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THE INFLUENCE OF ANTIOXIDANTS (SELENIUM AND VITAMIN E) ON DAILY BODY GAIN, REPRODUCTIVE PERFORMANCE AND SUBSEQUENT FERTILITY OF RAMS

(With 7 Tables and 4 Figures)

By

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تأثير مضادات الأكسدة على زيادة الوزن اليومي والكفاءة التناسلية مع الخصوبة التالية للكباش

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تم اختيار ٣٥ كبشا أوسيميا لدراسة تأثير مضادات الأكسدة على الزيادة اليومية في الوزن والكفاءة التناسلية مع الخصوبة التالية للكباش. وقسمت هذه الحيوانات إلى مجموعتين مجموعة المعاملات وعددها عشرون كبشا تم حقنها ب ٥ملى بمادة مضادات الأكسدة (فيتسلين – فيتامين هـ+ سيلينيوم) في العضل ومجموعة ضابطة وعددها خمسة عشر كبشا لم تحقن بهذه المادة. واستمر الحقن لمدة ستة عشر أسبوعا بواقع مرة في الأسبوع. تم تسجيل أوزان الحيوانات وأيضا مقاسات الخصية والمحيط الصفني مرة كل أسبوعين بينما تم أخذ عينات الدم وتجميع السائل المنوي لكل كبش على حدا مرة كل أسبوع. وتم تحديد مستوى الهرمون الخصوي (التستوستيرون) في عينات مصل الدم وكذلك تقييم بعض خصائص السائل المنوي لكل كبش سواء كباش المعاملات أو الضابطة أسبوعيا. وفى نهاية التجربة تم اختيار عشرة كباش من المجموعة الأولى لتلقيح ستة وخمسون نعجة وأيضا خمسة كباش من المجموعة الثانية لتلقيح تسعة وعشرون نعجة وذلك لقياس درجة الخصوبة وتم تسجيل معدلات الحمل في جميع النعاج الملقحة. وأوضحت النتائج أن الحيوانات التي تم حقنها بمادة الدراسة أظهرت زيادة معنوية في أوزان الحيوانات وكذلك زادت مقاسات الخصية مع المحيط الصفني زيادات معنوية عنها في المجموعة الضابطة. وارتفع مستوى الهرمون الخصوي ارتفاعا معنويا بالمقارنة بنفس الهرمون في المجموعة الضابطة. وبالنسبة لتقييم السائل المنوي– فكان لمضادات الأكسدة تأثيرا معنويا في زيادة حجم القذفة ونسبة الحركات الذاتية للحيوانات المنوية وأيضا زيادة معنوية في تركيز القذفة بينما أدت هذه المادة

إلى انخفاض معنويا في نسبة الحيوانات المنوية غير الطبيعية وكل هـــذا بالمقارنـــة بنفـــس المعدلات في المجموعة الضابطة. وأيضا تم ملاحظة ارتفاع معدلات الحمـــل فـــي النعـــاج الملقحة بكباش المجموعة المعاملة (٣٣,١٦±٢٩,٧٦%) عنـــها فـــي المجموعـــة الضابطـــة (٦٨,٩±٧١,٦٥%). ونستخلص من هذه النتائج أن حقن مادة مضادات الأكسدة (فيتامين هـــ + السيلينيوم) له تأثير فعال على زيادة الوزن مع زيادة في مقاسات الخصية مصحوبة بزيادة في المحيط الصنفي وكذلك تحسن ملحوظ في تقييم السائل المنوي وبالتبعية زيادة فى الكفـــاءة التناسلية ودرجة الخصوبة لكباش الأوسيمي.

SUMMARY

The effect of antioxidants (Selenium and vitamin E) on the body gain and reproductive performance with subsequent fertility of rams have been evaluated. Thirty-five Ossimi rams were assigned into two groups: treated group (n = 20) which received 5 ml Viteselen intramuscularly weekly for 16 weeks and control group (n = 15) which received 5 ml normal physiological saline. Every 2 weeks, the live body weight, testicular measurements and scrotal circumference were taken. Blood samples and semen collection were obtained Weekly from all animals for testosterone determination and semen evaluation. At the end of the experiment, ten rams from the treated group and 5 rams from the control group were allowed to mount 85 healthy normal ewes (56 ewes for treated rams and 29 for control) to evaluate the pregnancy rate for each group. The obtained results revealed that, antioxidants had a significant increasing effects on liveweight (kg) and daily body gain (g/day) in comparison to control rams. Testicular size and scrotal circumference increased significantly ($P<0.01$) in treated rams when compared with control rams. At the end of the experiment (16 weeks), the values of testicular size and scrotal circumference were 80.93±9.53 c.c, 61.57±6.95 c.c and 33.23±1.56 cm, 30.63±1.58 cm in treated and control rams respectively. Testosterone level (ng/ml) increased significantly ($P<0.01$) in treated rams (3.14±0.46 ng/ml) when compared with control rams (2.22±0.34 ng/ml) at the end of experiment. Moreover, the supplementation of antioxidant had a significant improving effects on semen quality. It has increasing effect on semen volume, individual motility, alive sperm and sperm concentration. However, the sperm total abnormalities decreased significantly as comparison to control. Pregnancy rate in this study, indicated that, treated group had a higher pregnancy rate (76.29±1.6.33%) than in control group (65.71±9.68%). It can be concluded that, supplementation of antioxidants (selenium and

