Plant Archives Vol. 20 Supplement 1, 2020 pp. 1859-1862 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF ARGININE AND SELENIUM WITH VITAMINE E ON PLACENTA OF IRAQI EWES

T. R. Mohammed[1*] A. F. Majeed[2] and Ibtehaj K. H. Alawiy[1]

[1]College Agriculture, University OF Anbar, Iraq.
[2]College veterinary Medicine, university of Fallujah, Iraq.

Abstract

The objective of the study to demonstrated the effect of arginine and selenium with Vitamin E or both on histological picture of ovine placenta in Iraqi ewes. The Study was conducted on 24 pregnant Iraqi Ewes aged between 2-4 years and weight of 40-50 Kg, presented in hit /Anbar during the period from 20 of April to 10 of October 2018. The animals were divided into four equal groups. Placentomes were taken within 6 hours post-partum from 4 ewes of each group. The Placentomes were taken manually per vagina following parturition. It is collected from the center of the pregnant horn. Directly after collection the samples were fixed in 10% buffered neutral formalin for 48 hours. The specimen of the tissue were dehydrated in a graded series of alcohol, then cleared with xylol and embedded in paraffin. Histological section were done with microtome using 4 µm thickness and then stained with hematoxylin and eosin. Each slide were examined under light microscope with low and high power magnification. Histological examination of sheep treated with arginine (T1) showed vascular changes includes vasodilation of blood vessels and lymph vessels. There was Congestion blood vessels and a hypertrophy of endothelial Cells. There were a mononuclear Cell infiltrations and hypertrophy in the Connective tissue of the maternal side. While sheep that treated with selenium plus vitamin E (T2) showed a high vascular change with presence of hemosiderin pigment. There is an enlargement of trophoblast. It is also noted that is few binucleated cell infiltration were seen. Histological picture of the third group (T3) treated with a combination of arginine with Se plus vitamin E showed vasodilation in blood vessels. There is an endothelial cell hypertrophy and trophoblastic hyperplasia. There is also extracellular matrix odema. These changes observed might be resulted from the treatment with the combination (arginine, Se + Vit E). Histologic section of Placentomes from ewes treated with normal saline (T4- Control) (Fig. 4) showed vascular changes, odema hyperemia and hemorrhage. There is a vasodilation and congestion in the blood vessels. There is a great hyperplasia of the trophoblast. Few number of binucleated cell were seen in the trophoblast. It was concluded from this study that arginine, Selenium and vitamin E increase placenta blood flow and increase immunity for both maternal and fetal side

Key words: Arginine, Selenium with Vitamin E, Placenta, Iraqi Ewes.

Introduction

Ovine placenta classified as cotyledonary epitheliochorial. The placenta is essential for normal pregnancy and fetal development. It plays an important role in immune responses, increase blood flow to allow more fetal nutritive demand and stimulate secretion of hormones for maternal recognition of pregnancy (Samin *et al.*, 2011). Placentomes consists of a Combination of a maternal caruncle and a fetal cotyledon. The blood vessels of chorio allntoic sac enter single placentome at its hilus (Soares and Hunt, 2006).

Arginine is an amine acid necessary for Small ruminants (crane, 2014). It is manufactured in the Kidney and liver but in Limited quantities (Grazul-Bilska *et al.*, 2013). It is has been reported that Arginine increases blood flow to the organ through the production of nitric oxide (No) that leads to relaxation of smooth muscle and to increase blood flow in blood vessels (Wu *et al.*, 2009, Wu *et al.*, 2016).

Arginine have an effect an Placental efficiency and angiogenic mechanisms, therefore might Lead to fetal development and placental growth (Bazer *et al.*, 2009, Bayraty *et al.*, 2012, Bazer *et al.*, 2018). Arginine regarded as a potent vasodilator that increase the weight of the fetus via Placental competence and fitness Bayatiy *et al.*, 2012).

***Author for correspondence :** E-mail: ag.thair.rasheed@uoanbar.edu.iq

Selenium (Se) is an essential trace elements in ruminants. It is play a role in maintaining Physiological functions and provides antioxidant sources (Sordillo, 2013). It is trans placental transfer factor essential for the fetas (Moeini *et al.*, 2011).

Vitamin E is a water soluble vitamin essential for ruminant to prevent white muscle disease and retention of the fetal membranes. It has also immune stimulatory effects (Allison and Laven, 2000, Ortunho *et al.*, 2016).

