REPRODUCTIVE INDEXES AND MILK PERFORMANCE OF EWES TREATED WITH DIFFERENT ANTIOXIDANT DURING TRANSIT PERIOD PRIOR TO BREEDING SEASON.

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ABSTRACT

Aim of this study was to evaluate the effect of treatment with vitamins A (VA), C (VC) and AD_3E, selenium (Se) or their combination before September mating season, on productive and reproductive performances of crossbred ewes and their offsprings. Total of 72 crossbred ewes (3-4 y of age and 44.19 kg live body weight, LBW) were used in this study. Ewes in the 1st group (G1) served as a control without treatmant, while those in treatment groups were treated orally with VA (5000 IU/ewe, G2) or injected with 1 ml of AD_3E (G3), Se (G4) or VC (G5), or with the same treatments of their combination (G6). LBW of ewes and lambs as well as placental weight (PW) were recorded. Milk yield was individually recorded and milk samples were taken for chemical analysis during 45 d of the suckling period. Blood samples were collected for determination of progesterone (P4) concentration during 50 post-mating in ewe serum, and of concentration of total proteins (TP), albumin (AL), globulin (GL) and cholesterol in lamb serum at weaning. Reproductive indexes including estrus rate (ER), fertility rate (FR), fecundity rate (FCR) and prolificacy rate (PR) were also calculated. Mortality rate (MR) of lambs was recorded at weaning.

Results show that the effect of treatment on LBW of ewes, PW of ewes or LBW of lambs at birth or weaning based on sex of lambs and type of birth, milk yield, milk contents, except fat percent, P4 concentration, and average LBW of lambs at lambing or weaning based on sex and type of birth was not significant. Average PW of all lambs was lower (P<0.05) in G3 and G2 than in G1, G4, G5 and G6. Milk fat percent increased (P<0.05) in G2 and G3 (5.04 and 5.43%), while it did not differ in G4, G5 and G6 (4.28, 4.62 and 4.29%) compared with G1 (4.47%). ER was 0.0, 25, 33.3, 16.7, 25 and 16.7% (P<0.05) during the transit period and 100, 83.4, 91.6, 100, 100 and 100% (P<0.05) at the end of breeding season in the treatment groups, respectively. FR was 83.3, 91.7, 75.0, 75.0, 91.7 and 91.7% (P<0.05), FCR was 125, 133, 100, 92, 108 and 108%, and PR was 150, 145, 133, 122, 118 and 118% in the six groups, respectively. Ewes in G2 showed the highest (P<0.05) twin births and the lowest single type (62.5:37.5%, P<0.05) versus an opposite trend in other treatment groups (G3-G6). MR of lambs at weaning was 0% in G1, G2 and G6 versus 8.33, 9.10 and 7.69% in G3, G4 and G5, respectively. Lambs of G6 showed the highest (P<0.05) concentration of TP, AL, GL and cholesterol.

In conclusion, The treatment with vitamins A (VA), C (VC) and AD_3E, selenium (Se) or their combination before September mating season improvement the productive and reproductive performances of crossbred ewes and their offsprings.

INTRODUCTION

Oxidative stress is experienced by the living organisms from both exogenous and endogenous sources in terrestrial as well as marine environments (Lesser, 2006). Production of oxidants together with the ability of organisms to respond to oxidative stress is intricately connected to ageing

and life span (Finkel and Holbrook, 2000). Oxidative stress is involved in the etiology of defective embryo development. Embryo metabolism generates reactive oxygen species (ROS) *via* several enzymatic mechanisms, which may alter most of cellular molecules and induce developmental block and retardation (Guérin *et al.*, 2001).

In mammals, ROS are essential factors of cell division, differentiation and growth, notably during prenatal stage. The ROS may be produced at early developmental stages, necessitating anti-oxidative defense mechanisms. Free radicals and ROS play significant and diverse roles in reproductive biology (Riley and Behrman, 1991) and have been implicated in fetal dysmorphogenesis, abortions and intrauterine growth restriction (Agarwal *et al.*, 2006). Under normal conditions, the developing embryo is capable of coping with oxidative stress but this may fail under various pathological conditions, leading to embryonic damage (Zaken *et al.*, 2000).

The corpus luteum (CL) at early pregnancy may be rescued from luteolysis through increasing activities of key antioxidant. The CL throughout pregnancy may be linked to ROS generated continuously in the steroidogenically active luteal cells, and may be involved in the maintenance of luteal steroidgenic activity and cellular integrity (Al-Gubory *et al.*, 2004).

It could be hypothesized that antioxidants and oxidative stress may influence the timing and maintenance of a viable pregnancy as threshold levels of female oxidative stress exist, depending on anatomic location and stage of preconception (Ruder *et al.*, 2008). The stress factors induce an inhibition of progesterone synthesis in repeat breeder cows (Rizzo *et al.*, 2007). Seasonal periods of heat stress reduce milk yields and fertility in lactating dairy cows. Heat stress during follicular recruitment suppresses subsequent growth to ovulation, accompanied by decreased LH receptor level and estradiol synthesis in the follicles (Özawa *et al.*, 2005; Roth, 2008). The antioxidant function could, at least in part, enhance immunity and increase animal production efficiency (McDowell, 2002). Short term supplementation of β-carotene to goats may result in enhanced ovarian function and progesterone synthesis (Arellano-Rodriguez *et al.*, 2008). It was reported that vitamin E-selenium injections given before ewe mating significantly increased the incidence of estrus response, fecundity rate and lamb body weight at 60 days of age (Koyuncu and Yerlikaya, 2007). Also, supplementation with vitamin E (VE) and selenium (Se) may be beneficial in reducing ROS in dystocia-affected buffaloes in the immediate post-partum period (Sathya *et al.*, 2007).

