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Effects of Organic and Inorganic Selenium Supplementation with Vitamin E during the Flushing Period on Reproductive Performance of Ghezel Ewes

Research Article

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ABSTRACT

Minerals and vitamins play an important role in animal nutrition with beneficial effects on animal reproductive performance. To investigate this issue, 44 Ghezel ewes weighing 55 ± 2 kg (Mean±SD) and 2-3 years old were randomly divided into groups (n=11), to investigate the effect of the selenium and vitamin E supplementation on blood hormones and metabolites and possible reproductive performance of Ghezel ewes. Treatments were consisted of group A: as control, B: flushing diet containing barley grain (73%), C: flushing diet + vitamin E (48 mg/day) + organic selenium (selenoproteins, 13.5 mg/day) and D: flushing diet + vitamin E (48 mg/day) + inorganic selenium (sodium selenite, 1.35 mg/day). The estrous cycles of the ewes were synchronized by application of a controlled internal drug release (CIDR) for 14 day. The results showed that the highest lambing rates (136.4%) and numbers of offspring (n=15) were in group C. Hormonal measurements including estrogen, progesterone and insulin revealed the relationship of these hormones with reproductive performance of ewes with the highest concentrations of estrogen and insulin (in estrus) and progesterone (in three weeks after mating) in groups C and D ($P<0.05$). Glucose and cholesterol levels of groups C and D increased significantly during the day prior to CIDR removal, estrus and three weeks after mating ($P<0.05$). Total protein and blood urea nitrogen (BUN) levels of B, C and D groups enhanced significantly in all times compared to the control group ($P<0.05$). Using inorganic and organic selenium in flushing diet had similar effects on blood metabolites and hormones related to reproductive performance.

KEY WORDS blood hormones, flushing, Ghezel ewes, reproductive performance, selenium, vitamin E.

INTRODUCTION

Reproductive performance of livestock is determined by four factors containing genetic merit, physical environment, management and nutrition. Nutritional factors are perhaps the most crucial ones, in terms of their direct effects on reproductive performance. Poor nutrition not only reduces performance below genetic potential, but also exacerbates detrimental environmental effects (Scaramuzzi *et al*. 2006). Moreover, nutritional factors are the most important factors because nutrition affects all aspects of the chain of reproductive events including gametogenesis, follicular development, ovulation and steroidogenesis in females. The reason for this close association between nutrition and reproduction is to ensure that reproduction is very closely aligned with the food supply (Scaramuzzi *et al*. 2006). One of the issues related to the optimization of reproductive performance is using flushing diets for 2 weeks before and 3 weeks after natural mating that may increase the lambing rate (Daghighkia and Asgari Safdar, 2015). Using nutritional supplements during the flushing period increases the ovulation rate and lambing rate in most of ewe breeds (Naqvi *et al*. 2011). One of the nutritional requirements is micronutrients including minerals and vitamins. Minerals

and vitamins play an important role in livestock nutrition. They have beneficial effects on animal reproductive performance. Cobalt, selenium, manganese, β-carotene and vitamins play an important role in the reproductive performance by affecting ovarian steroids (Harrison *et al*. 1984). Vitamin E and selenium deficiency has long been known to increase free radicals which disturb the ovulation rate (Goto *et al*. 1992), embryo survival and lamb growth after birth (Anke *et al*. 1989; Shkolnik *et al*. 2011).

However there is inadequate information about the effect of selenium and vitamin E on reproduction, especially sheep reproduction. It has been shown that selenium influences granulosa cells and stimulates 17-β-estradiol synthesis and finally increases ovulation and the number of live embryos in cattle (Basini and Tamanini, 2000). Some of the blood metabolites can influence steroid hormones; in fact cholesterol is used by luteinized ovarian cells as the precursor of progesterone synthesis. In a study in cattle with high level of cholesterol, follicular growth was improved and at the same time, estradiol, insulin and IGF-1increased (Bao *et al*. 1995). Increasing steroid in follicular fluid improves estrous properties, ovulation rate, fertility, cleavage and pregnancy rate in dairy cattle (Robinson *et al*. 2002).