There is a little information about the effect of arginine and selenium with Vitamin E or both on placenta of sheep. Therefore the aim of this study were designed to demonstrated the effect of arginine and selenium with Vitamin E or both on histological picture of ovine placenta in Iraqi ewes.

Materials and Methods

The Study was conducted on 24 pregnant Iraqi Ewes aged between 2-4 years and weight of 40-50 Kg, presented in hit /Anbar during the period from 20 of April to 10 of October 2018. The animals were divided into four equal groups. The first group (T1) were injected 160 µmol/Kg of arginine (us Bulk supplies) *i.m.* daily three times from day 5 to 20 mating (early pregnancy) and injected 300 µmol/Kg B.W. arginine daily three times from day 100 to 125 of Pregnancy (late Pregnancy). The 2nd group (T2) ewes were injected 2ml/ewe two times with Selenium Plus vitamin E (Norbrook, U.K.). The 1st dose were given at days 5 while, the 2nd dose were given on day 19 of early pregnancy. The ewes were injected with Selenium plus vitamin E in a dose of 2.5ml/ewe on day 100 and on day 114 at the late Pregnancy. The 3rd group (T3) ewes were treated with Selenium plus vitamin E (2 and 2.5ml / ewe early and at late Pregnancy), with arginine (160-300 µmol/Kg B.W. early and at late Pregnancy). The 4th group (T4) ewes were received a normal Saline *i.m.* and considered as a control group. Placentomes were taken within 6 hours post-partum from 4 ewes of each group.

The Placentomes were taken manually per vagina fallowing parturition. It is collected from the center of the pregnant horn. Directly after collection the samples were fixed in 10% buffered neutral formalin for 48 hours. The specimen of the tissue were dehydrated in a graded series of alcohol, then cleared with xylol and embedded in paraffin. Histological section were done with microtome using 4 µm thickness and then stained with hematoxylin and eosin (Culling *et al.*, 1985). Each slide were examined under light microscope with low and high power magnification.

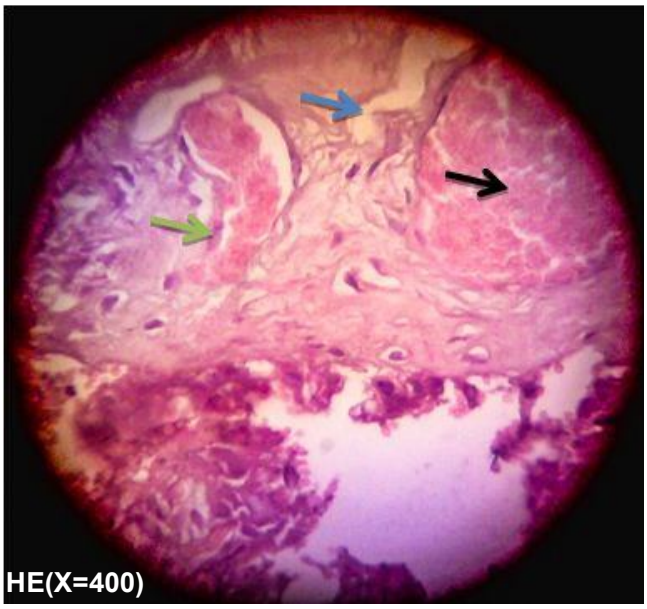

Fig. 1: Blood vessels congestion (black arrow) lymphatic vessels dilation (blue arrow) and endothelial cell hypertrophy (green arrow).

Results and Discussion

Histologic section of Placentomes taken from ewes treated with arginine (T1) (Fig. 1) showed vascular changes includes vasodilation of blood vessels and lymph vessels. There was Congestion blood vessels and a hypertrophy of endothelial Cells. There were a mononuclear Cell infiltrations and hypertrophy in the Connective tissue of the maternal side. Similar observation have been made by Neri *et al.*, (1995) and Reynolds *et al.*, (2010). This might be concentration of the arginine in

