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Effect of combination of vitamin E and selenium injections on reproductive performance and blood parameters of Ossimi rams

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ABSTRACT

The objective of this study was to determine the effect of the combination of vitamin E (Vit E) and selenium (Se) injection on semen quality, testes measures and some blood parameters of Ossimi rams. Fourteen mature healthy Ossimi rams were randomly divided into two equal groups (7 rams in each). The first group served as control (CG), while 2nd group served as treatment group (TG). Rams of the TG were treated twice weekly with 5 mg sodium selenite and 450 mg Vit E for 1 month. Semen quantity (semen volume, and concentration of the semen ejaculated) and quality (mass motility and percentage of live and dead cells) were recorded twice weekly. Blood samples collection, testes measurements (testis length (TL), breadth (TB); and scrotal circumference (SC)) using measuring tape and caliper were recorded. Sonar examination of the testes (mediastinum testes and tunica albugenia) and all accessory gland measurements were performed every 2 weeks. Semen quality and quantity were significantly affected by treatments: the ejaculate volume, mass activity and sperm concentration increased ($p < 0.01$) in treated rams in comparison with control ones. The percentages of dead and abnormal spermatozoa were reduced in the treated groups. Also ordinary testes measurements and sonar examination were mostly improved though the differences were not statistically significant. Pen libido test showed reduced reaction time for the first mount in treated rams. Serum testosterone, glutathione peroxidase and other blood parameters were elevated ($p < 0.05$) in treated group in comparison with the control one. The results of this experiment confirm that injections of the combination of Vit E and Se during the breeding season improved semen characteristics and the overall reproductive performance of Ossimi rams.

1. Introduction

There is physiological synergism between selenium and vitamin E. Previous reports have suggested that vitamin E and selenium (Se) are important nutrients that act synergistically and can affect many biological processes including spermatogenesis and semen quality (Marin-Guzman et al., 1997; Yousef et al., 2003), reproduction (Jerry, 1996; Koyuncu and Yerlikaya, 2007), metabolism (Awadeh et al., 1998), immunity (Hernken et al., 1998), and protecting against oxidative stress (Bernabucci et al., 2002). The association of Vit E deficiency with impaired male reproduction was established more than three decades ago, and traditionally it is called the "Anti-sterility Vitamin".

Selenium is an essential dietary trace element and is always of research interest required for the maintenance of male fertility by way of testosterone biosynthesis, formation and normal development of spermatozoa (Brown and Arthur, 2001). Both testis and epididymis require exogenously supplied Se in order to synthesize a variety of known

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Table 1
Composition and chemical analysis of the experimental basal diet of rams (%, as fed basis).

Item	%
Ingredient	
Yellow corn grain	55
SBM (44%)	18
Wheat straw	25
Limestone	1.2
Salt	0.5
Compound premix[a]	0.3
Chemical composition	
ME (Mcal/kg)	2.33
CP (%)	13.66
CF (%)	11.3
EE (%)	1.97
Ca (%)	0.51
P (%)	0.3
Se (ppm/kg)	0.11
Vitamin E (mg/kg)	15

[a] Compound premix each 3 kg contain: 1,250,000 IU, Vit A; 2,500,000 IU, Vit D3; 1000 mg, Vit E; 80,000 mg, Mn; 60,000 mg, Zn; 50,000 iron, 20,000 copper, 5000 iodine, 250 Se, 1000 Co mg tell 3 kg $CaCO_3$

selenoproteins, whose precise role in spermiogenesis and post testicular sperm maturation are not clearly defined (Ali et al., 2009). In many areas of the world plants do not provide levels of this element adequate to meet dietary requirements (Hogan et al., 1993). Requirements of selenium for sheep are 0.1–0.2 ppm/kg DM (NRC, 1985). In males bred on a low selenium diet, male hypogonadism was found as well as reduced production and deteriorated semen quality (Kleene, 1993). Supplementation with selenium has been reported to improve reproductive performance in sheep (Ali et al., 2009; Marai et al., 2009).

Since ruminants are not usually raised in confinement as poultry and swine, selenium supplementation of ruminant diets is more difficult. Measures of testicular size have received considerable attention as possible selection criteria for improving fertility in cattle and sheep, primarily because they are highly heritable (Ricordeau et al., 1986), Therefore, the aim of the present study is to evaluate the influence of the combination of vitamin E and Se injections on reproductive performance, testes measurements as well as the general health condition of rams.