vitamin E) had a beneficial effects on daily body gain and improve the reproductive performance and subsequent fertility of rams as comparison to control rams.

Key words: *Antioxidants, Body gain, Reproductive performance, Rams.*

INTRODUCTION

Sheep are believed to have been one of the first mammals to be domesticated and are known to have been closely associated with man from a very early date. These animals offer the potential of making an important and continuing contribution to providing food and fibre for a growing world population (Shelton, 1995). The efficiency of meat production from sheep can be increased by exploiting some of the unique advantages offered by this species. One of the most important advantages of sheep lies in its reproductive rate.

The success or otherwise of many controlled reproduction techniques is not alone a question of influencing the reproductive processes of the ewe, the outcome also depends on the capability of the ram (mating activity and semen quality) when natural service is the mode of breeding (Gordon, 1997). Improvement of the reproductive performance of the sheep is a major requirement in a wide variety of sheep enterprises. Indeed, the continuing existence of sheep farming in many countries is now dependent on producing sheep with a substantially increased reproductive rate. Furthermore, a higher reproductive rate is important because it results in a larger number of young sheep being available for selection and thus leads to accelerated genetic gain in other reproductive traits (Rae, 1986).

During the physiological process of oxidation of organic materials by molecular oxygen to produce energy, a number of reactive oxygen species (ROS) are formed. These free radicals are vitally important because they attack microorganisms invading the body (Woodford and Whitehead, 1998). They added that, ROS are so valuable that besides molecular oxygen, the body maintains stores of H_2O_2 and other prooxidants, which if suitably stimulated, will produce ROS to destroy both foreign bodies and dying or defective cells. A disturbance in the prooxidant / antioxidant system has been defined as "oxidative stress". It is associated with potential damage to the biological system.

Oxidative stress may be involved in processes such as membrane damage, lipid peroxidation and protein oxidation (Helmut, 1995).

The term antioxidant has been defined as any substance that delays or inhibits oxidative damage to a target molecule (Gutteridge and Halliwell, 1994). The antioxidant may be present within the cells, in the cell membranes or in the extracellular fluid. It may be lipophilic or hydrophilic and may be endogenously produced or derived from the diet (Rumley and Paterson, 1998). Selenium (Se) and vitamin E (vit.E) have complementory functions as antioxidants to minimize cellular damage. Se. by enhancing glutathione peroxidase activity, reduces peroxide levels which is essential to prevent the production of free radicals (Basini *et al.*, 1996). vit.E is localized in the cell membranes as a biological antioxidant and inhibits the formation of lipid peroxide as well as, accelerates the removal of free radicals from cellular membranes by donating a hydrogen atom to free radical species, resulting in the formation of a relatively stable vit.E radical which is then thought to be recycled either ascorbate or ubiquinol (Gutteridge and Halliwell, 1994).

Se and vit E enhance the phagocyte and macrophage function of cells and improve immune response (Weiss *et al.*, 1994; Finch and Turner, 1996 and Meydani *et al.*, 1997). The supplements of Se and vit. E improve the reproductive function of cattle (Arechiga *et al.*, 1998), sheep (Gabryszuk, 1994 and Megahed and Daghash, 1999) and buffalo (Mahmoud, 1994). Therefore, the present study was conducted to determine the influence of antioxidants (Se and vit E) administration on growth rate, reproductive efficiency (testicular measurments, scrotal circumference and semen quality with hormonal profile) as well as, fertility performance in Ossimi rams.

MATERIAL and METHODS

Animals, their feeding and management:

This study was conducted from January to April, 2000 at the Animal Production Experimental Farm of the Faculty of Agriculture, Al-Azhar University, Assiut, Egypt. Thirty-five Egyptian Ossimi rams with clinically normal genitalia were selected, from a total population of approximately 150 rams. All the animals grazed on Trifolium alexanderinum (Egyptian clover) beginning from December, 1999 until the beginning of January, 2000. Then, they were fed roughage and/or Egyptian clover together with wheat straw. The animals were housed

outdoors in sheltered dry lots and received daily maintenance rations, water, wheat straw and mineral salt licks were available ad-libitum.