The Se is the cofactor of glutathione peroxidase (GPx) which plays an important role in amino acids metabolism, normal growth and development of fetus, the optimal function of thyroid gland and stimulating immunity function (Moustafa *et al*. 2003). Vitamin E affects ovulation rate, fetus and embryo survival (El-Shahat and Abdel Monem, 2011). Therefore, the aim of this study was to investigate the effect of (in)organic selenium (sodium selenite and selenoproteins) and vitamin E supplementation E on reproductive performance of Ghezel ewes during the flushing period.

MATERIALS AND METHODS

Animals, diets, and experimental procedure

The experiment was carried out at the research Institute of Tabriz University. All of the animal procedures and protocols used in this study were approved by the college of Tabriz animal care and use committee. The experiment started in the breeding season (summer) with average temperatures of 29.3 ˚C and with 61.2% relative humidity. Forty-four Iranian Ghezel ewes, 2-3 years old with an average weight of 55 ± 2 kg (Mean±SD), were penned indoors in four equal groups (n=11). Treatments were divided into four groups; The ewes in each group were fed the same basal diet (Table 1) and received one of the following treatments: group A: the control group that did not receive the flushing diet (control group contained wheat bran (11%), wheat straw (40%), alfalfa (35%) and barley grain (14%), group B: flushing diet containing barley grain (73%), group C: flushing diet + vitamin E (DLalpha-tocopherol, 48 mg/d) + organic selenium (selenoproteins, 13.5 mg/d) and group D: flushing diet + vitamin E (48 mg/d) + sodium selenite (Na_2SeO_3, 1.35 mg/d) (Table 1).

The ewes' need for vitamin E and selenium were estimated according to NRC nutrient requirements for flushing duration. Vitamin E supplement and selenium were obtained from the Vetak Company (Tehran, Iran). Diets were formulated to meet the nutrient requirements of ewes according to NRC (1985) and the vitamin E and selenium were mixed with the concentrate portion of the total mixed ration.

Diets were weighed individually for each ewe (500 g in day for each ewe). The flushing period started on the day of CIDR insertion and continued for 3 weeks after CIDR removal, leading to a total period of 5 weeks over which the experimental diets were fed.

All ewes had average body condition score (BCS) of 2.5 and reached to approximately 3 at the time of mating (Daghighkia and Asgari Safdar, 2015). Ingredients and chemical analysis of diets are shown in Table 1.

The estrus synchronization of ewes was first accomplished using CIDR (EAZI BREED; Pfizer NEW Zealand LTD, Auckland, New Zealand) for 14 day; then the ewes received 400 units of eCG hormone [Bioniche Animal Health (LA Asia) Pty Ltp/Australia (preg-necol injection)]. The ewes were naturally mated using Ghezel rams that were introduced the day after the injection of eCG. The amount of selenium in basal diet was 0.09 mg/kg of dry matter diet. All the ewes had the same diet during pregnancy. Fertility, twining rate and lambing rate were recorded.

Blood sampling

During the experiment, blood samples were collected from the jugular vein at four times: start of the experiment or before of at CIDR application (T1), the day before CIDR removal (T2), the day after CIDR removal or estrus (T3) and three weeks after ram introduction (RI) (T4) using a venoject syringe. The blood samples were centrifuged for 12 min (1800×g at 18 ˚C) to separate the sera. All sera were stored in microtubes and frozen at -20 ˚C until time of analysis.

Analysis of blood metabolites and hormones

Concentration of glucose, total protein, cholesterol, and BUN were determined by commercial kits (Pars Azmun Laboratory, Tehran, Iran). Analysis of serum sample metabolites was done using a STAT Faz-2100 spectrophotometer. Serum sample hormones concentrations were measured using an ELISA reader (STAT-FAX 3200, USA).

