

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

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### Abstract

The objective of the study to demonstrated the effect of arginine and selenium with Vit 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 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 thicknesses and then stained with hematoxylin and eosin. Each slide was examined under light microscope with low and high power magnification. Histological examantion 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 extracellularmatrix 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) (Figure-4) showed vascular changes, odema hyperemia and hemorrhage. There is a vasodilation and congestion in the blood vessels. There is a great hyperplasiaof 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

**Keywords:** Arginine, Selenium, Vitamin E, Placenta, Iraqi Ewes

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### INTRODUCTION

Bovine 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 chorioallntoic 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 (Wuetal, 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 Bayaty et al, 2012). Selenium (se) is an essential trace element 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 fetus (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 Vit E or both on placenta of sheep. Therefore the aim of this study were designed to demonstrated the effect of arginine and selenium with Vit E or both on histological picture of ovine placenta in Iraqi ewes.

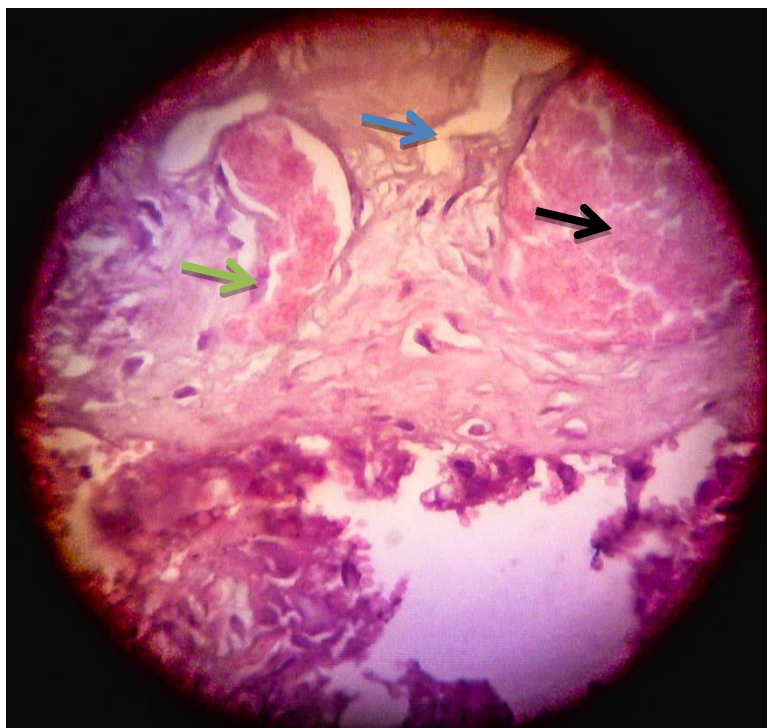
## **MATERIALS AND METHODS**

The Study was conducted on 24 pregnant Iraqi Ewes aged between 2-4 years and weight of 40-50 Kg presented in 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, UK). The 1<sup>st</sup> dose were given at days 5 while, the 2<sup>nd</sup> 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 third 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 4<sup>th</sup> 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 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 thicknesses and then stained with hematoxylin and eosin (Culling et al, 1985). Each slide was examined under light microscope with low and high power magnification.

## **RESULTS AND DISCUSSION**

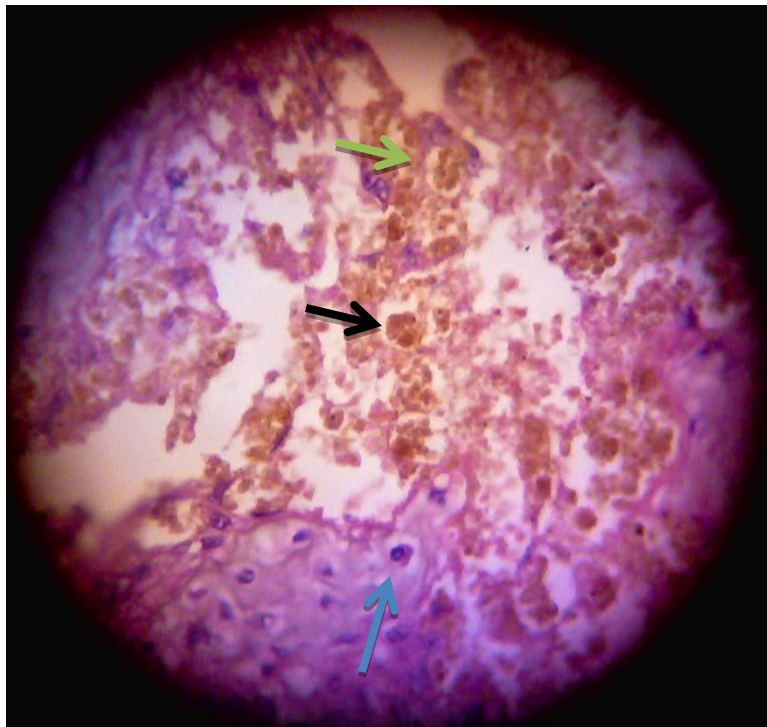
Histological section of Placentomes taken from ewes treated with arginine (T1) (Figure1) showed vascular changes includes vasodilatation of blood vessels and lymph vessels. There was Congestion blood vessels and a hypertrophy of endothelial Cells. There were a mononuclear Cell infiltrations and hypertrophy 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 maternal plasma of sheep were expected to increase placental angiogenesis and utero placental 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 thereafter (Reynolds et al, 2010), while the Vascular Changes of maternal Placental tissue side (caruncles) increased substantially (gradually) from day 40 untimed pregnancy then slowly there after (Reynolds et al, 2005). Histological picture of sheep treated with selenium plus vitamin E (T2) (Figure-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, 2010a). Also se