At the beginning of the study, the animals were 18-24 months old, weighed between 44 and 50 kg. The animals had previously been trained to mating occasionally. Whenever, the rams failed to show any interest to mounting, the introduction of another male into the mounting area stimulated the libido of the first ram which failed to show any interest to mounting within 5-10 min after the introduction of the second male. The animals were divided randomly into treated group (n=20) and control group (n = 15). Animals of treated group received 5 ml Viteselen (each 1ml contains 150 mg vitamin E and 1.67 mg sodium selenite, ADWIA Co.) intramuscularly weekly for 16 weeks. The control animals received 5 ml of normal physiological saline intramusculary.

Clinical measurements and Sampling:

The health status of all animals was monitored periodically by the farm veterinarian. Body weight and testicular measurements were recorded every 15 days. The scrotal circumference (SC) was measured at the widest scrotal diameter, with a flexible tape after the testes had been gently forced into the scrotum by applying pressure with the hand above the head of the epididymides. This ensures that the scrotal skin was smooth and tense. The testicular length, width and breadth were measured in the same by using caliper, always by the same operator. The testicular size (TS) was calculated as multiplying the testicular length, width and breadth with 0.52 (factor).

Blood samples and hormonal analysis:

Blood samples were collected weekly before injection from all animals by jugular venipuncture and kept in ice-box, then transported immediately to the laboratory. The samples were kept at 4°C for 6-12h and the serum was separated by centrifugation at 3000 rpm for 20 min, then stored at -20°C until analysed. Serum testosterone level was determined by using commercial ELISA kit (Biosource, Belgium). The minimum detectable value was 0.05 ± 0.02 ng/ml and the intra - and interassay coefficient variations were 6.2 and 6.4 % respectively.

Semen evaluation and pregnancy rate:

The semen was collected in the morning (8.00 to 9.00 a.m) once a week by using an artifical vagina (41-44°C) and restrained non-pregnant ewe. The semen parameters with respect to volume, individual motility, alive sperm %, total abnormalities % and sperm concentration were estimated according to the proccdures laid down by Zemjanis

(1970). Ten rams from the treated group and 5 rams from control group were allowed to mount 85 healthy normal ewes (56 for treated group and 29 for control group) during the breeding season (May). Pregnancy rate was calculated from results of pregnancy diagnosis made by abdominal palpation 4 months post-mating and/or after acutal lambing (West, 1986).

Statistical analysis:

The data were expressed as the means ± SE for all parameters, then analysed by using analysis of variance (ANOVA), and means ± SE were tested at least significant difference (LSD). Correlation coefficient was done between all parameters. All tests were done by using PC-stat comp-uter program. Results were considered significant only at $P<0.05$ or less.

RESULTS

The obtained results are presented in Tables 1-7 and Figures 1-4. The effect of antioxidants (Se and vit.E) upon body weight of rams is illustrated in Table 1. Liveweight was significantly ($P<0.01$) affected by administration of antioxidant. It had a significant increasing effect on liveweight (38.22%) as compared with the control (24.73%). It is also observed that, the increasing effect of antioxidant was noticed significantly ($P<0.05$) after 6 weeks from injection, thereafter, it had a significantly increasing effect at $P<0.01$ as compared with control. Furthermore, within the treated group, the body weight increased significantly every two weeks after injection as well as, the increase percent of body weight increased with the time of injection. However, within the control group, the increase in body weight was non-significant and the increase percent in body weight was lesser when compared with that of the treated group. Moreover, Table (2) revealed that antioxidants had an increasing significantly ($P<0.05$) effect on the total body gain (18.65 ± 1.39 kg) when compared with the control (11.87 ± 0.99 kg). The daily body gain in treated rams was higher significantly (155.35 ± 11.56 g/day) than in the control (98.92 ± 8.28 g/day).

The mean values of testicular size (TS) and scrotal circumference (SC) are shown in Table 3. The values of TS increased significantly ($P<0.05$) after 6 weeks post-injection of antioxidants (68.26 ± 9.04 c.c) as compared with control after the same period (58.11 ± 7.45 c.c). After 8 weeks, the TS continued increasing significantly ($P<0.01$) until the end

of the experimental period and reached to 80.93 ± 9.53 c.c in the treated group, while it became 61.57 ± 6.95 c.c in control rams. It is also observed that, the increase percent in TS within the treated group is higher than that within the control group.

The mean values of SC increased significantly from 6-10 weeks after treatment at 0.05 level of probability as well as, these values also increased significantly ($P<0.01$) after 12 weeks until the end of the study period. At the end of experiment, the SC values were 32.81±1.56 cm and 30.63 ± 1.58 cm in treated and control rams respectively. However, the increasing values of TS and SC in treated rams were non-significantly after 2 and 4 weeks as compared with control rams. The increase percent of SC continued greater in the treated group than that in control group at the same time post-injection of antioxidants.

