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The Effect of Arginine Supplementation on Some Blood Parameters, Ovulation Rate and Concentrations of Estrogen and Progesterone in Female Awassi Sheep

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Abstract: The hypothesis that arginine supplementation would increase antibodies production and concentrations of estrogen and progesterone-via stimulate Gonadotropin Releasing Hormone(GnRH)-in sheep was investigated. In experiment 1: Twelve Awassi ewes aged between 3.5-4.0 years weighing 48.0±2.5 kg were assigned randomly to four groups, to find whether the route of arginine administration would reflect different percentages of white blood cells, arginine and Immunoglobuline G (IgG) in the blood of ewes or not. Ewes in group one were fed ration supplemented with protected arginine (0.5 g kg^{-1} of body weight) (arginine group). Ewes in group tow were intravenous injected (IV) by arginine (IV group), whereas ewes in group three were intramuscular injected (Im group), group four was assigned as a control group. Percentages of white blood cells (Neutrophils, Monocytes and Lymphocytes) were recorded as well as concentrations of arginine and Immunoglobuline G (IgG) in blood of ewes were recorded. Percentage of neutrophils were increased ($p<0.01$) 6 h of treatment, whereas percentage of monocytes were increased ($p<0.01$) 12 h of treatment, meanwhile lymphocytes were decreased ($p<0.01$) 6 h of treatment. In general the increase and decrease of white blood cells were the highest in IV group, followed by arginine group, Im group, respectively in comparison with control group. Route of arginine administration affected the concentration of arginine in ewes blood. Levels of arginine were the highest in IV group (1697.67±945.25 mmol L^{-1}), followed by Im group (687.33±102.71 mmol L^{-1}), arginine group (335.00 ±17.90 mmol L^{-1}), respectively in comparison with control group (293.33±42.48 mmol L^{-1}). There were no significant differences between groups in IgG concentrations. In experiment 11: Twelve Awassi ewes aged between 3.5-4.0 years weighing 53.30±3.3 kg were used to find if that arginine supplementation would increase ovulation rate and concentrations of estrogen and progesterone. Experimental ewes were synchronized using intravaginal progestagen sponges. On day of sponges removal, ewes were divided into two groups. Ewes in group one were fed ration supplemented with protected arginine (0.5 g kg^{-1} of body weight) for 15 days; the second group was assigned as a control group. Concentrations of estrogen and progesterone in ewes blood beside the number of corpora lutea were recorded. Results showed that treatment with arginine increased the number of corpora lutea (2.38±0.67) in comparison with control group (1.00±0.58) which lead to increase lambing and twinning rate. Although of estrogen concentrations were fluctuated, but it was higher (5.92±0.33 pg mL^{-1}) in arginine group than control group (4.56±1.06 pg mL^{-1}). On the other hand progesterone concentrations were higher ($p<0.01$) (4.21±0.83 ng mL^{-1}) in arginine group in comparison with control group (1.79±0.31 ng mL^{-1}).

Key words: Arginine, immunity, Awassi sheep, ovulation rate

INTRODUCTION

Low reproduction efficiency of farm animals in Arabic World is one of the important reasons in lowering it's productivity (AL-Rawi *et al*., 1996; AL-Haboby *et al*., 1997).

In order to improve reproduction performance of these animals, many advanced techniques have been developed during the last decades, among these are Artificial Insemination (AI), Super Ovulation (Hafez, 1987), Estrous Synchronization, Embryo Transfer and *in vitro* Fertilization (Bearden and Fuquay, 1997). On the other hand, it was found that there is a specific nutrients play an important role in regulation of growth, development, reproduction (Barb *et al*., 1991) and immunity (Cunningham-Rundles, 1993). Among these

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nutrients a semi essential amino acid called arginine is considered as a metabolic signal in affecting of many biological activities in the body such as immune system (Cunningham-Rundles, 1993), stimulating the secretion of some hormones highly related to reproduction such as Growth Hormone (GH) (Recabareen *et al*., 1995), prolactin and insulin (Chew *et al*., 1984), gonadotropin releasing hormone (GnRH) and Luteinizing Hormone (LH) (Hall *et al*., 1992).

Lack of studies concerning the effect of the arginine treatment on some blood characteristics, ovulation rate and concentrations of estrogen and progesterone during the estrous cycle in Awassi ewes lead us to conduct this study.