The dietary and tissue balance of antioxidant nutrients is important in protecting tissues against free radical damage. Vitamins and trace minerals have dual effects: (a) control of metabolic pathways or gene expression and (b) ROS trapping activity. Their deficiency may induce high rates of ROS production resulting in different disorders of gestation (Aurousseau *et al.*, 2006). These deficiencies may also be due to different kinds of stress (McDowell *et al.*, 2007). Feeding calves on VE and/or vitamin C is associated with alterations in the concentrations of other antioxidants (Cusack *et al.*, 2005).

The aim of this study was to evaluate the effect of treatment with antioxidants such as vitamins A, C and AD_3E and trace mineral such as Se, or their combination during August, before September mating season, on productive and reproductive performances of crossbred ewes. Also, growth parameters, mortality rate and blood parameters of produced lambs were studied.

MATERIALS AND METHODS

This study was carried out at Sakha Experimental Station, located in the northern part of the Delta, belonging to the Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, in cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University.

Animals:

During August, before September mating season (according to the system of APRI), total of 72 crossbred (1/2 Finnish Landrace x 1/2 Rahmani) ewes having 3-4 years of age and averaged 44.19±0.19 kg live body weight (LBW) were used in this study. Ewes were kept under similar management conditions and housed in collective pens. Water and mineral salt were permanently available.

Feeding system:

Ewes were fed a basal ration consisting of 25% concentrate feed mixture (CFM) beside 75% fresh berseem (FB, *Trifolium alexandrinum*) during winter feeding (November-April) or 50% CFM and 50% berseem hay (BH) during summer feeding (May and June). The CFM consisted of cotton seed cake, linseed cake, yellow corn, wheat bran, molasses, calcium chloride and common salt. Ewes were fed according to NRC feeding requirements (**NRC, 2001**). The daily feed amounts of 1.250 kg CFM were offered once daily at 8 a.m. plus 4 kg FB or 1.3 kg BH were offered twice daily at 11 a.m. and 3 p.m. for each doe. Chemical composition of different feedstuffs is presented in Table (1). The amounts of CFM were adjusted according to the changes of body weight of ewes. The CFM and FB or BH were chemically analyzed according to the methods of AOAC (1990).

Table (1): Chemical composition of different feedstuffs.

Item	DM	Chemical composition (on DM basis)					
		OM	CP	CF	EE	NFE	Ash
CFM	89.85	87.77	14.42	7.18	2.42	63.78	12.54
Fresh berseem	17.26	88.79	16.68	21.98	2.38	48.65	11.44
Berseem hay	86.49	89.39	12.75	27.84	3.43	45.38	10.87

CFM: Concentrate feed mixture.

Treatments:

At middle of August, the experimental ewes (n=72) were divided into six groups (12 in each) according to their age and LBW. Ewes in the 1st group (G1) served as a control group without treatment. However, ewes in the 2nd, 3rd, 4th and 5th groups were treated orally with vitamin A (A-VETONE, capsule of 5000 IU of vitamin A, G2) or injected with AD_3E (ADEVET, each 1 ml

contained 100.000 IU vitamin A, 20.000 IU vitamin D_3 and 20 mg vitamin E, G3), selenium (Tocosel-Forte, each 1 ml contained 150 mg dl-alpha tocopheryl acetate and 1.1 mg, sodium selenite, G4) and vitamin C (Sevagi, each 1 ml contained 0.2 g vitamin C and 0.2 g Anlagin, G5). While, ewes in the 6th group were received a combined of oral vitamin A plus injections of vitamin AD_3E, selenium and vitamin C (G6).

Body and placental weights:

Ewes in all groups were weighed at August, September (breeding season) and post-lambing. Also, placental weight was recorded at lambing. Lambs produced from each group were weighed at birth and weaning according to sex and type of births.

Milking and milk samples:

During the day of milking, lambs were removed from their dams and allowed to suckle other ewes. During the suckling period, 5 ewes of each group were milked by hand every two weeks. Hand milking was carried out twice at the day of milking (6 a.m. and 5 p.m.). Milk yield was individually recorded and milk samples were taken for chemical analysis. The total milk yield for a ewe and chemical composition of milk at the day of milking was considered to represent average daily milk yield (ADMY) during the previous two weeks (15, 30 and 45 days of the suckling period), then ADMY and milk composition was calculated for the first 45 days of post-partum for each ewe.

The morning milk from ewes of each breed group cooled at 5^oC, added to the evening milk, well mixed and representative samples were taken. Milk samples were stored at -5^oC till analyses for fat, protein, lactose, total solids and solids not fat using Milko-Scan (133B N. Foss Electric, Denmark).

Blood sampling:

On day 5, 30, 40 and 50 post-mating, blood samples were taken from the jugular vein of all ewes in each group into vacotainer tubes. After clotting, blood samples were centrifuged at 15 g for 15 min, and then blood serum was separated and stored at $-20\ ^oC$ until progesterone (P4) assay.