Serum concentrations (means ± SE) of testosterone (ng/ml) were significantly affected by injection of antioxidant. The variation in serum testosterone throughout the experimental period is shown in Table 4 and Figure 1. Serum levels of testosterone showed changes i.e., they increased but non-significantly after 1-2 weeks when compared with the control. Moreover, they increased significantly ($P<0.05$) after 3-4 weeks, as well as increased significantly at 0.01 level of probability from 6 weeks to the end of the experiment as compared with the control. At the end of the study, the testosterone levels were 3.14 ± 0.46 ng/ml in treated rams and 2.22 ± 0.34 ng/ml in control rams. The increase percent of testosterone level was higher within treated group than that within control group.

The effects of antioxidants administration upon the semen quality and pregnancy rate of treated and control rams are presented in Tables 5,6 and Figures 2-4. Se and vit.E had a significantly increasing effect on the ejaculate volume ($P<0.05$) after 8-10 weeks post-treatment and at $P<0.01$ after 12 weeks to the end of the experiment (Table 5 and Fig.2). At the end of experiment, the ejaculate volume of treated ram was higher (2.05 ± 0.65 ml) than that in control ram (1.28 ± 0.45 ml). Moreover, the injection of antioxidant had a noticable increasing effect upon volume of ejaculate thereby the increase percent when compared with it in the control group. In addition, individual motility of sperm in treated rams (Fig.2) increased significantly ($P<0.05$) after 6-8 weeks and at $P<0.01$ after 10 weeks to the end of experiment when compared with control. Individual motility % was higher significantly ($P<0.01$) at the

end of experimental period in treated rams (84.75 ± 3.28%) than in control rams (72.37 ± 5.12%). The increase percent of individual motility is elevated within the treated group than that in the control group.

Table 6 and Figure 3 reveal the changes of sperm alive % and sperm total abnormalities % in treated and control rams. Sperm alive % increased significantly ($P<0.01$) as comparison with control and it was 85.76 ± 1.09% in treated rams and 81.84 ± 2.73 % in control rams at the end of experiment. The increase percent of alive sperm % within the treated group was higher than that within the control group. Moreover, sperm total abnormalities % in treated group decreased significantly ($P<0.01$) when compared with control group and it was 19.53 ± 1.75 % and 22.64 ± 1.39% in treated and control rams respectively at the end of study. However, the increase percent in total abnormalities was lower in treated group than that in control group after 4 weeks post injection till the end of study.

Sperm concentration and pregnancy rate improved after supplementation with antioxidants (Se and vit.E) as shown in Figure 4. Sperm concentration increased significantly ($P<0.05$ after 6-10 weeks and $P<0.01$ after 12 weeks to end of the experiment). Generally, the increase percent of sperm concentration in treated animals is higher than that in the control group. Sperm concentration was higher (3.853 ± 0.52 x $10^6/ml^3$) in treated rams than in control rams (2.714 ± 0.39 x $10^6/ml^3$). The pregnancy rate was higher (76.29 ± 16.33%) in ewes which mated by the treated rams than that in ewes serviced by control rams (65.71 ± 9.68%).

Concerning of correlation coefficients (Table 7) in this study, testosterone, TS, SC and liveweight were significantly correlated ($P<0.001$). Moreover, testosterone level was correlated with volume of ejaculate, individual sperm motility, sperm alive and sperm abnormalities % ($P<0.001$) as well as, it correlated significantly ($P<0.001$) with sperm concentration.

DISCUSSION

The present study monitored the variations in liveweight, testosterone levels, TS and SC, as well as, the variation in semen quality and pregnancy rate after the administration of antioxidants to growing

rams. It demonstrated the increasing effect in the liveweight, TS, SC and serum concentrations of testosterone. It has been postulated that, the liveweight and daily body gain of treated rams would be increased significantly ($P<0.01$) when compared with the control. These findings are in agreement with that reported by Kolb *et al.* (1997) and Wichtel (1998) who reported that, the supplementation of Se and vit.E was important for growth and development of sheep. These results are supported by the finding of Reffet *et al.*, (1988) who concluded that, antioxidant (Se and vit.E) cause the enhancement of immune response and increase the concentrations of natural antioxidants in the blood which may be useful to maintain host defense against disease and protected immune response from the immune-suppressive effects (Hogan *et al.*, 1992; Bendich, 1993 and Abdel-Salam *et al.*, 1995). Moreover, it could enhance neutrophil bactericidal activity (Eicher *et al.*, 1994) and protect neutrophils from the destructive action of free radical (Hogan *et al.*, 1992).