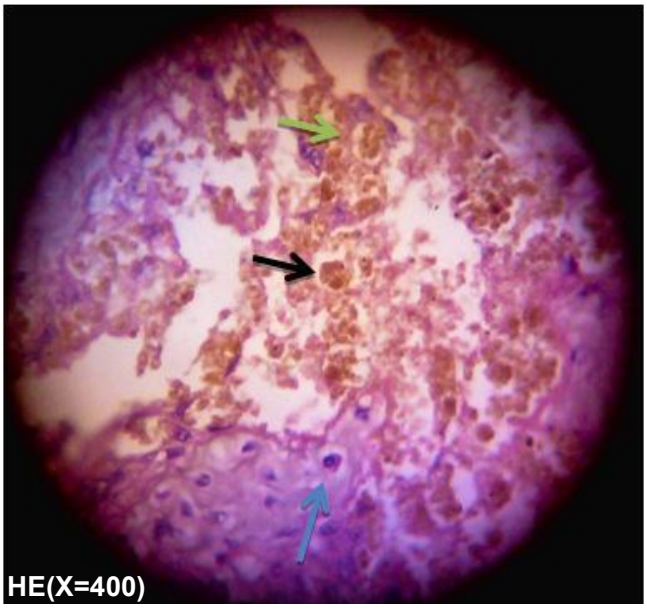

Fig. 2: Presence of hemosidrosis (black arrow) trophoblasts enlargement (blue arrow) and few binucleated cell infiltration (green arrow).

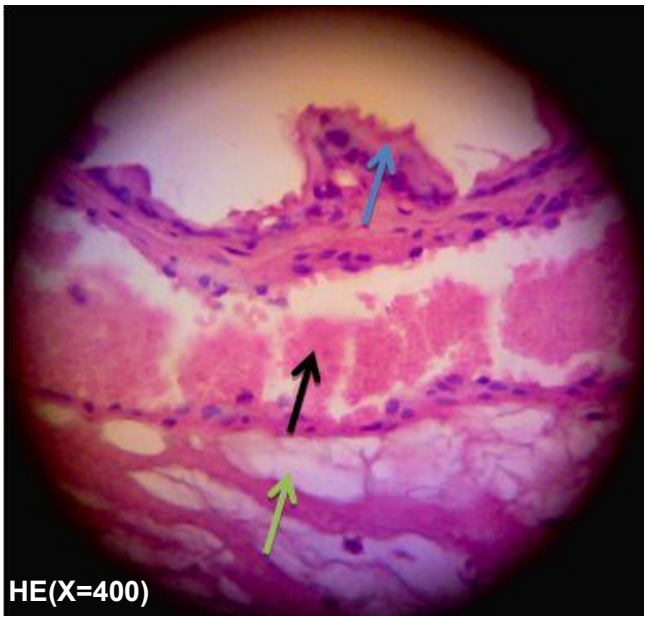

Fig. 3: Vasodilation (black arrow) trophoblasts hyperplasia (blue arrow) and extracellular matrix edema (green arrow).

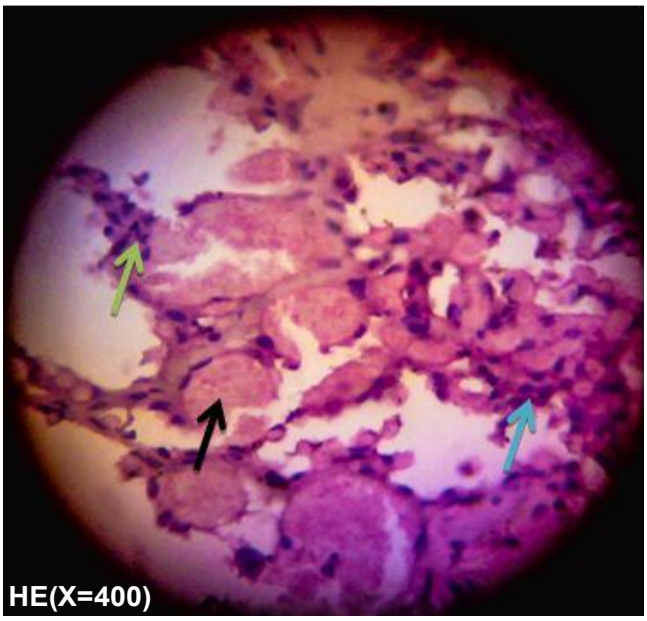

Fig. 4: Vasodilation and congestion of blood vessels (black arrow) hyperplasia of trophoblasts (blue arrow) and few number of Binucleated cell seen (green arrow).