Quantitative determination of P4 in blood serum was carried out using progesterone radioimmunoassay kit (Catalog No. 1188 manufactured by Immunotech, France). The assay is based on competition reaction (Bojanic *et al.*, 1991). Samples (50 μl) were incubated 1 h with I^{125} labeled progesterone (500 μl), as tracer, in antibody-coated tube. After incubation the liquid contents of the tubes are aspirated and bound radioactivity is measured to determine progesterone in serum using automatic Mini-Gama counter (LKB 1275, USA). The sensitivity of the P4 assay is 0.03 ng/ml, while coefficient of variation is 4.3% for both progesterone intra- and inter-assay, respectively.

Also, blood samples were taken from 5 lambs of each group at weaning for determination of total proteins (Henry, 1964), albumin (Hill and Weels (1983) and total cholesterol (Rolschlau, 1974) concentrations in blood serum. However, globulin concentration was calculated.

Suckling period:

After parturition, lambs were allowed to suckle their dams up to reach a minimum age of 8 weeks and a minimum body weight of 11-12 kg when

they have been weaned. Newborn male and female lambs produced from each experimental group were kept at the same feeding system of their dams. Lambs were fed according to the NRC (2001) allowances on concentrate feed mixture (CFM) and fresh berseem (FB) up to May 2013 and CFM and berseem hay (BH) up to the end of the experiment. Mortality rate of lambs was recorded at weaning.

Reproductive performance:

Reproductive traits of ewes in response to different treatment were recorded including estrus rate (ER), fertility rate (FR), fecundity rate (FCR) and prolificacy rate (PR) as the following:

ER%=No. of ewes showing estrus during season/joined ewes x 100.

FR%= No. of lambed ewes / joined ewes x 100.

FCR%=No. of born lambs/ joined ewes x 100.

PR= No. of born lambs /lambed ewes x 100.

(Janadeen and Hafez, 1993)

Statistical analysis:

Data obtained in this study were subjected to statistical analysis using General Linear Models Procedures (GLMP) adapted by SPSS for windows (2004) for user's guide. Duncan Multiple range test of SPSS programme was done to determine the degree of significance between the means at P<0.05.

RESULTS AND DISCUSSION

Changes in live body weight of ewes:

Data presented in Table 2 show that the effect of treatment on LBW of ewes in August and September, and post-lambing was not significant. During the interval from August and September, LBW of ewes was higher in G1 than in other treatment groups.

Such result indicated no effect of different treatments on LBW of ewes pre- or during breeding season.

Table (2): Effect of treatment on live body weight (kg) of ewes in August and September, and post-lambing.

Treatment group	August	September*	Post-lambing
G1 (Control)	44.75±1.85	47.50±2.20	47.00±2.46
G2 (VA)	43.25±1.28	44.91±1.11	44.87±2.15
G3 (AD$_3$E)	44.83±1.08	45.75±1.29	45.33±1.41
G4 (Se)	42.83±1.22	44.33±1.42	43.50±1.88
G5 (VC)	44.08±1.27	45.85±1.59	44.90±1.29
G6 (Combination)	45.41±0.92	46.58±0.97	43.33±1.52

* Breeding season. All group differences are not significant at P≥0.05.

Placental weight at lambing:

Results shown in Table 3 revealed significant effect of treatment on average placental weight of ewes at lambing. Only, placental weight of ewes in G3 treated with vitamin AD$_3$E was significantly (P<0.05) lower than that in

the control group (G1), G4, G5 and G6, but did not differ significantly from that in G2.

Table (3): Effect of treatment on placenta weight (g) of ewes at lambing.

Item	G1 (Control)	G2 (VA)	G3 (AD$_3$E)	G4 (Se)	G5 (VC)	G6 (Combin.)
Mean	512±51.1[a]	378±34.5[ab]	310±52.7[b]	505±28.2[a]	457±53.4[a]	496±45.7[a]
Placental weight (g/ewe) as affected by sex of born :						
Male	533±36.2	415±82.1	347±81.4	542±37.8	490±85.1	591±64.7
Female	487±101	349±19.7	281±74.8	468±18.1	412±66.5	437±47.4
Placental weight (g/ewe) as affected by type of birth :						
Single	416±102	312±0.00	347±82.1	495±34.4	386±46.2	523±19.8
Twins	560±58.2	397±41.0	281±74.9	535±0.00	600±111	480±1.43

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05.

The observed reduction in placental weight of ewes treated with AD$_3$E was not explained in the literature. However, this reduction was associated with decreasing of lamb body weight produced from ewes in G3, which will be discussed latter in Table (3).

It is of interest to note that the effect of treatment on placental weight of ewes was not significant when placental weight was compared among groups based on sex of lambs and type of births (Table 3). This finding may indicate poor relationship between placental weight of ewes and each of sex of lambs and type of births, regardless treatment.

Milk production:

Results presented in Table 4 revealed insignificant effect of treatment on average daily milk yield of ewes. However, among all milk contents, only fat percent in milk of ewes in G2, G3 significantly (P<0.05) increased as compared to the control milk (G1). Meanwhile, milk of ewes in G4, G5 and G6 did not differ significantly than in G1.

It is worthy noting that ewes in G3 treated with AD$_3$E showed the highest milk yield and fat content, indicating the highest fat yield from ewes in this group as compared to other treatment and control groups. Such trend may be due to synergistic effect of vitamins A, D and E.

Estimates of the amount of milk produced by lactating ewes provide information for the implementation of optimum management and feeding strategies for ewe and their lambs (Cardellino and Benson, 2002). Generally, milk yield of dairy ewes was affected by number of suckling lambs during pregnancy or lactation (Treacher, 1978), type of birth, single, twins or triplets (Snowder and Glimp, 1991), lambing season (Hamdon, 2005) or lactation period length (Morsy, 2002).