Table 1 Ingredients and nutrient composition of experimental diets (dry matter basis) fed during the flushing period

Ingredient (%)	Treatment A	Treatment B	Treatment C	Treatment D
Wheat bran	11	11	11	11
Wheat straw	40	-	-	-
Soybean meal	-	8.5	8.5	8.5
Limestone (Ca and P)	1	1	1	1
Barley grain	14	73	73	73
Salt	-	0.5	0.5	0.5
Molasses	-	6	6	6
Alfalfa	34	-	-	-
Dietary supplements				
Inorganic selenium (sodium selenite, mg/day)	-	-	-	1.35
Organic selenium (Selenoprotein, mg/day)	-	-	13.5	-
Vitamin E (mg/day)	-	-	48	48
Nutrient composition				
Crude protein (CP) (g/kg of DM)	81	132	132	132
Ca (g/kg of DM)	3.15	3.78	3.78	3.78
P (g/kg of DM)	2.23	2.64	2.64	2.64
Digestible energy (Mcal/kg)	2.38	3.41	3.41	3.41
Metabolizable energy (Mcal/kg)	2.14	3.01	3.01	3.01

Treatment A: control group; Treatment B: flushing with barley grain; Treatment C: flushing with barley + vitamin E + organic selenium and Treatment D: flushing with barley + vitamin E + inorganic selenium.

Serum insulin level was determined by Monobind insulin microplate enzyme-linked immunosorbent assay (ELISA) kit no 2425-300 manufactured by Monobind Inc., USA and estrogen and progesterone were determined by Monobind insulin microplate enzyme-linked immunosorbent assay (ELISA) kit no 4925-300A and 4825-300 manufactured by Monobind Inc., USA.

Statistical analysis

This study was conducted in a completely randomized design (CRD). SAS (2003) software was used for data analysis. Blood hormones and metabolites concentrations were analyzed using MIXED procedures and data are presented as mean ± SE. Feed intake levels were the same in all the treatments; therefore its effect was ignored from the model. Only after CIDR removal and during estrus, dry mater intake reduced; however, it was similar among all the treatments. The statistical model used is as follows: The statistical model used is as follows: that in the model effect of weight as a covariate and $(Treat*Time)_{ij}$ = treatment by time interaction.

$$Y_{ijkl} = \mu + Treat_i + Time_j + (Treat \times Time)_{ij} + B(X_{ijk} - X\ldots)_k + Animal_l + e_{ijkl}$$

Where:
Y_{ijkl}: animal's performance.
μ: population mean.
$Treat_i$: *i* treatment effect.
$Time_k$: effect of the kth time.
$B(X_{ijk}-X\ldots)_k$: effect of body weight as a covariate.
$(Treat \times Time)_{ij}$: treatment by time interaction.
$Animal_l$: *l* animal effect.
e_{ijkl}: residual or error.

General linear mode (GLM) procedure was used to analyze pregnancy rate and birth weight and Logistic procedure was applied to analyze type of parturition. Mean comparison of treatments was done by the Tukey-Kramer test.

RESULTS AND DISCUSSION

Reproductive performance

The results of the present study showed great increase in the birth weight and percentage of fertility, lambing and twinning rate. According to this investigation, adding organic selenium and vitamin E (treatment C) and inorganic selenium and vitamin E (treatment D) to the diet during reproductive season increased the number of offsprings ($P<0.05$; Table 2). Also, twinning and lambing rates in treatments C and D were higher than those of B and control groups.

However, fertility rate in treatment B was the lowest among the treatments (Table 2). The lambs in treatments C and D showed the highest birth weight which were significantly different from treatment B and the control group ($P<0.05$; Table 2). Treatment C with four twinning (Table 3) had the highest twinning rate compared to the others (Table 2). The number of born male lambs was higher than the females. The lowest and highest ratio of male/female new-born lambs were belong to the control and group D, respectively (Table 3).

Table 2 Reproductive performance of Ghezel ewes fed four different diets (number of ewes=11 per treatment)

Treatment	Total offspring	Estrus response (%)	Fertility (%)	Twining rate (%)	Lambing rate (%)	Birth weight (kg)
A	11	100	100	0	100	3.65±0.15[c]
B	12	90.9	90.9	20	120	4.01±0.15[b]
C	15	100	100	36.4	136.4	4.52±0.14[a]
D	14	100	100	27.3	127.3	4.44±0.14[a]

Treatment A: control group; Treatment B: flushing with barley grain; Treatment C: flushing with barley + vitamin E + organic selenium and Treatment D: flushing with barley + vitamin E + inorganic selenium.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 3 Characteristics of born lambs in different treatments (number of ewes=11 per treatment)