The obtained results can be attributed to antioxidant (Se and vit.E) adequate for normal growth and production in the animals, as well as preventing tissue breakdown (mainly through peroxidation) and body loss (Kolb *et al.*, 1997). In addition, Se leads to activation of thyroid hormone (tri-iodothyronine) through thyroxine deiodinase enzyme and secretion of this hormone by the thyroid gland (Bender, 1993 and Kolb *et al.*, 1997). Thyroid hormones are necessary for the normal body growth and development through improvement of the metabolic activity, protein metabolism and muscle growth (Horst *et al.*, 1989 and Baruah *et al.*, 1993). Thyroid hormones concentration were positively correlated with body weight of male and female (Baruah *et al.*, 1993). This is supported by the finding of Ganong (1995) who concluded that, the increase in the basal metabolic rate is accompained by increase in the animal appetite food intake and subsequent with increases the body weight. Intake of protein with the presence of antioxidant had a general relationship among weight and growth rate (Ferrell, 1991). This interrpretation can be supported by that reported by Pinzani *et al.*, (1998) and Kankofer (2001). They concluded that, ROS cause tissue damage by a variety of different mechanisms, which include DNA damage, lipid peroxidation, protein damage and loss of biological activity.

Furthermore, deficiency of antioxidant may result in retarded testicular growth and reduced androgen production (Ferrell, 1991 and Thwaites, 1995). These supported the obtained results which concluded

that testicular size and scrotal circumference increased significantly in rams treated with Se and vit.E as compared with control rams.Concerning of the effect of antioxidant on testicular hormonal function, the obtained result indicated that testosterone levels increased significantly in treated rams. This finding is in agreement with that reported by Ealy *et al.*, (1994) and Gabryszuk (1994). They mentioned that supplementation of Se and vit.E improved the reproductive functions of the male and female genital organs. The increase of testosterone level might be attributed to the prevention of protein damage by antioxidant cause more activation of hypothalamic – pituitary axis, which leads to more stimulation to the release of pituitary gonadotrophis and subsequent increase the activation of the leydig cells (Ferrell, 1991 and Martin *et al.*, 1994).

Regarding the effect of antioxidants on semen quality, the results in this study indicated that the volume of the ejaculate, individual motility and sperm concentration were improved significantly as well as, sperm abnormalities decreased significantly which compared with the control. The obtained results are in agreement with that reported by D'Yachenko and Kartoshenko (1990) and Upreti *et al.*, (1997). They concluded that vit.E had a beneficial effects on semen output and sperm motility. Se and vit.E improved the semen quality through maintaining sperm integrity, moreover, antioxidants improve the motility of ram sperm and integrity of their acrosomes (Maxwell and Stojanov, 1996). Dead spermatozoa are a substantial source of ROS due to the breakdown of their membrane and the continued availability of unsaturated membrane fatty acid to peroxidation (Kessopoulov *et al.*, 1992) which leads to loss of sperm motility and reduced fertilizing ability of many species (Aitken, 1994 and Lenzi, *et al.*, 1996). The peroxidation of sperm plasma membrane plays an important role in the pathophysiology of male infertility (Aitken *et al.*, 1993 and Smith *et al.*, 1996). ROS increase DNA fragmentations (Lopes *et al.*, 1998) and affect the sperm axonema development (De Lamirande and Gagnon, 1992). The antioxidant system of the cells is not potent enough to prevent lipid peroxidation compeletely. Although, the main beneficial effects of antioxidant supplement may delay the sperm membrane destabilization associated with sperm ageing (Maxwell and Watson, 1996) and maintenance of spermatozoal motility (Aitken, 1995).

The noticable changes in B.W, TS, SC and seven quality in the control group can be attributed to the effect of age in these animals. The

body weight increased from time to another to represent the natural growth curve. There is a positively correlation between live weight and testicular size and scrotal diminsions (Osinowa *et al.*, 1992). Moreover, scrotal circumference of the ram increased gradually and positively correlated with body weight (Cartee *et al.*, 1990). It is believed that, semen quality; especially semen volume and sperm motility was positively correlated with testicular size and scrotal circumference (Mukasa and Ezaz, 1992; Nowakowski and Cwikla, 1994 and Wahid and Yunus, 1994). It is evident that, the testicular growth may also reflect the testicular activity of the testosterone production (Martin *et al.*, 1994).

Concerning the effect of antioxidants on the pregnancy rate in ewes which were mated with the treated rams, the injected rams had a higher pregnancy rate. This finding coinced with that reported by D'Yachenko and Katroshenko (1990), Maxwell and Stojanov (1996) and Hemingway (1999). They reported that, the ewes which were mated with the rams supplemented with Se and vit.E had a higher pregnancy rate than those mated with the control rams. They added that, the field fertility trial after addition of antioxidants increase the conception rate. The obtaind results might be attributed to the improved viability of spermatozoa which reflected to improve fertilizing capacity of sperm and fertility (Maxwell and Stojanov, 1996).

