

The Effects of Supplemental Vitamin E on Reproductive Development of Prepubertal Karya Male Lambs

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ABSTRACT

The objective of this study was to determine the effect of dietary vitamin E supplementation on testicular and behavioural development, and histological appearance of testes in Karya male lambs. Thirteen lambs weaned at 2.5 months of age were used in the experiment. Experiment was carried out over a period of 70 days and all lambs were fed ad libitum on concentrate and 100 g/day clover hay per lamb. In addition, the experimental group (n=6) received a supplement of 45 mg/day vitamin E (*dl- α -tocopherol acetate*) per lamb. Testicular measurements and blood testosterone concentrations were recorded every second week. Behavioural tests were carried out four times per animal at three days intervals during the last 12 days of the experiment. After slaughtering, testicular tissue samples of all animals were collected and evaluated for the histological appearances. Except for mount attempts ($P<0.05$), sexual behaviour and testicular growth characteristics of the lambs were similar. The mounting parameters, testosterone concentrations and testes and epididymides weights were similar between the two groups. However, the mean of mounting weight was found lower ($P<0.05$) with Vitamin E lambs compared to control (28.78 ± 3.48 vs. 33.98 ± 3.64 kg, respectively). The area of seminiferous tubules was statistically different ($P<0.001$) between the two groups. However, there were no significant differences between the two groups for most of the reproductive characters. These findings suggest that supplementation of Vitamin E during early prepubertal age of

Karya male lambs does not have considerable effects on the reproductive characteristics.

Key words: *Vitamin E, testicular growth, testicular histology, Karya male lambs, testosterone, sexual behaviours.*

INTRODUCTION

Natural mating is the major breeding protocol applied for sheep breeding in most developing countries. Reproductive performance of rams during mating is very important to have maximum pregnancy rate in the flock. Reproductive activity of rams depends on the quantity and quality of sperm and libido performances (Perkins et al., 1992). Testicular development and libido performance of rams or ram lambs are affected by genotype, ewes' experience, ram lamb/ewe to body size ratio, season, age and feeding level (Price et al., 1991; Mukasa-Mugerwa and Ezaz, 1992; Lindsay et al., 1993; Godfrey et al., 1998; Bielli et al., 2000; Simitzis et al., 2006).

Different levels of correlations have been found among feeding levels, testis size, sperm production, hormone levels, and onset of puberty in male lambs. Production and quality of sperm, testicular growth and libido performances can increase with the aid of special feeding programs (Erdoğan et al., 1987; Mukasa-Mugerwa and Ezaz, 1992; Lindsay et al., 1993; Brigelius-Flohé et al., 2002; Kheradmand et al., 2006; Swanepoel et al., 2008).

Vitamins have significant effects on the reproductive performance and some

biochemical parameters in ewes and rams (Erdoğan et al., 1987; Robinson et al., 2006). Vitamin E seems to act as the principal chain-breaking antioxidant in biological systems (Brigelius-Flohé et al., 2002). It is well known that vitamin E functions as a lipid-soluble antioxidant in cell membranes and it effectively control lipid oxidation. It is definitely required for growth, development and prevention of chronic diseases. In its absence, tissue degeneration related problems occur (Jensen et al., 1995; McDowell et al., 1996). Vitamin E has been also known as an essential nutrient for reproductive functions that share in (or interfere in) development and protection of germ cells, tissue development of testis and ovary, fertilization, placentation thanks to its antioxidant and protective functions. In the absence of Vitamin E, the most prominent effects appear in pituitary and sex gonads leading to infertility in animals (Ersoy and Bayşu, 1986; Brigelius-Flohe and Traber, 1999). Some studies mentioned above have shown that absence of this vitamin causes abnormalities of male sexual development. However, the effects of supplemental vitamin E on prepubertal testis growth, histological appearance and sexual behaviour of lambs have not been documented. There is a need information with regard to effects of Vitamin E supplementation on reproductive characteristics of prepubertal male lambs. The objective of this study was to determine the effects of dietary vitamin E supplementation on sexual behaviour, histological traits and growth of testes in prepubertal Karya male lambs.

