0.076 ± 0.754 a	N.S**
Ten day	A 1.34 ± 2.42 a	A 0.507 ± 3.31 a	A 0.443 ± 3.53 a	A 1.20 ± 5.13 a	N.S.
Fifteenth day	A 1.02 ± 1.83 a	A 0.243 ± 3.41 a	A 0.505 ± 3.56 a	A 0.763 ± 4.69 a	N.S.
Eighteen Day	A 1.05 ± 2.11 a	A 0.227 ± 3.00 a	A 0.217 ± 3.40 a	A 0.729 ± 4.45 a	N.S.
Significant Level	N.S.	0.0001	0.0001	0.0048	

* Values=Means ± SE

** N.S = Mean No significant difference ($P \leq 0.05$).

a. b. c. small letters within one row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level ($P \leq 0.05$).

Table – 3: the effect of different treatments different days on estrogen (pg/ml)

Days	treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 5.66 ± 29.7 a	A 5.86 ± 36.2 a	A 6.18 ± 21.5 a	A 8.23 ± 45.2 a	N.S.**
Ten day	A 3.42 ± 26.3 a	A 5.74 ± 31.6 a	A 6.45 ± 17.2 a	A 8.49 ± 34.1 a	N.S.
Fifteenth day	A 3.38 ± 25.6 a	A 5.29 ± 30.0 a	A 4.85 ± 14.8 a	A 7.63 ± 29.1 a	N.S.
Eighteen Day	A 3.75 ± 25.9 a	A 3.22 ± 27.5 a	A 3.31 ± 14.3 a	A 5.92 ± 27.9 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means ± SE

** N.S = Mean No significant difference (P≤0.05).

Table – 4: the effect of different treatments different days on Prolactin (ng/ml)

Days	treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A ± 0.0472 0.0002 a	A ± 0.0470 0.0005 a	A ± 0.0472 0.0002 a	A ± 0.0494 0.0021 a	N.S.**
Ten day	A ± 0.0472 0.0004 a	A ± 0.0470 0.0001 a	A ± 0.0472 0.0001 a	A ± 0.0472 0.0002 a	N.S.
Fifteenth day	A ± 0.0470 0.0003 a	A ± 0.0470 0.0002 a	A ± 0.0470 0.0003 a	A ± 0.1338 0.0865 a	N.S.
Eighteen Day	A ± 0.0472 0.0002 a	A ± 0.0470 0.0003 a	A ± 0.0470 0.0001 a	A ± 0.0472 0.0002 a	N.S.
Significant Level	N.S.	N.S.	N.S.	N.S.	

* Values=Means ± SE

** N.S = Mean No significant difference (P≤0.05).

Table – 5: the effect of different treatments different days on growth hormone (ng/ml)

Days	treatments				Significant Level
	T4	T3	T2	T1	
fourth day	A 0.0021 ± 0.0300 a	A 0.002 ± 0.0300 a	A 0.0024 ± 0.0300 a	A *0.0212 ± 0.0512 a	N.S.**.
Ten day	A 0.0054 ± 0.0354 a	A 0.002 ± 0.0300 a	A 0.0020 ± 0.0300 a	A 0.0021 ± 0.0300 a	N.S.
Fifteenth day	A 0.0020 ± 0.0320 a	A 0.002 ± 0.0300 a	A 0.0021 ± 0.0300 a	A 0.0012 ± 0.0300 a	N.S.
Eighteen Day	A 0.0021 ± 0.0300 a	A 0.0021 ± 0.0300 a	A 0.0012 ± 0.0300 a	A 0.0023 ± 0.0300 a	N.S.
Significant	N.S.**	N.S.	N.S.	N.S.	

* Values=Means ± SE

** N.S = Mean No significant difference (P≤0.05).

4- Growth hormone concentration (Table -5)

The results of the present study showed no significant difference between the treated groups and control. It was also noted that there were no significant differences between the different withdrawal periods within the same treatment. The results of the study agreed with Yunta et al., (18) that between giving the Arginine during pregnancy did not stimulate growth hormone secretion. While the results differed with Zeitoun et al., (19) who reported that injecting arginine pregnant ewes led to a decrease in growth hormone concentration during pregnancy. Bass et al., (23) confirmed that injecting arginine into mature ewes in anestrus phase resulted in a significant increase in growth hormone concentration within 15-30 minutes after injection

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