It is apparent from the obtained results, that the administration of antioxidants (Se and vit.E) had a beneficial effects on daily body gain and improve the reproductive performance of rams. These antioxidants improved significantly the liveweight, testicular size and scrotal circumference. Moreover, supplementation of Se and vit.E had an increasing significantly effect on testosterone level as well as, improved significantly the semen quality accompanied by increase the ram's fertility.

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Table 1: Effect of antioxidants administration upon body weight (kg) of rams.

Treatment period (wks)	Control (n = 15)	Treated (n = 20)
0	48.00 ± 1.71	48.80 ± 1.97
2	$49.36 \pm 1.82^{1,a}$ (2.83 %)	$50.16 \pm 2.16^{1,a}$ (2.79 %)
4	$51.04 \pm 1.95^{1,a,b}$ (6.33 %)	$52.51 \pm 2.24^{1,b}$ (7.60 %)
6	$52.46 \pm 1.98^{1,b,c}$ (9.29 %)	$54.85 \pm 2.34^{2,c}$ (10.66 %)
8	$54.16 \pm 1.80^{1,b,c}$ (12.83 %)	$57.11 \pm 2.30^{2,d}$ (17.03 %)
10	$55.79 \pm 1.64^{3,d,e}$ (16.23 %)	$59.63 \pm 2.65^{4,e}$ (22.19 %)
12	$57.34 \pm 1.48^{3,e,f}$ (4.00 %)	$62.27 \pm 2.89^{4,f}$ (4.00 %)
14	$58.68 \pm 1.46^{3,f,g}$ (22.25 %)	$64.79 \pm 3.06^{4,g}$ (32.77 %)
16	$59.87 \pm 1.31^{3,g}$ (24.73 %)	$67.45 \pm 3.17^{4,h}$ (38.22 %)

- LSD is 2.19 at P<0.05 and 2.96 at P<0.01.
- Means in the same column with the same superscript letters are not significantly different.
- Means in the same row with the same superscript number are not significantly different. ^{1,2} P<0.05 and ^{3,4} P<0.01.
- Figures in parentheses indicate increase percent in body weight in treated and control over the values at time zero.

Table 2: Effect of antioxidant administration upon body weight (B.W) of the rams.

Item	Control (n = 15)	Treated (n = 20)
Initial B.W (kg)	48.0 ± 1.71	48.80 ± 1.97^{ns}
Final B.W (kg)	59.87 ± 1.31	67.45 ± 3.17 **
Total B. gain (kg)	11.87 ± 0.99	18.65 ± 1.39 *
Daily B. gain (g)	98.92 ± 8.28	155.35 ± 11.56 **

ns = non-significant * = P< 0.05 ** = P< 0.01

Table 3: Effect of antioxidants administration upon testicular size (TS) and scrotal circumference (SC) of the rams (means + SE).

Treatment Period (wks)	TS (c.c)		SC (cm)	
	Control (n = 15)	Treated (n = 20)	Control (n = 15)	Treated (n = 20)
0	55.89 ± 7.36	56.50 ± 9.63	25.83 ± 1.72	25.92 ± 1.52
2	56.69 ± 7.24^{1,a} (1.58 %)	61.29 ± 9.43^{1,a} (8.48 %)	26.47 ± 1.66^{1,a} (2.08 %)	26.83 ± 1.55^{1,a} (2.19 %)
4	57.46 ± 7.44^{1,a} (2.96 %)	65.45 ± 9.12^{1,a,b} (15.84 %)	27.06 ± 1.54^{1,a,b} (4.36 %)	27.78 ± 1.42^{1,a} (7.18 %)
6	58.11 ± 7.45^{1,a} (4.12 %)	68.26 ± 9.04^{2,a,b,c} (20.81 %)	27.60 ± 1.59^{1,a,b} (6.44 %)	29.23 ± 1.65^{2,a,b} (12.77 %)
8	58.96 ± 7.29^{3,a} (5.16 %)	70.47 ± 9.40^{4,b,c,d} (24.73 %)	28.1 ± 1.55^{1,b,c} (8.37 %)	29.65 ± 1.66^{2,b} (26.58 %)
10	59.54 ± 7.31^{3,a} (6.68 %)	72.84 ± 9.35^{4,b,c,d} (28.92 %)	28.57 ± 1.65^{1,b,c,d} (10.18 %)	30.19 ± 1.68^{2,b,c} (16.47 %)
12	60.31 ± 7.26^{3,a} (8.06 %)	75.59 ± 9.13^{4,c,d,e} (33.79 %)	29.17 ± 1.58^{3,c,d,e} (12.49 %)	31.29 ± 1.7^{4,c,d} (20.72 %)
14	60.92 ± 7.12^{3,a} (9.14 %)	77.93 ± 9.21^{4,d,e} 37.79 %)	29.96 ± 1.65^{3,d,c} (15.54 %)	32.10 ± 1.62^{4,d,e} (23.84 %)
16	61.57 ± 6.95^{3,a} (10.32 %)	80.93 ± 9.53^{4,e} (43.24 %)	30.63 ± 1.58^{3,e} (18.13 %)	32.81 ± 1.56^{4,e} (26.58 %)