Table (4): Effect of treatment on average daily milk yield (g) and milk composition of ewes during up to 45 days of lactation.

Treat. group	Milk yield (g/h/d)	Milk composition (%)				
		Fat	Protein	Lactose	SNF	Total solids
G1 (Control)	615 ±0.012	4.47 ±0.507[b]	4.66 ±0.253	5.47 ±0.664	10.71 ±0.500	15.19 ±0.832
G2 (VA)	625 ±0.015	5.04 ±0.960[a]	4.51 ±0.497	5.30 ±0.470	10.48 ±0.928	15.50 ±1.497
G3 (AD$_3$E)	638 ±0.009	5.43 ±0.681[a]	4.54 ±0.452	5.10 ±0.669	10.27 ±0.807	15.50 ±1.194
G4 (Se)	614 ±0.011	4.28 ±0.736[b]	4.48 ±0.441	5.20 ±0.665	10.28 ±0.651	14.60 ±1.263
G5 (VC)	615 ±0.011	4.62 ±0.584[b]	4.45 ±0.287	5.59 ±0.315	10.68 ±0.258	15.27 ±0.730
G6 (Combin.)	613 ±0.013	4.29 ±0.457[b]	4.28 ±0.435	5.66 ±0.475	10.63 ±0.882	14.71 ±0.452

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05.

Ovarian and estrous activity:
Estrous rate:

Results presented in Table 5 revealed that ewes in all treatment groups showed estrous activity during the transit period prior to breeding season (16-30 August), while none of the control ewes exhibited estrus signs. Comparing among treatment groups, ewes in G3 treated with vitamin AD$_3$E showed significantly (P<0.05) higher estrous rate (33.3%) than those treated with Se (G4, 16.7%) or with a combination of vitamins and Se (G6, 16.7%), but did not differ significantly from those in G2 treated with vitamin A or C (25% for each).

During the 1st half of the breeding season (1-15 September), only ewes in G6 (75%) and G4 (66.7%) showed significantly (P<0.05) higher estrus rate than in the control ewes (G1, 50%). However, ewes in other treatment groups did not differ in estrus rate from that in the control (G1). This means that, up to the 1st half of breeding season, ewes in all treatment groups showed higher estrus rate than the control ewes, being 91.7, 83.3, 82.7, 83.3 and 66.7% in G6, G5, G4, G3 and G2 as compared to 50% in G1, respectively.

During the 2nd half of the breeding season (16-30 September), the control ewes showed the highest estrus rate (50%) as compared to 16.7% in G2, G4, and G5, and 6.3% in G3 and G6. Interestingly to recorded that, all ewes (100%) in G1, G4, G5 and G6 exhibited estrous activity and mated at the end of breeding season versus 83.4 and 91.6% in G2 and G3, which may suggest inverse effect of vitamin A or AD$_3$E on estrous activity of ewes during breeding season

Table (5): Effect of treatment on estrus/mating rate of ewes pre- and during breeding season.

Treatment group	N	Ewes showing estrus/mating						Mated ewes	
		16-30 Aug.		1 -15 Sept.		16-30 Sept.		16 Aug.-30 Sept.	
		n	%	n	%	n	%	n	%
G1 (Control)	12	0	0.00[c]	6	50.0[bc]	6	50.0[a]	12	100[a]
G2 (VA)	12	3	25.0[ab]	5	41.7[c]	2	16.7[bc]	11	83.4[b]
G3 (AD$_3$E)	12	4	33.3[a]	6	50.0[bc]	1	8.30[c]	11	91.6[ab]
G4 (Se)	12	2	16.7[b]	8	66.7[a]	2	16.7[bc]	12	100[a]
G5 (VC)	12	3	25.0[ab]	7	58.3[b]	2	16.7[bc]	12	100[a]
G6 (Combin.)	12	2	16.7[b]	9	75.0[a]	1	8.3[c]	12	100[a]

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05. N: total number of ewes in each group.

In sheep, there is a significant seasonal variation in the responsiveness of the hypothalamic-hypophysis axis to the negative feedback action of estrogen on LH secretion, which appears to be mainly dictated by the photoperiod (Herbison, 1995). During seasonal anestrus, LH continues to be released but with lower frequency than during the follicular phase of the cycle during the breeding season (Rosa and Bryant, 2003). The low level of estrogen was observed during seasonal anestrus, resulting in the short and long-term suppression of GnRH and gonadotrophin release (Clarke et al., 2009) and a marked change of hypothalamic responsiveness to estradiol alone can be observed (Webster and Haresign, 1983).

The transition from anestrus to reproductive activity is gradual with the occurrence of an initial short cycle as the first CL regresses prematurely 5-6 days after its formation. It is only after the 1st ovarian cycle that behavioural estrus is exhibited (Rosa and Bryant, 2003). Estrogen is also responsible for estrous behaviour in all species including sheep; however, small amounts of progesterone priming are necessary for the induction of estrus in sheep during the first reproductive cycle of the breeding season (Herbison, 1995; Stellflug et al., 1997). Absence of ovulation during seasonal anestrus is thought to be due to reduced frequency of LH pulses (Wallace et al., 1986).