MATERIALS AND METHODS

This study was conducted at AL-Khanasry Research Station at the National Centre for Agricultural Research and Extension (NCARE), Mafraq-Jordan, during the period between March, 1999 to August, 2000. In experiment 1: The effect of arginine supplementation on some blood characteristics in Awassi ewes was studied. Twelve Awassi ewes aged between 3.5-4.0 years, with average weight (X±SE) 48.0±2.5 kg were divided randomly into four equal groups. Ewes in group one were fed individually a ration supplemented with protected arginine (0.5 g kg^{-1} body weight) (arginine group). Ewes in group tow were intra venous (IV) injected by arginine solved in 120 mL of saline solution (IV group), injection process was done by 10 interval period, 2 h between one dose and the other. Whereas ewes in group three were intramuscular injected arginine (Im group) in doses equal to those did in IV group. Group four was assigned as a control group injected with saline solution only in the same times and quantities given to ewes in second and third group. All ewes were placed on individual pens in a barn before a week of the beginning of the experiment as a preliminary period to insure that ewes got their amount of feed requirements recommended by NRC (1985).

For the purpose of differential account of neutrophils, lymphocytes and monocytes, blood samples were taken from all ewes in deferent groups in tubes containing anticoagulant at the beginning of arginine administration, then after each 2 h.

To determine the arginine and IgG concentrations in ewes blood, blood samples were taken from all experimental ewes in special tubes (Ependorf tubes) before an hour and after 12:20 h of arginine treatment. Serum separation was done by a centrifuge at a 3000 cycle min^{-1} for 10 min, serum samples were kept in -20°C until chemical analysis.

In experiment 2 the effect of arginine administration on ovulation rate and concentrations of estrogen and progesterone was studied. Twelve Awassi ewes aged between 3.5-4.0 years weighing 53.30±3.3 kg were synchronized using intravaginal progestagen sponges (Medroxy progesterone acetate, MAP) for 14 days. On day of sponges removal, ewes were divided into two groups. Ewes in group one were fed ration supplemented with protected arginine (0.5 g kg^{-1} of body weight), whereas ewes in group two were assigned as a control group fed a ration met the requirements (NRC, 1985) for 15 days.

To measure the change in concentration levels of estrogen and progesterone during the estrous cycle using Enzyming Linked Immunosorbant Assay (ELISA)way, blood samples were taken from three ewes of each group on the day of sponges removal (0 day) followed by on other samples on days 1, 3, 5, 7, 9, 11, 13 and 15. Serum of these samples was separated and stored until chemical analysis on the same way mentioned on experiment 1. For measuring the ovulation rate, three ewes from each group were randomly slaughtered after 6 days of the beginning of arginine treatment to calculate the number of corpora lutea.

Statistical analysis: The effect of treatment on concentrations of estrogen , progesterone, ovulation rate, arginine, IgG and differential account of WBC. were analysed as a Completely Randomized Designe (CRD) using General Linear Model Procedure (GLM) of SAS, 1992. Duncan's Multiple Range Test was used to compare the significant differences between means.

RESULTS

Experiment 1

Differential account of white blood cells: Percentages of Neutrophils were the highest ($p<0.01$) in IV group (69.67±3.18%) followed by arginine group (58.67±2.19%), Im group (55.67±5.70%) and control group (43.67±1.33%), respectively, 6 h after treatment, no significant differences ($p>0.05$) between groups in other different times (Fig. 1).

Figure 2 showed the percentages of lymphocytes in animal's blood which was the highest ($p<0.01$) in control group (55.67±1.67%), followed by Im group (43.33±5.67%), arginine group (41.00±5.52%) and IV group (30.33±3.18%), respectively, 6 h after treatment, there were no significant differences ($p>0.05$) between groups in other different times.

Figure 3 showed that the percentages of monocytes were the highest ($p<0.01$) after 12 h of treatment in IV group (1.33±0.33%), followed by arginine group (0.67±0.33%), control group (0.33±0.33%), but there was no effect of treatment ($p<0.01$) at the percentages of monocytes in Im group (0.00±0.00%).

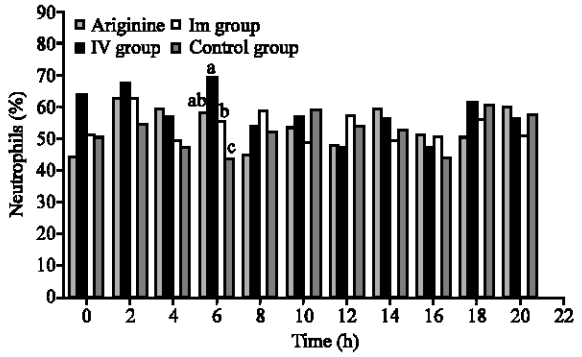

Fig. 1: Neutrophil cells percentages in the blood of different animal groups. Different superscripts within one time in different animals groups shows significant different at ($p<0.01$)