maternal plasma of sheep were expected to increase placental angiogenesis and uteroplacental flow (Neri *et al.*, 1995, Reynolds *et al.*, 2010). It has been observed that arginine stimulate and regulate nitric oxide synthesis in the endothelial cells (Wu and Meininger, 2002). Also has been reported that in sheep, the vascular density of the fetal placental tissue (Cotyledons) remains relatively constant between day 40 and day 80 of pregnancy and with an exponentially increases there after (Reynolds *et al.*, 2010), while the Vascular Changes of maternal Placental tissue side (caruncles) increased substantially (gradually) from day 40 until mid of pregnancy then Slowly there after (Reynolds *et al.*, 2005). Histologic picture of sheep treated with selenium plus vitamin E (T2) (Fig. 2) showed a high vascular change with presence of hemosiderin pigment. There is an enlargement of trophoblast. It is also noted that is few binucleated cell infiltration were seen. Similar results have been reported by Lekatz *et al.*, (2010a, 2010b). It has been observed that se have an effects on placental development especially the cotildonary tissue more than (greater) Caruncular tissue (Lekatz *et al.*, 2010 a,b). This might be due to there is an increase cellular proliferation and DNA concentration in cotildonary tissue. But not have an effect on placentome number, mass and Carunculas weight treated ewes (Lekatz *et al.*, 2610a). Also se have an a antioxidant activity through increase glutathione peroxidase activity in Cotiledonary and Caruncular tissue than in normal Level fed to sheep (Lekatz *et al.*, 2010a). It has been found that Se plus vitamin E increase immunity to sheep through increase the levels of immunoglobulin's that Prevents mastitis and Diseases (palmieri and Szarek, 2011). It is also reported in cattle that Se with vitamin E injection decrease the incidence of retained fetal membranes (palmieri and Szarek, 2011). This might be due to se and vitamin E increase the mechanism of sloughing and necrosis between fetal and maternal tissues that enhance the detachment of placenta (Noakes *et al.*, 2009).

Histological picture of the third group (T3) (Fig. 3) treated with a combination of arginine with Se plus vitamin E showed vasodilation in blood vessels. There is an endothelial cell hypertrophy and trophoblastic hyperplasia. There is also extracellular matrix odema. These changes observed might be resulted from the treatment with the combination (arginine, Se + Vit E). It has been reported that arginine regulates Nitric oxide synthesis in endothelial cells (wu and Meininger, 2000) and increase placental angiogenesis (Reyneolds *et al*., 2010) and utero placental blood flow (Neri *et al.*, 1995). It is also observed that Se plus vitamin E act as antioxidant stimulate glutathione peroxidase that enhance he Development of Cotiledonary and Caruncular tissue via increase blood flow and increases immunity (Palmieri and Szarek, 2011).

Histologic section of Placentomes from ewes treated with normal saline (T4- Control) (Fig. 4) showed vascular changes, odema hyperemia and hemorrhage. There is a vasodilation and congestion in the blood vessels. There is a great hyperplasia of the trophoblast. Few number of binucleated cell were seen in the trophoblast. Similar observation have been found by Wooding (1983) and william *et al.*, (1987). Wooding (1992) showed that the

uterine epithelium persist in a modified sate. Fetal chorionic binucleated cell specific to ruminant species migrate and fuse with the uterine epithelium to form a hybrid fetal maternal syncytium at the internal membrane. It was concluded from this study that arginine, Selenium and vitamin E increase placenta blood flow and increase immunity for both maternal and fetal side.

References

Allison, R.D. and R.A. Laven (2000). Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. *Vet Rec.*, **147:** 703-708.

AlBayaty, M.A., F.J. AL-Tahan and H.F. Hasan (2012). Influence Of Protein Extract From *Helarthus Annuus* L. Seeds on Blood Volume of Reproductive Organs in Pregnant Mice, *J.P.C.S.*, **6:** 62.

Bazer, F.W., T.E. Spencer, G.A. Johnson, R.C. Burghardt and G. Wu (2009). Comparative aspects of implantation. *Reproduction.*, **138:** 196-209.

Bazer, F.W., R.C. Burghardt, G.A. Johnson, T.E. Spencer and G. Wu (2018). Mechanisms for the establishment and maintenance of pregnancy: synergies from scientific collaborations. *Bio. of Reprod*., **99:** 225-241.

Crane, A. R. (2014). Impacts of Supplemental Arginine on Ewe Reproductive Performance. Thesis, Agric and Appl. Sci., N Dakota SU.