The beneficial effects of different treatments were pronounced on exhibiting estrous activity during transited period ewes. In particular ewes treated with vitamins A or AD$_3$E. In accordance with the present results, Beta-carotene supplementation positively affects ovarian activity in goats (Arellano-Rodriguez *et al.*, 2007). Also, vitamin E-selenium injections given before mating significantly increased the incidence of estrus in ewes (Koyuncu and Yerlikaya, 2007).

Progesterone (P4) profile:

Data of P4 concentration in blood serum of ewes during different days post-mating (Table 6) showed significant (P<0.05) increase in P4 concentration with advancing post-mating day, being the highest on day 50 post-mating. This trend of increase was similar in all groups with similar P4 concentration in all groups at each sampling day.

Table (6): Effect of treatment on progesterone concentration in serum of ewes at different days post- mating during breeding season.

Treatment group	Post-mating (day)			
	5	30	40	50
G1 (Control)	1.103±0.02[d]	12.651±0.145[c]	14.813±0.314[b]	18.317±0.214[a]
G2 (VA)	1.313±0.01[d]	12.632±0.278[c]	14.212±0.242[b]	18.738±0.252[a]
G3 (AD$_3$E)	1.214±0.05[d]	12.264±0.124[c]	14.333±0.258[b]	18.812±0.334[a]
G4 (Se)	1.402±0.02[d]	12.581±0.226[c]	14.462±0.365[b]	18.621±0.462[a]
G5 (VC)	1.211±0.03[d]	12.862±0.157[c]	14.126±0.275[b]	18.701±0.341[a]
G6(Combin.)	1.121±0.05[d]	12.131±0.148[c]	14.135±0.285[b]	18.562±0.225[a]

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05.

It was observed that P4 concentration was above 1 ng/ml starting from 5 days post-mating, which may indicate incidence of pregnancy or ovarian activity in term of presence of CL at this early period post-mating in all groups even in the control ewes.

The positive relationship between P4 level and post-insemination day confirmed the statement of Mukasa-Mugerwa and Viviani (1992), who recorded positive correlation between P4 levels and the gestation stage. They observed that P4 values on the 40th day of pregnancy were higher than the peak values during the lutheal stage of the sexual cycle, which is due to an extraovarian source of progesterone (placental synthesis). Also, in support of this notion are the established significantly higher (P<0.01) P4 concentrations in pregnant vs. non-pregnant sheep. Also, Davies et al. (2006) found a positive correlation between the P4 levels and the surface of CL in sheep with different ovulation values.

During seasonal anestrus, P4 levels remain virtually undetectable, while FSH concentrations do not seem to vary from those found during the breeding season. Although P4 levels are low and no gonadotrophin surge occurs, neither the ovary nor the hypothalamus are inactive. The LH pulse system, although active, is compromised as GnRH and LH pulse frequencies are low. The mature follicles present during anestrus are capable of producing estrogen which now has greater ability to suppress LH release. (Rosa and Bryant, 2003).

Reproductive performance:

The superiority of ewes in G6 treated with the combination treatment in term of estrous/mating rate during transit period and during breeding season (Table 7) was indicated also by expressing significantly (P<0.05) the highest fertility rate (100%), whereas all ewes in this group showed estrus, mated and lambed (Table 7).

Maximizing the fertility rate of ewes in G2 was associated with fecundity rate (133%) and significantly (P<0.05) higher prolificacy rate (145%) than that of the other treated groups. Ewes in G2, G5 and G6 had the similar fertility rate (91.67%), with significantly differences than G1, G3 and G4 (83.33, 75.0 and 75%, respectively) and showed lower fecundity rate (92%, P≥0.05) on G4 and lower prolificacy rate (122%, P<0.05) (Table 7).

It is worthy noting that ewes in G3 and G4 treated with vitamin AD_3E and Se showed similar fertility rate (75%), and increased fecundity rate (100% and 92% P≥0.05) and prolificacy rate (133 and 122%, P<0.05).

Table (7): Effect of treatment on reproductive traits of ewes during breeding season.

Group	N[1]	Mated ewes	Lambed ewes[2]	Fertility rate %[2/1]	Born lambs[3]	Fecundity rate (%)[3/1]	Prolificacy rate (%)[3/2]
G1 (Control)	12	12	10	83.33[b]	15	125[ab]	150[a]
G2 (VA)	12	11	11	91.67[a]	16	133[a]	145[a]
G3 (AD₃E)	12	11	9	75.00[c]	12	100[bc]	133[b]
G4 (Se)	12	9	9	75.00[c]	11	92[c]	122[c]
G5 (VC)	12	12	11	91.67[a]	13	108[b]	118[c]
G6 Comb.)	12	12	11	91.67[a]	13	108[b]	118[c]

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05. N: total number of ewes in each group.

On the other hand, treatment of ewes in G3 (AD_3E) and G4 (Se) failed to increase fertility and fecundity rates compared with controls (G1) and other treatment groups, but significantly (P<0.05) increased prolificacy rate as compared to that in G5 and G6. It is of interest to note that increasing prolificacy rate in G2 than in G6, inspit the nearly similar number of borns was attributed to increasing fertility rate in G6 than in G2 (Table 7).

The oxidative stress may occur at different sites, different times, and by different mechanisms in the animal system. Levels of the antioxidant vitamins are associated with fertility and production levels in ruminants including sheep. These antioxidants need to be replenished/regenerated or supplemented at appropriate timings in order to optimize the ruminant health and productive/reproductive performance (Nayyar and Jindal, 2010). The reactive oxygen species production during the oxidative stress is responsible for the ovarian pathology and uterine disorders affecting the female reproduction, with the poor fertility outcome (Qureshi *et al.* (2010). Also, antioxidant defenses in the female reproductive tract may have some regulatory role in fertility (Jean-François and Marc-André, 2001).