MATERIALS AND METHODS

This study was performed on 13 Karya male lambs born in January. The Karya sheep was created in the last decade by backcrossing of native fat-tailed Çine Çaparı sheep with Kıvırcık X prolific Chios (Sakız) rams by local breeders of Aydın Province in Turkey (Karaca and Cemal, 2002). The animals in this study were purchased from a breeder's farm and maintained in Adnan Menderes University, Çine Vocational School Practice and Research

Unit. All lambs, weaned at 2.5 months of age, were fed for 70 days on concentrate (90% dry mater, 167.7 g/kg crude protein, 60.9 g/kg ash, 24.4 g/kg crude fat and 2407 ME Kcal/kg) *ad libitum* and 100 g clover hay/animal/day and had free access to drinking water. Additionally, 45 mg vitamin E (*dl- α -tocopherol acetate*) per animal was added daily to the ration of treatment group (n=6).

Testicular and scrotal measurements were measured and blood samples were collected to determine the testosterone concentrations every second week. Blood samples were taken via venipuncture and centrifuged at 3000 r/m for 15 min. Serum was stored at -20 °C until testosterone assay with radioimmunoassay.

Oestrus was induced in the adult ewes by progestagen impregnated sponges (Chronogest, Intervet) plus 500 IU eCG injection for evaluating sexual behaviour of male lambs. Reproductive behaviour trials were performed, during the last 12 days of the experiment, for 4 times at three days intervals (Crichton et al., 1991; Godfrey et al., 1998). Behavioural trial was performed in an 4x6 m yard for all trials. The average temperature of the test days was 23.16 °C, and average daylight length was 15.48 hours.

Behaviour trials were performed at 08.00-10.30 am without considering groups in choosing animals. Ten minutes were allowed to each animal with one in-heat ewe in the yard during the trial (Crichton et al., 1991; Auclair et al., 1995). Sexual behaviours were recorded with a video camera and recorded activities were evaluated using the techniques described by Winfield and Kilgour (1977), Crichton et al. (1991), Perkins et al. (1992), Auclair et al. (1995) and Godfrey et al. (1998). Accordingly, the investigated traits of sexual behaviour were number of flehmen responses (FRE), number of anogenital investigation (sniffing or licking) (SNL), number of rapid lapping of the tongue normally associated with low-pitched vocalizations (tongue flicks) (TF), number of mount attempts (MA), number of mounts with and without ejaculation (mounting) (MO), number of foreleg kicks and nudge with shoulder (kicking and nudging) (KNN), and latency to first mount (LFM,

minute) and latency to first ejaculation (LFE, minute). First mount weight and first mount age were recorded at the time of the first mount of the lambs. Total physical activity was calculated as the sum of the number of foreleg kicks, attempted mounts, and mounts with and without ejaculation.

At end of the experiment all animals were slaughtered and testes with the epididymises were weighed. At the same time a small tissue samples from each testis (7 control and 6 vitamin E) were taken and fixed in 10% neutral buffered formalin solution. After tissue processing, serial sections of 6 μ thickness at 50 μ intervals apart were cut from the paraffin blocks. Sections were stained with Crossman's triple stain for general histological examination. Prepared sections were examined under a light microscope (Leica DMLB) and by computer with images transferred through a video camera (Leica DC200) compatible with the microscope. All measurement and cell counts were performed using computer-assisted image analysis software (Leica QWin Standard Version 2.8). Forty tubular profiles that were round or nearly round were measured. The epithelium height was obtained in the same tubules. Thickness of *Tunica albuginea* were measured in 5 sections from each animal. Also Leydig cell numbers were counted in 6 consecutive cross-section per animal.