Treatment	Number of offspring		Lamb sex		Male/female ratio
	Singletons	Twins	Male	Female	
A	11	0	5	6	0.83
B	8	2	6	6	1
C	7	4	9	6	1.5
D	8	3	10	4	2.5

Treatment A: control group; Treatment B: flushing with barley grain; Treatment C: flushing with barley + vitamin E + organic selenium and Treatment D: flushing with barley + vitamin E + inorganic selenium.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Hormones and metabolites

Table 4 shows the variation of blood estrogen levels at the different stages of the experiment. Treatments D and C had the highest estrogen concentration during the estrus (P<0.05). There were no significant differences between treatments in serum progesterone concentrations at the beginning of the experiment but the differences were significant (P<0.05) three weeks after mating. Progesterone concentration in the treatments receiving inorganic selenium with vitamin E and organic selenium with vitamin E were significantly different with the control group (P<0.05) but not with B group. The highest progesterone concentration was related to three weeks after mating probably because of embryo formation and ewe's pregnancy. Also its concentration was significantly different among flushing treatments (P<0.05). Treatment D and treatment C had the highest progesterone concentrations which were significantly different from the control and flushing groups. Also, its concentration in the flushing treatment was significantly different from the control group (P<0.05).

The results presented in Table 4 show that experimental treatments affected serum insulin concentration at all times except for the beginning of the experiment. The lowest and highest level of insulin at the day before CIDR removal were observed in treatments A and C, respectively. The highest level of insulin at the day after CIDR removal among treatments was observed in treatment D which was not significantly different with treatment C but was higher than with treatments A and B (P<0.05). Insulin level for treatment D was higher than the other treatments three weeks after mating, but it was just significantly different from control group (P<0.05; Table 4).

The serum glucose concentrations were similar for all treatments at the first sampling. The results presented in Table 5 show that serum glucose of groups C and D increased significantly at the day before CIDR removal, estrus and three weeks after mating compared to A and B (P<0.05).

The highest level of glucose was observed in the treatment D at all the times except the beginning of the experiment (Table 5).

The serum cholesterol concentrations were not significantly different among the treatments at the first sampling. The results presented in Table 5 show serum cholesterol level at day before CIDR removal, estrus and three weeks after mating in treatment B compared to the control group (P<0.05).

Also, cholesterol level increased significantly in treatments C and D compared to treatment B at all times of blood sampling (P<0.05;Table 5).

The serum BUN concentrations were similar for all the treatments at the first sampling. Investigations showed that serum BUN concentration increased significantly at the day before CIDR removal, during estrus and three weeks after mating compared to the control group (P<0.05). But there was no significant difference between treatments C and D compared to treatment B (Table 5).

The serum total protein concentrations were similar for all the treatments at the first sampling. Serum total protein level increased significantly at day before CIDR removal, estrus and three weeks after mating in all the experimental treatments compared to the control group (P<0.05). But there was no difference between treatments C and D compared to treatment B (Table 5).

Table 4 Effects of different diets during the flushing period on serum hormones concentration (Mean±SE)

Variable	Treatment	T1	T2	T3	T4
Estradiol-17β (pg/mL)	A	-	8.09±0.45^{b}	25.98±0.45^{c}	8.63±0.43^{b}
	B	-	7.60±0.49^{b}	31.20±0.59^{b}	8.46±0.47^{b}
	C	-	10.51±0.45^{a}	37.66±0.45^{a}	8.35±0.43^{b}
	D	-	11.33±0.45^{a}	37.43±0.47^{a}	10.20±0.43^{a}
Progesterone (ng/mL)	A	1.23±0.07	1.39±0.09^{b}	0.61±0.08^{b}	3.31±0.13^{c}
	B	1.31±0.07	1.47±0.09^{ab}	0.97±0.09^{a}	4.11±0.15^{b}
	C	1.34±0.06	1.68±0.08^{a}	0.74±0.08^{ab}	5.12±0.13^{a}
	D	1.35±0.06	1.69±0.08^{a}	0.78±0.08^{ab}	5.20±0.13^{a}
Insulin (ng/mL)	A	0.47±0.01	0.48±0.01^{b}	0.55±0.02^{b}	0.41±0.02^{b}
	B	0.45±0.01	0.50±0.01^{b}	0.58±0.03^{b}	0.49±0.01^{a}
	C	0.47±0.01	0.59±0.01^{a}	0.69±0.02^{a}	0.54±0.02^{a}
	D	0.46±0.01	0.55±0.01^{ab}	0.74±0.02^{a}	0.53±0.02^{a}