2. Materials and methods

2.1. Animals and experimental design

The present study was carried out in the Experimental Farm of Animal Production Department, Faculty of Agriculture, Assiut University, Egypt. The work was carried out from 30th April 2011 to 30th June 2011. A total number of fourteen sexually mature Ossimi rams aged between 1.5 and 2 years and averaging 46.6 kg body weight were chosen healthy and clinically free of external and internal parasites. Rams were randomly divided into two equal groups (7 per each). The first group with no supplementation was considered as a control (CG); however the second group served as a treated group (TG). The rams of TG were injected with the combination of selenium and vitamin E [3 ml IM from Viteselen 15®, Adwia Company, Egypt]. So each ram from the treated group injected with 5 mg sodium selenite and 450 mg vitamin E two times/week for 1 month. All rams were fed on the experimental diet which formulated according to the requirement for mature ram according to NRC (1985) for sheep. The diet consists of 25% wheat straw and 75% concentrate mixture as presented in Table 1. The experimental diet for both group contained 0.1 ppm Se and 15 mg Vit E/kg. Foods were prepared weekly and dry matter intake (DM1, kg/day) was recorded daily till the termination of the experiment. Animals had a free access to water all the day.

2.2. Training rams for semen collection

Observation of the sexual behavior for each ram was carried out daily between 8:00 a.m. and 9:00 a.m. Libido was evaluated by introducing rams to a teaser ewe selected as random. All rams lambs were allowed to go out to the collection area without restraint to observe the sexual activity toward the ewe. Rams, which achieved erection and extrusion of the penis out of the sheath were trained for semen collection using an artificial vagina. One false mount rams were allowed to do before semen collection and semen was collected during the subsequent mounting. Semen collection started in the fourth week (2 times weekly) after injections and continued for 5 weeks.

2.3. Testis measurements

Scrotal circumference was measured by measuring steel tape (Ahmed and Noakes, 1995). The length (TL) and the breadth (TB) of the testes were measured using a pair of metal calipers (Islam and Land, 1977). Testis length was measured from top of the tail to the head of the epididymis for each testis. The breadth of the testes measured between the lateral surface of the testes and scrotal raphi. The testicular thickness was measured between the anterior and posterior surface. All testes dimensions were done for both right and left testes.

2.4. Ultrasonographic (sonar) examinations

The testes and accessory genital glands including seminal, prostate and bulbourethral glands were scanned per rectum using ultrasound scanner with 6/8 MHz linear array transrectal probe (Pie Medical 100 LC, Holland) twice weekly for 8 weeks (1 month during and 1 month after treatment). The transducer was fitted in a self-manufactured connector to favor its manipulation per rectum. All examinations were done by same operator. The thicknesses of tunica albugenia and mediastinum testes were recorded for each side. The measurements of all accessory genital glands were recorded.

2.5. Semen analysis

Two ejaculates per week were collected from each ram using an artificial vagina. During initial evaluations semen was maintained in 37 °C water bath. Semen was evaluated immediately upon collection for general characters (volume, color, concentration, pH, mass movement, live, dead sperm and abnormal spermatozoa). Semen volume (ml) was measured using a graduated collection tube to the nearest 0.1 ml. Initial pH of semen samples was measured by means of comparative nitrating pH paper. Mass movement of sperm was assessed at a low magnification (10×), scored on a scale from 0 (no motility) to 5 (excellent motility). The percentages of live and dead sperms were determined from fixed-smear stained with eosin. Two hundred sperms were calculated from different fields in the stained smear, the colored head sperm was calculated as a dead sperm, while a colorless sperm was considered as a live sperm. Sperms abnormalities were classified into two categories according to Blom (1950). Defects which occur during spermatogenesis are considered a primary and those developing subsequent to spermiation considered as secondary. Sperm concentration was determined by the use of the Hemocytometer. Total number of spermatozoa per ejaculate was calculated by multiplying the ejaculate volume × sperm concentration per ml. Reaction time for ram recorded as the time needs for mounting an ewe until complete ejaculation. It was measured in seconds using stopwatch.

2.6. Collection of blood samples

Blood samples were collected from the jugular vein at 8.0 a.m for measuring some biochemical parameters and testosterone. Blood samples were allowed to clot at 4 °C for 10 h in the refrigerator, then centrifuged and harvested sera stored at −20 °C until subsequent analysis. Serum testosterone concentration was determined using ELISA kits (Bio Check, Foster City, CA 94404, USA) using the micro-well method. The kit had a sensitivity of 0.05 ng/ml. Blood parameters (total protein, albumen, glucose, cholesterol, Ca and P) analyses were carried out by spectrophotometer using commercial test kits (Spinreact, Spain). Serum glutathione