Activities of antioxidant enzymes, superoxide dismutase, nitric oxide synthase, glutathione reductase and glutathione-S-transferase in sheep CL are subjected to major changes during the estrous cycle (Al-Gubory *et al.*, 2005). The mRNA expression and distribution of enzyme activities e.g. glutathione peroxidase, superoxide dismutase and catalase in the bovine oviduct during the estrous cycle indicated their physiological role in the events leading to successful fertilization and implantation *in vivo* (Jérôme and Jean-François, 2003).

The obtained enhancement of fertility of ewes in G6 may be attributed to the synergestic effects of the combined of differet vitamins and SE. The naturally occurring antioxidants: vitamin E, β-carotene and vitamin C are known to ameliorate the oxidative stress generated during various physiological and pathological conditions (Nayyar and Jindal, 2010). Since vitamin C works in cooperation with other antioxidants, its administration in disease which are followed by oxidative stress is more effective when used in

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combined preparations. Qureshi et al. (2010) reported that with combined treatment of vitamin C+E there was complete protection in the entire component of the ovaries and there was also significant increase in number of different types of developing follicles in deficient animals. Also, vitamin C has been associated with fertility in human and small range of other mammals (Millar 1992). Moreover, Se has been amply documented to significantly enhance animal development, a number of metabolic processes, and the reproductive success (Grela and Sembratowicz 1997). Administration of Se improves reproductive performance in ewes (Gabryszuk and Klewiec, 2002).

In agreement with the obtained improvement in fecundity rate of ewes in G2 treated with vitamin A, Arellano-Rodriguez *et al*. (2007) found that β-carotene supplementation, as a source of vitamin A, positively affects ovarian activity in goats. In this respect, there is feed dependent accumulation of β-carotene in bovine luteal tissue (Arikan and Rodway, 2001) and β-carotene is spread over the entire volume of luteal cells with higher levels occurring at distinct sites, including the surface (Arikan *et al*., 2002). Also, Vitamin E-selenium injections given before mating significantly increased fecundity rates in ewes (Koyuncu and Yerlikaya, 2007).

Lambing performance:

Sex ratio, type of births and mortality rate:

Results presented in Table 8 showed that the effect of treatment on sex ration and type of birth of produced lambs was significant (P<0.05). Ewes G6 significantly (P<0.05) produced more less males and more females (38.4:61.5%).

Regarding the type of birth, ewes in G2 showed significantly (P<0.05) the highest twin births and the lowest single type (62.5:37.5%) versus an opposite trend in other treatment groups (G3-G6). However, the control ewes showed 50% for each type of birth (Table 8).

Table (8): Effect of treatment on sex ratio, type of birth and mortality rate of lambs.

Treatment group	N	Born lambs		Type of birth		MR (%)
		♂ n(%)	♀ n(%)	Single (%)	Twine (%)	
G1 (Control)	15	8 (53.3)^{ab}	7 (46.7)^{bc}	5 (33.3)^{b}	5 (66.7)^{b}	0.00
G2 (VA)	16	8 (50.0)^{b}	8 (50.0)^{b}	6 (37.5)^{b}	5 (62.5)^{a}	0.00
G3 (AD$_3$E)	12	5 (41.6)^{bc}	7 (58.3)^{ab}	6 (50.0)^{ab}	3 (50.0)^{c}	8.33
G4 (Se)	11	5 (45.5)^{bc}	6 (54.5)^{ab}	7 (63.6)^{a}	2 (36.4)^{cd}	9.09
G5 (VC)	13	8 (61.5)^{a}	5 (38.4)^{c}	9 (69.2)^{a}	2 (30.8)^{d}	7.69
G6(Combin.)	13	5 (38.4)^{c}	8 (61.5)^{a}	9 (69.2)^{a}	2 (30.8)^{d}	0.00

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05. N: Number of lambed ewes. MR (%): Mortality rate.

It is of interest to note that mortality rate of produced lambs at weaning was 0% in G1, G2 and G6 versus 8.33, 9.09 and 7.69% in G3, G4 and G5, respectively (Table 7).

Lamb survival until weaning reflect milk production ability of ewes (Snowder and Glimp, 1991). The neonatal period is very decisive in the

rearing of lambs and during this period, mortality is a major factor limiting profitability in sheep farming. Immediately after birth, lambs are characterized by homeostatic physiological variability, as shown in previous study on different physiologic parameters in these specie during the first 4 weeks postpartum (Piccione et al., 2006; Piccione et al., 2007).

Basser and Gay (1994) found that the mortality rate was lower in calves with a high than low serum immunoglobulin (IgG) concentration. Also, Solones et al. (1995) reported a higher mortality rate and lower serum immunoglobulin concentration in lambs fed artificial colostrum compared to those fed maternal colostrum. Colostrum is an important source of IgG , which is 100-fold higher in colostrum than that in normal milk.

Live body weight of lambs:

Concerning LBW of lambs, data presented in Table (9) showed that the differences in average LBW of lambs at lambing between all treatment groups and the control one were not significant, but the differences were recorded between treatment groups. Lambs in G4, G5 and G6 were significantly heavier than those in G2 and G3. However, the differences in average LBW of lambs among groups based on sex and type of birth at lambing or weaning were not significant (Table 9).