Culling, C.F.A., R.T. Allison and W.T. Barr (1985). Cellular pathology technique 9th ed. Butter worth, London, 155-163.

Grazul-Bilska, A.T., M.L. Johnson, P.P. Borowicz, L. Baranko, D.A. Redmer and L.P. Reynolds (2013). Placental development during early pregnancy in sheep: effects of embryo origin on fetal and placental growth and global methylation. *Theriogenology.*, **79:** 94-102.

Lekatz, L.A., J.S. Caton, J.B. Taylor, L.P. Reynolds, D.A. Redmer and K.A. Vonnahme (2010a). Maternal selenium supplementation and timing of nutrient restriction in pregnant sheep: effects on maternal endocrine status and placental characteristics. *J. Anim. Sci*., **88(3):** 965-973.

Lekatz, L. A., M.A. Ward, P.P. Borowicz, J.B. Taylor, D.A. Redmer, A.T. Grazul-Bilska and K.A. Vonnahme (2010b). Cotyledonary responses to maternal selenium and dietary restriction may influence alterations in fetal weight and fetal liver glycogen in sheep. *Anim. Reprod. Sci*., **117(3-4):** 216-225.

Moeini, M.M., A. Kiani, H. Karami and E. Mikaeili (2011). The effect of selenium administration on the selenium, copper, iron and zinc status of pregnant heifers and their newborn calves. *J. Agric. Sci. Tech.,* **13:** 53-59.

Neri, I., G.C. Di Renzo, G. Caserta, A. Gallinelli and F. Facchinetti (1995). Impact of the L-arginine/nitric oxide system in pregnancy. *Obstet. and Gynecol. Surv*., **50:** 851-858.

Noakes, D.E., T.J. Parkinson and G.C.W. England (2009). Vet. Reprod. Obstetrics. Saunders, London.

Ortunho, V.V., M. Beline, M.E.D. Souza Carli, P. Carvalho and G. De-Souza (2016). The use of selenium in the sheep industry: A review. *Sanidade Ani*., **10(3):** 529-540.

Palmieri, C. and J. Szarek (2011). Effect of maternal selenium supplementation on pregnancy in humans and livestock. *J. Elementol.*, **16(1):** 143-155.

Reynolds, L.P., P.P. Borowicz, K.A. Vonnahme, M.L. Johnson, A.T. Grazul Bilska, D.A. Redmer and J.S. Caton (2005). Placental angiogenesis in sheep models of compromised pregnancy. *J. Physiology.*, **565:** 43-58.

Reynolds, L.P., P.P. Borowicz, J.S. Caton, K.A. Vonnahme, J.S. Luther, D.S. Buchanan and D.A. Redmer (2009). Uteroplacental vascular development and placental function: an update. *Inter. J. Dev. Bio*., **54:** 355-365.

Sammin, D., B. Markey, H. Bassett and D. Buxton (2009). The ovine placenta and placentitis-a review. *Vet Microbio*., **9:** 54.

Soares, M. J., & Hunt, J. S. (Eds.). (2006). Placenta and trophoblast: methods and protocols. Vol.1, Humana press, Totowa, Newjersy.þ

Sordillo, L.M. (2013). Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. *Vet. Med. Inter*., 2013.

Williams, W.F., M.J. Margolis, J. Manspeaker, L.W. Douglass and J.P. Davidson (1987). Peripartum changes in the bovine placenta related to fetal membrane retention. *Theriogenology.*, **28:** 213-223.

Wooding, F.B. (1983). Frequency and localization of binucleate cells in the placentomes of ruminants. *Placenta.*, **4:** 527-539.

Wooding, F.B.P. (1992). The synepitheliochorial placenta of ruminants: binucleate cell fusions and hormone production. *Placenta.*, **13:** 101-113.

Wu, G. and C.J. Meininger (2002). Regulation of nitric oxide synthesis by dietary factors. Annual review of nutria., **22:** 61-86.

Wu, G., F.W. Bazer, T.A. Davis, S.W. Kim, P. Li, J.M. Rhoads and Y. Yin (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino acids*, **37(1):** 153-168.

Wu, Z., Y. Hou, S. Hu, F.W. Bazer, C.J. Meininger, C.J. McNeal and G. Wu (2016). Catabolism and safety of supplemental L-arginine in animals. Amino acids, Doi, 10.1007/s 00726-016-2245-9.