- LSD is 8.24 at P<0.05 and 11.09 at P<0.01 in Ts while, it become 1.52 and 2.05 at P<0.05 and P<0.01 in SC respectively.
- Means in the same column with the same superscript letters are not significantly different.
- Means in the same row with the same superscript numbers are not significantly different .^{1,2} P<0.05 and ^{3,4} P<0.01.
- Figures in parentheses indicate increase percent in TS and SC in treated and control over the values at time zero.

Table 4: Effect of antioxidants administration upon testosterone levels (means ± SE) in rams.

Treatment period (wks)	Control (n = 15)	Treated (n = 20)
0	1.66 ± 0.37	1.66 ± 0.33
1	1.69 ± 0.36^{1,a} (1.81 %)	1.73 ± 0.34^{1,a} (4.22 %)
2	1.71 ± 0.37^{1,a} (3.01 %)	1.76 ± 0.39^{1,a} (6.03 %)
3	1.72 ± 0.37^{1,a} (3.62 %)	2.09 ± 0.36^{2,a} (25.90 %)
4	1.74 ± 0.36^{1,a} (4.82 %)	2.13 ± 0.36^{2,b} (28.13 %)
5	1.77 ± 0.37^{3,a} (6.63 %)	2.28 ± 0.37^{4,b,c} (37.35 %)
6	1.80 ± 0.36^{3,a,b} (6.63 %)	2.39 ± 0.37^{4,b,c,d} (43.98 %)
7	1.83 ± 0.37^{3,a,b,c} (10.24 %)	2.42 ± 0.35^{4,b,c,d} (45.78 %)
8	1.86 ± 0.35^{3,a,b,c,d} (12.05 %)	2.52 ± 0.36^{4,c,d,e} (51.81 %)
9	1.89 ± 0.35^{3,a,b,c,d,e} (13.86 %)	2.59 ± 0.35^{4,c,d,e} (56.02 %)
10	1.92 ± 0.34^{3,a,b,c,d,e} (15.66 %)	2.61 ± 0.35^{4,c,d,e,f} (57.23 %)
11	1.95 ± 0.33^{3,a,b,c,d,e} (17.47 %)	2.68 ± 0.36^{4,d,e,f} (61.45 %)
12	1.99 ± 0.36^{3,a,b,c,d,e} (19.87 %)	2.73 ± 0.35^{4,d,e,f} (64.46 %)
13	2.13 ± 0.35^{3,b,c,d,e} (28.31 %)	2.79 ± 0.36^{4,e,f} (68.07 %)
14	2.16 ± 0.34^{3,c,d,e} (30.12 %)	2.86 ± 0.37^{4,e,f,g} (72.29 %)
15	2.19 ± 0.33^{3,d,e} (31.19 %)	2.94 ± 0.41^{4,f,g} (77.11 %)
16	2.22 ± 0.34^{3,e} (33.73 %)	3.14 ± 0.46^{4,g} (89.16 %)

- LSD is 0.35 at $P<0.05$ and 0.47 at $P<0.01$.
- Means in the same column with the same superscript letters are not significantly different.
- Means in the same row with the same superscript number are not significantly different.
  ^{1,2} $P<0.05$ and ^{3,4} $P<0.01$.
- Figures in parentheses indicate increase percent in testosterone levels in treated and control over the values at time zero.

Table 5: Effect of antioxidants upon volume of ejaculate and individual motility % of ram semen.