Data were analysed by SAS (2005) program using least squares technique. Generalized linear models were used for the analysis of sexual behaviours. Because the distributions were not normal, a logarithmic transformation was performed to achieve an approximately normal distribution. Testosterone concentration, mounting and ejaculation time values were subjected to fitting test for normal distribution. Data for testicular histology were evaluated by t test.

RESULTS AND DISCUSSION

Testes length, testes diameter, scrotal length and scrotal circumference of the two groups (Fig. 1) show a steady increase throughout the study. Testicular and scrotal growth

characteristics of vitamin E animals were not differed from control animals from 76 to 146 days of age. Similarly, testes + epididymides weights after slaughter and testosterone concentrations throughout the study of control and vitamin E supplemented male lambs were not statistically different during the experiment (Table 1). Hong et al. (2009) reported that vitamin E supplementation increased epididymis weight whereas this supplementation did not affect testis weight in Boer goats. Wilson et al. (2003) reported that lack of vitamin E decreased body and testis weight in rats, but it did not affect testosterone concentrations.

First mount age and total physical activity of the two groups are also presented in Table 1. Except for the first mount weight ($P < 0.05$), the investigated parameters were similar in the vitamin E and control groups. First mount weight and first mount age of male lambs in vitamin E group were determined as 28.78 ± 3.48 kg and 144.75 ± 4.10 days, respectively. In the control animals, the same traits were determined as 33.98 ± 2.64 kg and 144.50 ± 11.84 days, respectively.

Ram lambs have the ability to mount for the first time at 7-9 months age. But, this is depend on body weight much better than age as puberty in a ram may represent 40-45 % of the adult body weight of its breed (Abdel Rahim et al., 1989; Mukasa-Mugerwa and Ezaz, 1992; Kridli et al., 2006). In this study, first mount weights of all lambs were positively affected by good level of nutrition. In addition to, vitamin E lambs exhibited 5.2 kg lighter first mount weight compared with that of control lambs.

The male lambs were tested four times at 3 days intervals during the last 12 days of the experiment. Latency from lambs exposed to the in-heat ewe to first mount were found as 1.30 ± 0.29 min and 1.45 ± 0.17 min for the vitamin E and control groups, respectively. During the 10-min exposure to ewes, lambs in vitamin E exhibited 1.13 ± 0.35 min latency to first ejaculation. Control lambs ejaculated after

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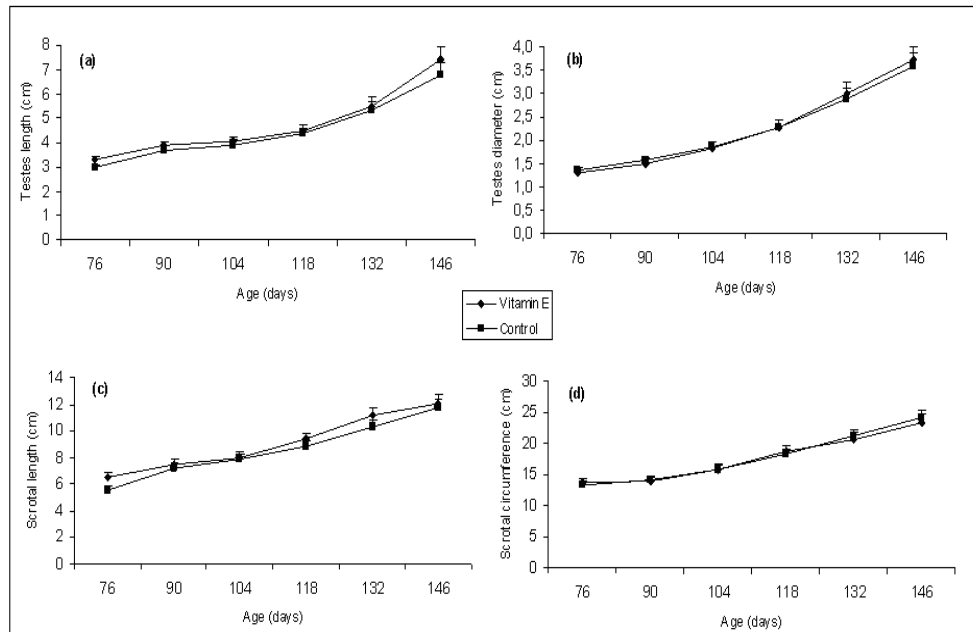


Figure 1. Testes length (a), testes diameter (b), scrotal length (c) and scrotal circumference (d) of vitamin E and control lambs during the experiment.