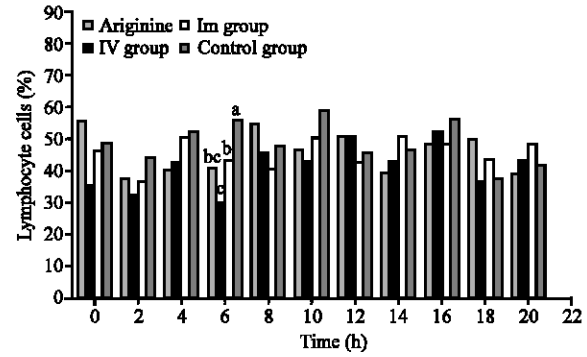

Fig. 2: Lymphocyte cells percentages in the blood of different animal groups. Different superscripts within one time in different animals groups shows significant different at ($p<0.01$)

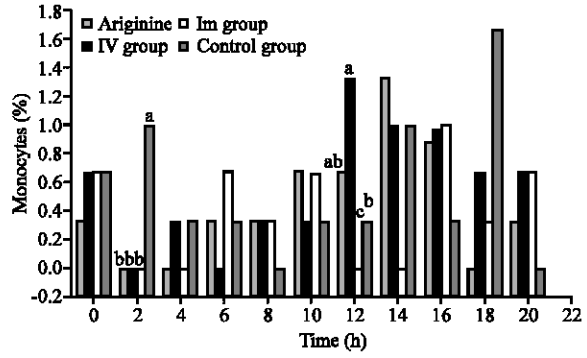

Fig. 3: Monocyte cells percentages in the blood of different animal groups. Different superscripts within one time in different animals groups shows significant different at ($p<0.01$)

Arginine concentration in the blood serum: Figure 4 showed that concentration of arginine in the blood

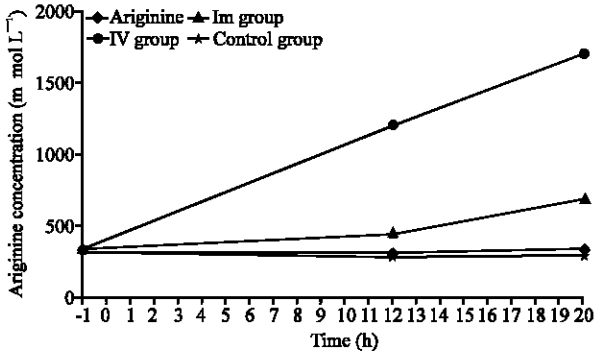

Fig. 4: Arginine concentration (mmol L^{-1}) in the blood of different animals groups

Table 1: Immunoglobuline IgG concentration (mg mL^{-1}) (X ±SE) in the blood serum of different animal groups

	Time of treatment (h)		
Animal groups	-1	12	20
Arginine group	15.67±0.92	13.67±0.98	13.33±0.95
IV group	12.67±0.93	13.67±0.90	14.00±0.67
Im group	14.00±0.62	13.00±0.65	15.67±2.56
Control group	12.33±0.21	12.33±0.85	11.33±0.54

serum of different animal groups which was the highest ($p<0.01$) in IV group after 12 h (1198.67±770.16 mmol L^{-1}) and 20 h (1697.67±945.25 mmol L^{-1}), followed by Im group (438.00±64.71 mmol L^{-1}), (687.33±102.71 mmol L^{-1}), arginine group (307.00±24.00 mmol L^{-1}), (335.00±17.90 mmol L^{-1}) and control group (289.33±41.87 mmol L^{-1}), (293.33±42.48 mmol L^{-1}). There was no significant differences in IgG concentrations among different animal groups at 1, 12 and 20 h of arginine treatment.

IgG concentration in the blood serum: Results of this experiment showed that there is no significant differences ($p>0.05$) among groups in IgG concentrations by different times of measurements as it is clear in the Table 1.

Experiment 2: The average number of corpora lutea were higher in arginine group (2.38±0.67) than control group (1.00±0.58), but these differences were not significant ($p>0.05$). Estrogen levels during 15 days of measurement were variable in ewes of the two groups as shown in Fig. 5, but it was obviously noticed a higher concentration in this hormone in arginine group (5.92±0.33 pg mL^{-1}) than control group (4.56±1.06 pg mL^{-1}), but these differences were not significant ($p>0.05$).

On the other hand, changes in the concentration levels of progesterone during the estrous cycle is shown in Fig. 6 which were similar in arginine group (1.40±2.30 ng mL^{-1}) and control group (1.83±1.24 ng mL^{-1})

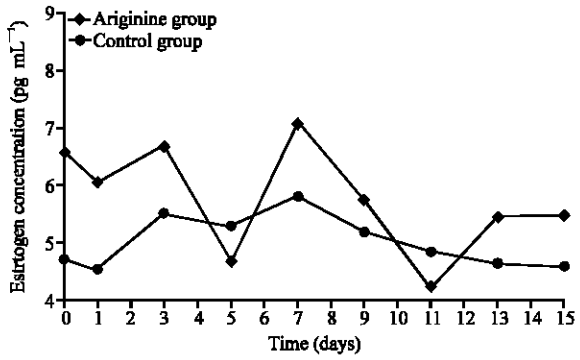

Fig. 5: Estrogen concentration (pg mL^{-1}) in the blood serum of animals in arginine and control groups