Treatment A: control group; Treatment B: flushing with barley grain; Treatment C: flushing with barley + vitamin E + organic selenium and Treatment D: flushing with barley + vitamin E + inorganic selenium.
T1: at CIDR application; T2: day before CIDR removal; T3: day after CIDR removal (estrus) and T4: three weeks after mating.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 5 Effects of different diets during the flushing period on serum metabolites concentration (Mean±SE)

Variable	Treatment	T1	T2	T3	T4
Glucose (mg/dL)	A	58.4±1.13	57.4±1.04^{c}	56.6±1.04^{c}	54.8±1.04^{c}
	B	57.4±1.07	61.2±1.04^{b}	64.0±1.04^{b}	59.6±1.04^{b}
	C	59. 1±1.04	66.4±1.04^{a}	69.2±1.04^{a}	65.8±1.04^{a}
	D	58.2±1.09	67.9±1.04^{a}	71.8±1.04^{a}	66.7±1.04^{a}
Cholesterol (mg/dL)	A	66.2±0.84	67.0±0.92^{c}	67.4±0.92^{d}	70.0±0.92^{c}
	B	66.2±0.63	73.0±0.92^{b}	74.1±0.92^{c}	83.0±0.92^{b}
	C	66.1±0.27	93.4±0.92^{a}	95.3±0.092^{a}	90.2±0.92^{a}
	D	66.4±0.71	92.8±0.92^{a}	91.0±0.92^{b}	89.4±0.92^{a}
BUN (mg/dL)	A	17.2±0.40	17.1±0.40^{c}	18.8±0.40^{c}	18.4±0.40^{b}
	B	17.6±0.40	21.6±0.40^{b}	21.1±0.40^{a}	21.2±0.40^{a}
	C	17.1±0.40	19.7±0.40^{a}	20.3±0.40^{ab}	20.5±0.40^{a}
	D	17.4±0.40	19.4±0.40^{a}	19.9±0.40^{b}	21.6±0.40^{a}
Total protein (mg/dL)	A	7.28±0.15	7.31±0.14^{b}	7.66±0.14^{b}	8.26±0.14^{b}
	B	7.31±0.15	8.91±0.14^{a}	8.14±0.14^{a}	9.00±0.14^{a}
	C	7.01±0.15	8.44±0.14^{a}	8.12±0.14^{a}	9.38±0.14^{a}
	D	7.23±0.15	8.72±0.14^{a}	8.89±0.14^{a}	9.21±0.14^{a}

Treatment A: control group; Treatment B: flushing with barley grain; Treatment C: flushing with barley + vitamin E + organic selenium and Treatment D: flushing with barley + vitamin E + inorganic selenium.
T1: start of study; T2: day before CIDR removal; T3: day after CIDR removal (Estrus) and T4: three weeks after mating.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Some nutritionists assume that reproductive performance will not be limited when animals are fed diets meeting the NRC levels. However, little is known about the effects of vitamin E and selenium supplementation on specific reproductive events in sheep. In the current study, supplementation with Se and vitamin E increased estrogen concentration in plasma and consequently the number of offspring.

Sen *et al.* (2011) demonstrated that inorganic selenium with vitamin E supplementation in Suffolk sheep increased estrogen concentration before ovulation compared to the control group. The high concentration of estradiol during follicular phase causes surge of gonadotropin secretion. The increase of estrogen in follicular fluid improves estrus properties, ovulation rate, fertility, cleavage, pregnancy rate in dairy cattle (Robinson *et al.* 2002).