In general, lamb body weight gains until weaning reflect milk production ability of their dams (Snowder and Glimp, 1991). It was reported that Se is transported to the offspring (lambs) along two pathways: via the placenta during the foetal stage, and with the colostrum at the neonatal phase. Selenium, even at low concentration in the ewe's body, is efficiently passed on to the foetus (Koller et al. 1984). Also, Cuesta et al. (1995) found that parenteral administration of Se significantly increases its concentrations in the colostrum and milk.

According to some authors (Horton, et al. 1986), Se improves health and increases body weight of the neonatal lambs. This was proved in lambs of ewes treated with Se alone (G4) or Se in combination with vitamins A, AD_3E and C (G6). Also, Mauka et al. (1998) demonstrated the blood serum Se content in the sheep fed Se+vitamins-enriched feed to be by 25% higher (increase from 0.51 to 0.63 µmol/l), the increase in the liver amounting to 35%. The Se level increase was translated into lamb body weight gains: in month 5 of the experiment, the treatment lamb body weight was by 28.6 % higher than that of the untreated lambs. Moreover, administration of Se improved daily weight gain of lambs (Gabryszuk and Klewiec, 2002) and vitamin E-selenium injections given before mating significantly increased lamb body weight at 60 days of age (Koyuncu and Yerlikaya, 2007).

Table (9): Effect of treatment on average live body weight (kg) of lambs at lambing and weaning.

	G1 (Cont.)	G2 (VA)	G3 (AD$_3$E)	G4 (Se)	G5 (VC)	G6 Combin.
Average LBW (kg) of total born lambs:						
At lambing	3.10^a ±0.20	2.96^{ab} ±0.16	2.77^b ±0.21	3.50^a ±0.25	3.19^a ±0.16	3.26^a ±0.28
At weaning	15.73 ±0.95	14.16 ±0.97	15.27 ±1.20	16.87 ±0.51	16.46 ±1.07	14.67 ±1.11
Average LBW (kg) of ram lambs:						
At lambing	3.25 ±0.28	3.23 ±0.00	3.40 ±0.24	3.62 ±0.37	3.31 ±0.26	3.30 ±0.48
At weaning	15.75 ±1.35	14.50 ±1.65	17.40 ±1.24	17.75 ±0.70	17.40 ±1.65	15.37 ±1.01
Average LBW (kg) of ewe lambs:						
At lambing	2.92 ±0.29	2.70 ±0.23	2.25 ±0.11	3.37 ±0.37	3.00 ±0.00	3.25 ±0.36
At weaning	15.71 ±1.45	14.00 ±1.28	13.50 ±1.70	16.00 ±0.70	15.87 ±1.65	13.25 ±2.80
Average LBW (kg) of single lambs:						
At lambing	3.14 ±0.12	3.53 ±0.15	3.24 ±0.11	3.16 ±0.14	3.01 ±0.14	3.37 ±0.12
At weaning	16.00 ±1.81	16.66 ±185	17.40 ±1.24	16.83 ±0.47	16.88 ±1.39	17.60 ±1.02
Average LBW (kg) of twin lambs:						
At lambing	2.14 ±0.23	2.39 ±0.22	2.10 ±0.12	2.04 ±0.10	2.11 ±0.11	2.12 ±0.14
At weaning	14.60 ±1.18	13.33 ±1.07	13.50 ±1.70	15.00 ±2.00	14.50 ±1.65	12.57 ±1.28

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05.

Blood parameters of lambs:

Results presented in Table 10 showed that the effect of treatment on some blood biochemicals in blood serum of produced lambs was significant (P<0.05). Briefly, lambs of G6 showed significantly (P<0.05) the highest concentration of total proteins, albumin and globulin versus the lowest values in lambs of the control group and moderate values in other treatment groups (G2-G5), being significantly (P<0.05) higher than those of the control group and lower than those of G6. Cholesterol concentration significantly (P<0.05) increased in G6 and G2 as compared to the controls, being significantly higher in G6 than in G2.

Table (10): Effect of treatment on some blood biochemicals in serum of ewes at weaning.

Treatment	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Cholesterol (mg/dl)
G1 (control)	6.15±0.02^c	2.809±0.002^b	3.809±0.002^b	62.0±0.70^c
G2 (VA)	6.94±0.09^b	2.906±0.002^b	3.906±0.002^b	67.0±0.70^b
G3 (AD$_3$E)	6.82±0.13^b	2.905±0.001^b	3.905±0.001^b	64.2±0.37^c
G4 (Se)	6.62±0.21^b	2.905±0.002^b	3.937±0.031^b	65.6±0.92^{bc}
G5 (VC)	6.88±0.04^b	2.902±0.001^b	3.903±0.001^b	64.0±0.70^c
G6(combin.)	7.66±0.08^a	3.232±0.182^a	4.200±0.192^a	77.8±0.37^a

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05.

Determining concentrations of blood total proteins is more practical and more economical for measuring animal immunity. There is a close relationship between serum total protein concentrations and serum IgG concentrations (Donovan et al. 1986). Concentrations of plasma total proteins and globulin can be used as an indicator for monitoring passive transfer and estimating the amount of circulating IgG in newborns. In addition, total protein concentrations in serum and colostrum are important for lamb growth (O'Brien and Sherman, 1993). Based on these findings, increasing concentration of albumin and globulin, and consequently total proteins in lambs of ewes (G6) may indicate high immunity and high tolerance of different stress factors, reflecting high viability of lambs in G6.