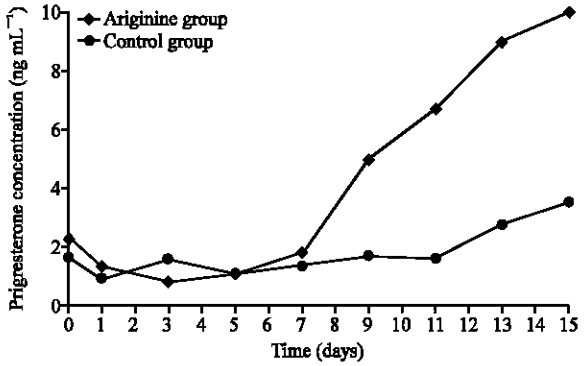

Fig. 6: Progress concentration (pg mL^{-1}) in the blood serum of animals in arginine and control groups

in the day of sponges removal, then there was a decrease in the concentration level one day after sponges removal to (1.30±0.28 ng mL^{-1}) and (0.92±0.5 ng mL^{-1}) in arginine and control groups, respectively. The continuous depression in the concentration level of progesterone in the two groups was until the 7th day of the experimental treatment, but the differences were not significant ($p>0.05$) during this period. Start release in concentration levels of progesterone were in the day 9 of the experimental treatment, reached the peak in arginine and control group (10.00±1.15 ng mL^{-1}) and (3.52±0.82 ng mL^{-1}), respectively in the 15th day. Differences were significant ($p<0.01$) during days 11, 13 and 15 of the experimental treatment in the concentration level of progesterone between ewes in arginine and control groups.

DISCUSSION

Supplemented arginine play an important roll in immune function through increased Thymic weight and cellularity as well as enhanced nitrogen-stimulated lymphocyte proliferation (Barbul *et al*., 1980a, 1981b). On another hand it was observed that L-arginine supplementation depressed circulating lymphocytes number and total IgG concentrations as a result of it's roll in inhibiting humoral immune (Fligger *et al*., 1997).

In this study, it was observed a significant decrease ($p<0.01$) in lymphocytes percentages, an increase ($p<0.01$) in neutrophils and monocytes specially after 6 h of arginine injected. The decrease and increase in number of these cells was in consistently with the increase of arginine concentration in the blood serum, because iv injection is consider as the highest way in elevating concentrations of the injected material in the blood, according to it's direct absorb to the blood cycle (Ganong, 1983). The slight increase in arginine concentration in animal's blood fed ration supplemented with arginine resulting in slight change in number of WBC may be due to the insufficient time for passing digested and undigested food included arginine to the small intestine, then absorbing digestive nutrients to the blood. Hassan (1986) mentioned that the optimal time of measuring the rate of passage of the material is 48 h after feeding the animal, as well as the efficiency of arginine absorption via intestine wall is less than absorb efficiency via IV or muscle injection (Hogan, 1973; Beever *et al*., 1977). There were slightly differences in arginine concentration in animal's blood during different times and the way of treatment, but these differences was not significant which was may be due to the low number of animals used in each group and the big variation in arginine concentration in the same animal's group after treatment in comparison with it's concentration before injection.

Other studies in laboratory animals showed an increase in lymphocyte cells type T as a result of Thyme weight increase when fed a ration supplemented with arginine (Barbul, 1985; Daly *et al*., 1990). In another study by Takagi *et al*. (1994) there was an inhibition in humoral immunity related to lymphocyte cells type B which are producing immunoglobulines such as IgG. But there was no significant differences ($p<0.01$) in IgG concentrations between different animal's groups in this study, which is may be due to the shortage period of measurement taken after treatment.

In experiment 11 there was an increase in the rate of corpora lutea number by 58% in arginine group comparing with control group, which is may be due to the stimulation effect of arginine in LH secretion (Recabarren *et al*., 1995b), but this increase wasn't significant which was may be due to the lower number of animals used in this

experiment. There was an increase in progesterone level in arginine group comparing with control group reaching the peak in the day 15 of the estrous cycle as a result of the increase in the ovulation rate, which was consistently with the normal secretion of progesterone after ovulation (Hafez, 1987), but the increase in the concentration level of this hormone was higher in arginine group (4.21±0.83 ng mL^{-1}) than control group (1.79±0.31 ng mL^{-1}), which is a normal refractory to the increase in the number of corpora lutea obtained in arginine group. The consistently secretion of estrogen was variable in the two groups, but there was an obvious direction in increasing the estrogen level in arginine group coincided with the increase in ovulation rate and progesterone levels. The variability in estrogen secretion may be due to the growth, development and atratia in ova before reaching the full maturity (Savio *et al*., 1988; Ginther *et al*., 1989).

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