Van Niekerk *et al.* (1996) showed that using selenium supplementation in synchronized ewes decreases lambing rate to 19% which contrasts with to the results of this study. However, using selenium and vitamin E supplements in diets may have different results due to the level of the selenium and vitamin E used before, during and after mating (Rooke *et al.* 2004). The study of Makkawi *et al.* (2009) on Awassi sheep showed that selenium and vitamin E supplementation increases the number of leukocyte which protects receptors of gonadotropin against oxidants leading to significant improvements in estrogen secretion (Segerson and Libby, 1982). Selenium and vitamin E stimulate estrogen process, secretion and releasing gonadotropin from anterior pituitary which increases folliculogenesis in the ovaries (Ha and Smith, 2003; Meshreky and Metry, 2000).

The Se is a cofactor of the GP_X enzyme which has the main role in removing hydrogen peroxide (H_2O_2). Decrease in the levels of selenium reduces GP_X enzyme activity in follicular fluid and aggregation of reactive oxygen species (ROS) which directly leads to unfertilized oocytes in humans (Paszkowski *et al*. 1995). ROS aggregation affects different physiological activities of oocyte fertilization, steroidogenesis in the ovary, ovulation, fertilization and formation of blastocyst (Sugino *et al*. 2000). High amounts of ROS cause oxidative stress, damaging oocyte and granulosa cells (Taniguchi *et al*. 2009). The ovarian granulosa cells are steroidogenic cells which are responsible for folliculogenesis and have a role in follicle growth and oocyte maturation (Albertini and Barrett, 2002).

Lukasz *et al*. (2010) demonstrated that increasing dietary selenium during mating and pregnancy of ewes increases serum progesterone concentration on days 50, 90, 106 of pregnancy compared to the control group, leading to fetal protection. In fact, pregnancy rate increased 1.44% per 1 ng increase in plasma progesterone (Staples *et al*. 1998).

Progesterone plays an important role in fetal transfer from oviduct to uterine and implantation; meanwhile it causes increasing endometrial thickness, histotroph secretion and decreasing uterine contractions (Cam and kuran, 2004). Grazul-Bilska *et al*. (2014) showed that using organic selenium in ewe's diet decreases progesterone concentration compared to the control group, leading to abortion which is in contrast to our results. Progesterone prepares the uterine for embryo implantation and helps pregnancy protection by supplying fetal nutrition. Almost 25 to 55% of mammals 'embryos are lost in early pregnancy and most of them are because of corpus luteum dysfunction in progesterone production (Cam and Kuran, 2004). ROS increases $PGF_{2\alpha}$ production and high concentration of $PGF_{2\alpha}$ causes luteolysis and fetus abortion (Lekatz *et al*. 2010).

ROS causes damage of luteinizing hormone (LH) receptors and inhibits cholesterol transfer to mitochondria for progesterone synthesis (Kato *et al*. 1997). Selenium increases GP_X enzyme activity in follicular fluid and reduces ROS (Lekatz *et al*. 2010). In treatments of C and D, increase in progesterone levels after the ovulation period (three weeks after mating) were evident, increase in blood progesterone concentration may be due to an increase in levels of available cholesterol which is a precursor for progesterone biosynthesis by corpus luteum.

In fact, cholesterol is used as precursor of progesterone and increases follicle growth and levels of estradiol, insulin and IGF-1 (Scaramuzzi *et al*. 2006). Ziaei (2014) showed that selenium and vitamin E supplementation have significant effect on lipoprotein profiles and plasma cholesterol of Raeini goats. Whereas Baiomy and Suliman (2012) showed that interactions between vitamin E and selenium inhibit fatty acids oxidation, leading to the decrease of plasma cholesterol.

Insulin is a known factor for follicular performance in some animal species and has some important roles such as increasing steroidogenesis in granulosa cell, cell mitosis, morphological differentiation in follicles and increasing progesterone concentration in follicular fluid (Scaramuzzi *et al*. 2006). The addition of a growth hormone and insulin to bovine granulosa cells increases progesterone production (Savion *et al*. 1981).

Increased insulin can be harmful for oocyte competence and blastocyst survival (Adamiak *et al*. 2005). Increasing insulin concentration causes growth and development of follicles, increases ovulation rate and finally improves offspring (Scaramuzzi *et al*. 2006).