According to Piccione et al. (2011) the albumin's medium half-life ranged from 14 to 16 days in ruminants, after which period the liver is responsible for albumin synthesis (Thrall, 2004). Therefore, increasing albumin level in lambs of G6 indicated high liver function and good healthy status of these lambs. These results could be explained by the first intake of colostrum during the first hours of life of lambs. It is well demonstrated that newborn physiological protein concentration and globulin fractions are variable during the first month of life as adaptive response to various environmental factors after birth, including nutrition. In particular, the levels of these parameters are closely related to newborn ingestion of colostrum within the first hours of life (Piccione et al., 2009).

Bornez et al. (2009) reported an increase in concentration of total proteins and albumin in blood serum of lambs with the advanced age. This finding indicated high total protein content in lambs of G6 after birth as a result of increasing serum immunoglobulin content (Baranowski et al., 2000). Also, the higher cholesterol concentration in lambs of G6 may reflect the degree of stress (Schaffer et al., 1981). Therefore, lower cholesterol might be expected from stress (particularly by weaning lambs) in lambs of other groups.

CONCLUSION

Treatment of ewes prior to breeding September breeding season with antioxidant such as vitamins (A, AD_3E or C), selenium or their combination had impact on lamb and milk production during breeding season, with the best results for ewes treated with vitamin A.

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الأداء الانتاجى والتناسلى للنعاج المعامله بأنواع مختلفة من مضادات الأكسدة خلال الفترة الانتقالية وصولا لموسم التلقيح

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تهدف هذه الدراسة الى دراسة تأثير المعاملة بفيتامين أ ، فيتامين سى ، فيتامين أد3هـ ، السلينيوم ، وكلها معا فى الفترة قبل موسم تناسل سبتمبر على الاداء الانتاجى والتناسلى فى الاغنام. استخدم 72 نعجة خليط (عمرها 3-4 سنوات ووزن جسم حى 44.19 كجم). نعاج المجموعة (ج 1) كانت مجموعة ضابطة بدون معامله بينما النعاج فى المجموعات المعامله كانت (ج 2) أعطيت حبوب فيتامين أ(5000 وحدة دولية\نعجة) عن طريق الفم ، ج 3 حقنت ب 1 مل أد3هـ ، ج4 بالسيلينيوم و ج5 بفيتامين سى و ج 6 كل المعاملات معا . تم تسجيل وزن النعاج والحملان ووزن المشيمة . سجل انتاج اللبن الفردى وكذا تركيب اللبن خلال 45 يوم اثناء فترة الرضاعة . اخذت عينات الدم لتقدير هرمون البروجسترون خلال 50 يوم بعد الولادة من النعاج وكذا تم تقدير البروتين الكلى ، الالبيومين ، الجلوبيولين ، الكولسترول فى سيرم الحملان عند الفطام . تم عمل القياسات التناسلية مثل معدل الشبق ، الخصوبة ، الانتاج الفعلى ، prolificasy ، fucndity وكذا معدل النفوق فى الحملان حتى الفطام .

أظهرت النتائج تأثير المعاملات على وزن الجسم ووزن المشيمة فى النعاج والحملان عند الولادة وعند الفطام حسب جنس المولود وحسب نوع الولادة وإنتاج وتركيب اللبن وكذا تركيز البروجسترون ولم يكن متوسط وزن الحملان عند الفطام معنويا حسب الجنس والسلالة. كان متوسط وزن المشيمة للحملان منخفضا معنويا فى ج2، ج3 عن باقى المجموعات (ج1 ، ج4 ،ج5 ،ج6) زاد محتوى اللبن من الدهن بفروق معنوية ف ج2، ج3 (5.04، 5.43%) ولم يختلف فى كل من ج 4 ، ج5 ، ج6 (4.28 ، 4.62 ، 4.29 %) مقارنة ب ج 1 (4.47 %) . معدل الشبق كان معنويا 25 ، 33.3 ، 16.7 ، 25 ، 16.7 % خلال الفترة الانتقالية ووصل الى 100، 83.4، 91.6 ،100،100 ، 100% فى نهاية موسم التلقيح فى المجموعات المعاملة على التوالى . كان معدل الخصوبة 83.3 ، 91.7 ، 75 ، 75 ، 91.7 ،91.7% وكانت fecundity 125 ، 133 ، 100 ، 92 ، 108 ، 108 وكانت prolificacy 150 ، 145 ، 133 ، 122 ، 118 ، 118 فى المجموعات الستة على التوالى . المجموعة ج 6 كانت الاقل فى انتاج الذكوروكانت المجموعة ج2 الاعلى فى انتاج التوائم عن باقى المجموعات المعاملة. كان معدل النفوق فى الحملان عند الفطام صفر فى كل من ج 1 ، ج2 ، ج6 مقارنة ب 8.33 ، 9.10 ، 7.69 لكل من ج 3 ، ج4 ، ج5 على التوالى .حملان المجموعة ج6 كانت الاعلى فى تركيب كلا من البروتين الكلى ، الألبيومين ، الجلوبيولين والكوليسترول.

تخلص هذه الدراسه الى ان معامله النعاج بمضادات الاكسدة (فيتامين أ، سى ، أد 3هـ ، السيلينيوم او كلها معا) فى الفترة الانتقالية قبل موسم تناسل سبتمبر حسنت من الاداء الانتاجى والتناسلى للنعاج وحملانها.

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