Table 1. Mounting parameters, mean testosterone concentrations and testes+epididymides weights of Vitamin E and Control ram lambs (Mean±SE).

Traits	Groups	
	Vitamin E (n=6)	Control (n=7)
First mount weight	*28.78±3.48	33.98±3.64
First mount age	144.75±4.10	144.50±11.84
Latency to first mount (min.)	1.30±0.29	1.45±0.17
Latency to first ejaculation (min.)	1.13±0.35	1.32±0.20
Mean testosterone concentrations (ng/ml)	1.33±0.04	1.40±0.04
Testes+epididymides weight (kg)	0.168±0.026	0.171±0.024
Total physical activity	38.30	37.85

*: (P<0.05)

1.32±0.20 min of exposure to ewe. But the differences were not significant (Table 1).

Libido characteristics are important factors to be considered especially when natural mating is performed (Godfrey et al., 1998). The mean values of the sexual activity parameters tested four times in vitamin E and control lambs are shown in Figure 2. Except for mount attempts, there were no differences in the mean number of behavioural responses to the in-heat ewe during 10 minutes in each test between the two groups. Similarly, mean mounting activity of control lambs tended to be higher than that of the vitamin E lambs (P>0.05). Conversely, mean number of FRE, SNL, TF and KNN were

more frequent in the vitamin E group than in the control group (Fig. 2). But the differences were not significant. In the present study, although the differences were not significant, the responses related to precopulatory behaviours of the lambs showed that vitamin E supplemented lambs were more active than that of control lambs. But the responses of copulatory behaviours of the lambs were higher in the control lambs compared to vitamin E lambs. This is because the rest period (sexual inactivity period) is required for lambs to exhibit other sexual behaviour after ejaculation. (Price et al., 1992). Each lamb in the present study was tested for a fixed time period.

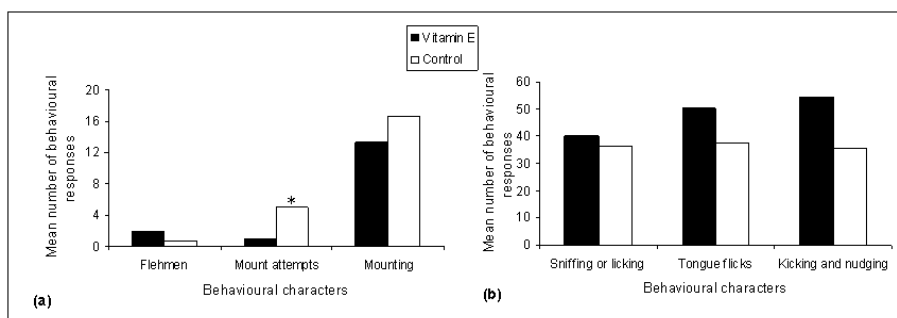


Figure 2. Mean number of behavioural responses of vitamin E and control lambs. *: (P<0.05)

Table 2. Means of area and epithelium height of seminiferous tubules, thickness of tunica albuginea and leydig cell numbers in testes tissues of Vitamin E and Control lambs at 146 days of age (Mean±SE).

Traits	Vitamin E (n=6)	Control (n=7)
Seminiferous tubule area (mm ²)	1.6±0.05***	1.4±0.03
Epithelium height (µm)	27.15±0.56	26.96±1.00
Thickness of Tunica albuginea (µm)	692.95±47.77	652.10±27.89
Leydig cell numbers	2.03±0.14	1.78±0.12

Within same rows asteriks indicates a significant difference (P<0.001) from control.