Flushing diet activates insulin receptors by increasing blood glucose, which can increase glucose metabolism and ROS production. This increase in ROS causes insulin resistance, and so decreases the insulin level and glucose absorption (Fridlyand and Philipson, 2006).

Juniper *et al*. (2006) showed that using selenium in diet increases selenium concentration of blood, milk and tissues. It is defined that selenium has a role in thyroideroxidase as selenoenzyme which acts in iodinating globulin and prevents damaging thyroid epithelial membrane (Hefnawy and Tortora-Perez, 2010). Selenium is necessary for some biological activities related to thyroxin and thyroid for metabolism regulation (Hefnawy and Tortora-Perez, 2010). Iodothyronine deiodinase is another important selenoproteins. This enzyme regulates thyroxin conversion to biologically active 3, 3´, 5-triiodothyronine or to reverse triiodothyronine. Thyroid hormone increases gluconeogenesis, therefore increases serum glucose concentration (Brenta, 2011). Glucose is one of the most important ingredients for a suitable reproductive performance (Hess *et al*. 2005). Glucose infusion is effective on estradiol secretion in follicular phase and is accompanied by folliculogenesis stimulation and increasing ovulation rate (Scaramuzzi *et al*. 2006). It has been shown that high glucose concentration during estrus especially at the beginning of reproduction cycle has high correlation with the number of offspring and ovulation rate (Scaramuzzi *et al*. 2006; Daghighkia *et al*. 2012).

It seems that increasing blood total protein level is not owing to selenium and vitamin E supplementation, because total protein level also increased in group B. Therefore, increasing protein was due to flushing diet. The result of this study contrasts with the results of Ziaei (2014) and El-Shahat and Abdel Monem (2011) which showed that selenium and vitamin E supplementation as a mixture increased blood total protein, albumin and globulin concentration.

Ziaei (2014) reported that increasing total protein increased reproduction efficiency as changing mating season

to an earlier time, decreasing unfertile ewes, increasing estrus and lambing birth weight. Hoon *et al*. (2000) reported that diets containing protein supplements affected the fertility rate at 400 g/d and high levels of protein absorption increased follicle-stimulating hormone (FSH) pulses and improved the fertility rate.

Naqvi *et al*. (2012) reported that a significant increase in the levels of blood urea was related to the increased rate of protein catabolism in the body. However, it seems that increasing blood urea level is not due to selenium and vitamin E supplementation, because BUN level also increased in group B (Table 5).

These results are consistent with those of El-Shahat and Abdel Monem (2011) that reported selenium and vitamin E supplementation as a mixture did not have significant effect on blood urea level compared to control group. However, Jack and Desai (1977) reported that adding selenium and vitamin E to diet increases blood urea level. Increasing urea may increase energy consumption for urea genesis. Increasing BUN through decreasing uterine pH leads to $PGF_{2\alpha}$ releasing and pregnancy failure and decrease ovulation rate (Scaramuzzi *et al*. 2006).

Balicka-Ramisz *et al*. (2006) study on Polish Merino ewes showed that sodium selenite increases birth weight of lambs. Lekatz *et al*. (2010) showed that increasing dietary selenium increases cell proliferation of cotyledon tissue of placenta and thus reduce the possibility of intrauterine growth restriction (Mistry *et al*. 2008). On the other hand, it is demonstrated that selenoproteins increases conversion of thyroxin to triiodothyronine, so affects the metabolism of mother and fetus and finally increases fetus weight (Pappas *et al*. 2008). However, Sanchez *et al*. (2008) showed that selenium did not have significant effect on birth weight.

CONCLUSION

It is concluded that the inclusion of organic and inorganic selenium with vitamin E in the flushing diets of Ghezel ewe increased metabolites and hormones related to the reproductive performance especially cholesterol, glucose, progesterone and estrogen with a consequent improvement in their fertility and lambing rate compared to the control and barley flushing groups. But it seems that the inclusion of inorganic and organic selenium supplementation in flushing diet has similar results on blood metabolites and hormones related to reproductive performance.

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