have an 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 (Palmieriand Szarek , 2011).It is also reported in cattle that Se with vitamin E injection decrease the incidence of retained fetal membranes (Palmieriand 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)(Figure3) treated with a combination of arginine with Se plus vitamin E showed vasodilatation in blood vessels. There is an endothelial cell hypertrophy and trophoblastic hyperplasia. There is also extracellularmatrix 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 (Wuand Meininger, 2000). and increase placental angiogenesis (Reyneolds et al,2010) and utero placental blood flow (Neriet al, 1995). It is also observed that Se plus vitamin E act as antioxidant stimulate glutathione peroxidase that enhance the development of Cotiledonary and Caruncular tissue via increase blood flow and increases immunity (Palmieri and Sszarek, 2011). Histological section of Placentomes from ewes treated with normal saline (T4- Control) (Figure-4) showed vascular changes, odema hyperemia and hemorrhage. There is a vasodilatation 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 has 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.



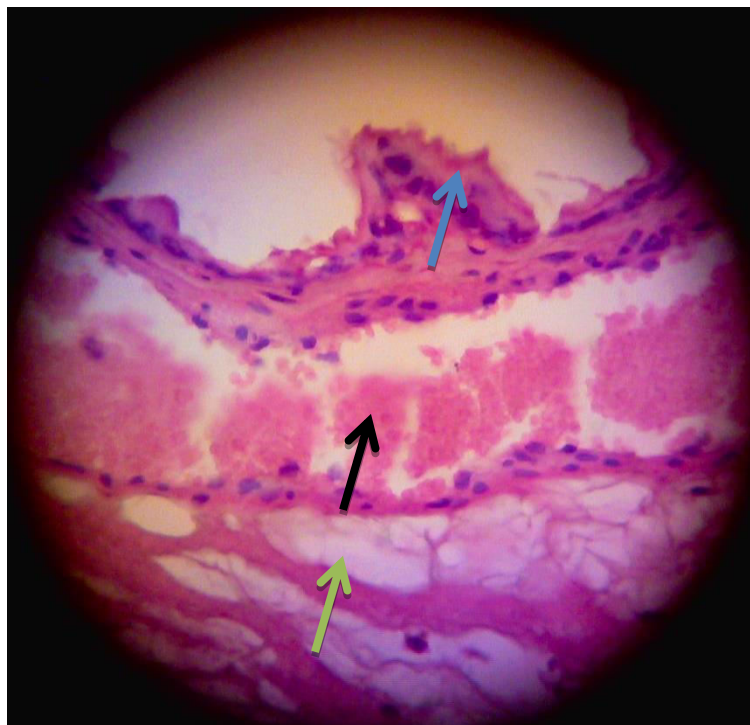
HE(X=400)

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



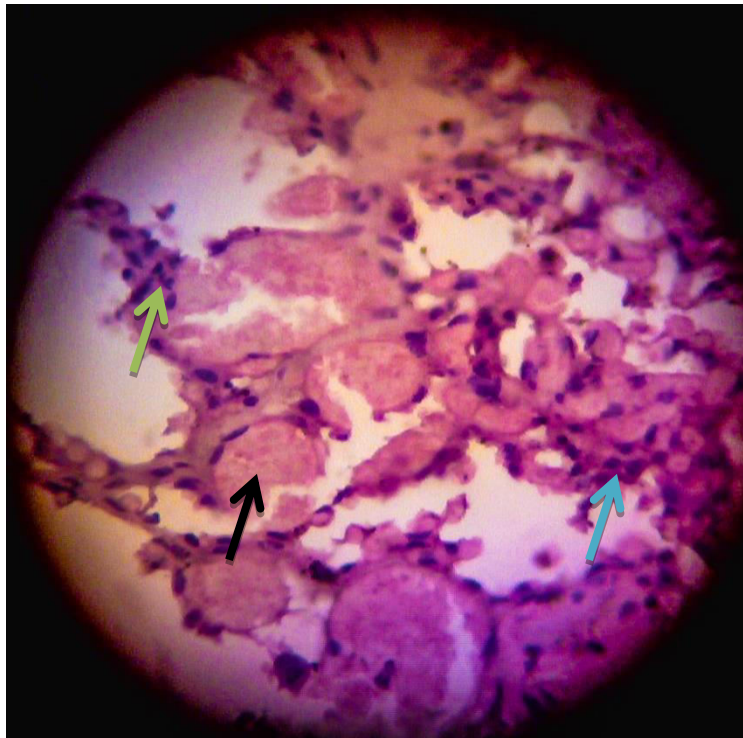
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Fig (2): Presence of hemosiderosis (black arrow) trophoblasts enlargement (blue arrow) and few binucleated cell infiltration (green arrow).



HE(X=400)

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



HE(X=400)

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

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