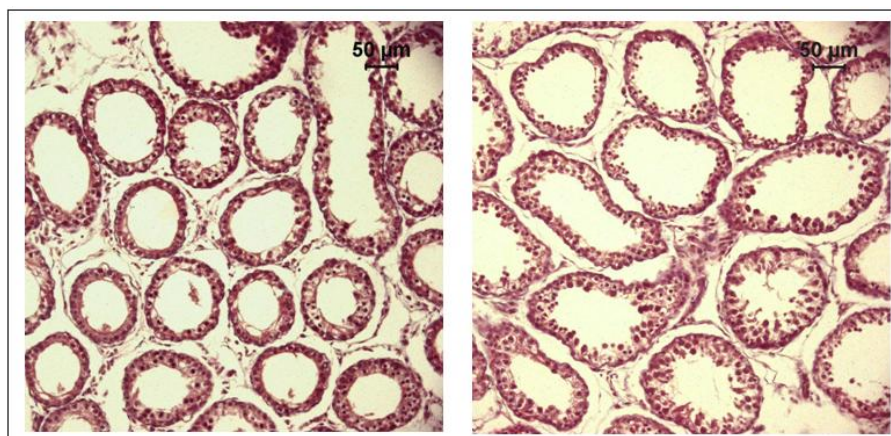


Fig. 3. Histological appearances of tubulus seminiferous contortus of lambs in control (shown at left) and group Vitamin E group (shown at right).

In the histological investigation of testes samples it was observed that vitamin E and control lambs had a similar epithelium height, *tunica albuginea* thickness and leydig cell numbers. But, vitamin E animals had a larger seminiferous tubules' area in testicular tissue than that of control animals (P<0.001) (Table 2). Hong et al. (2009) reported the similar findings for tubules' wall thickness in vitamin E supplemented kids. Similarly, they reported an insignificant increase in leydig cell number in vitamin E supplemented kids.

Histological appearances of testes samples are shown in Fig. 3. It is seen that the seminiferous tubules' area in testis tissues of vitamin E lambs are larger than that of control lambs. Other structures shown in Figure 3 do not show different shapes between groups. Other histological characters are similar between the groups.

All stages of spermatogenesis in seminiferous tubules were not determined in the histological specimens taken from either control or vitamin E group's testes in the present study.

In some tubules and in tubules' walls primary spermatocytes and spermatogonia were observed.

The adequacy of studies regarding the reproductive characteristics in vitamin E supported lambs is not enough. However, effectiveness of vitamin E supplementation for reproductive functions of livestock species has been investigated from various aspects. These studies are based on the fact that germ cells are affected from oxidative damages and require anti-oxidant protection (Castellini et al., 2007). Hong et al. (2010) reported that vitamin E supplementation increased vitamin E content in serum and testis, and thus the antioxidant abilities of testis. It was determined in the same study that vitamin E prevented free radicals' adverse effect on the tissue via improving antioxidant efficiency in the testis. Considering the spermatologic findings in their study on rams, Erdinç et al. (1987) proposed that vitamin E supplementation to the ration could improve fertility of the flock.

CONCLUSIONS

In some countries artificial insemination in sheep is not common, except experimental studies, therefore rams used in natural mating need to have appropriate libido and fertility characteristics. The use of ram lambs for natural breeding of ewes requires that the lambs express adequate levels of body and testicular growth, libido and fertility. In this study, reproductive characteristics of the lambs except first mount weight and the seminiferous tubules area were not affected by dietary vitamin E supplementation. Presently there has not been work done to evaluate the prepubertal reproductive characteristics of male lambs supported with dietary vitamin E. As no attempt was made, in this study, to investigate the semen characteristics of the lambs, therefore, further studies are required to determine the effects of dietary vitamin E supplementation on testicular, behavioural and spermatological characteristics of male lambs in different breeds.

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