Treatment Period (wks)	Volume of ejaculate (ml)		Individual motility %	
	Control (n = 15)	Treated (n = 20)	Control (n = 15)	Treated (n = 20)
0	0.78 ± 0.32	0.78 ± 0.34	65.21 ± 5.77	65.36 ± 5.35
2	$0.82 \pm 0.34^{1,a}$ (5.13 %)	$0.86 \pm 0.33^{1,a}$ (10.26 %)	$65.87 \pm 5.32^{1,a}$ (1.01 %)	$66.43 \pm 5.34^{1,a}$ (1.64 %)
4	$0.88 \pm 0.33^{1,a}$ (12.82 %)	$0.96 \pm 0.39^{1,a}$ (23.08 %)	$66.71 \pm 5.25^{1,a,b}$ (2.30 %)	$69.28 \pm 5.14^{1,a,b}$ (5.99 %)
6	$0.94 \pm 0.34^{1,a,b}$ (20.51 %)	$1.04 \pm 0.39^{1,a,b}$ (33.33 %)	$67.86 \pm 3.93^{1,a,b,c}$ (4.06 %)	$72.69 \pm 5.80^{2,b,c}$ (11.22 %)
8	$1.02 \pm 0.40^{1,a,b}$ (30.76 %)	$1.39 \pm 0.42^{2,b,c}$ (78.21 %)	$69.57 \pm 3.35^{1,a,b,c}$ (6.68 %)	$75.0 \pm 4.79^{2,c,d}$ (14.75 %)
10	$1.09 \pm 0.34^{1,a,b}$ (39.74 %)	$1.48 \pm 0.47^{2,c}$ (89.74 %)	$70.51 \pm 3.56^{3,b,c}$ (8.13 %)	$77.5 \pm 4.27^{4,d,e}$ (18.57 %)
12	$1.12 \pm 0.21^{3,a,b}$ (43.58 %)	$1.59 \pm 0.53^{4,c}$ (103.85 %)	$71.12 \pm 3.45^{3,b,c}$ (9.06 %)	$80.35 \pm 5.71^{4,e,f,g}$ (22.94 %)
14	$1.18 \pm 0.23^{3,a,b}$ (51.28 %)	$1.79 \pm 0.61^{4,d}$ (129.49 %)	$71.95 \pm 4.87^{3,c}$ (10.34 %)	$82.43 \pm 5.75^{4,f,g}$ (26.12 %)
16	$1.28 \pm 0.45^{3,b}$ (64.10 %)	$2.05 \pm 0.65^{4,d}$ (162.82 %)	$72.37 \pm 5.12^{3,c}$ (10.98 %)	$84.75 \pm 3.28^{4,g}$ (29.67 %)

- LSD is 0.41 at $P<0.05$ and 0.55 at $P<0.01$ in volume of ejaculate while, it become 4.93 and 6.64 at $P>0.05$ and $P<0.01$ in Individual motility % respectively.
- Means in the same column with the same superscript letters are not significantly different.
- Means in the same row with the same superscript number are not significantly different. ^{1,2} $P<0.05$ and ^{3,4} $P<0.01$.
- Figures in parentheses indicate increase percent in volume of ejaculate and individual motility % in treated and control over the values at time zera.

Table 6: Effect of antioxidants upon sperm alive % and total abnormalities % in ejaculate of rams.

Treatment Period (wks)	Sperm alive		Total abnormalities	
	Control (n = 15)	Treated (n = 20)	Control (n = 15)	Treated (n = 20)
0	77.18 ± 3.56	77.95 ±3.89	16.25 ± 1.83	16.09 ± 1.63
2	77.36 ± 3.46^{1,a} (0.23 %)	79.03 ± 3.89^{1,a} (6.22 %)	17.26 ± 1.86^{1,a} (6.22 %)	17.09 ± 1.72^{1,a} (6.22 %)
4	78.07 ± 3.36^{1,a,b} (1.15 %)	79.87 ± 3.64^{1,a,b} (2.46 %)	17.79 ± 1.71^{1,a} (9.48 %)	17.52 ± 1.71^{1,a,b} (8.89 %)
6	78.79 ± 3.59^{1,a,b,c} (2.09 %)	80.54 ± 3.24^{1,a,b,c} (3.32 %)	18.40 ± 1.62^{1,a,b} (13.23 %)	17.58 ± 1.73^{1,a,b} (9.26 %)
8	78.87 ± 3.42^{1,a,b,c} (2.19 %)	81.39 ±2.97^{2,a,b,c} (4.41 %)	19.27 ± 1.48^{1,b,c} (18.58 %)	17.63 ± 1.74^{2,a,b,c} (9.57 %)
10	79.48 ± 3.19^{1,a,b,c,d} (2.98 %)	82.39 ± 2.67^{2,b,c,d} (5.69 %)	20.13 ± 1.65^{1,c,d} (23.87 %)	18.34 ± 1.76^{2,a,b,c,d} (13.98 %)
12	80.27 ± 3.11^{1,b,c,d} (4.00 %)	83.19 ± 2.44^{2,c,d,e} (6.72 %)	21.08 ± 1.51^{3,d,e} (29.72 %)	18.73 ± 1.71^{4,b,c,d} (16.41 %)
14	81.62 ± 2.89^{1,c,d} (5.75 %)	84.57 ± 1.69^{2,d,e} (8.49 %)	21.81 ± 1.56^{3,e} (10.34 %)	19.19 ± 1.72^{4,c,d} (19.27 %)
16	81.84 ± 2.73^{3,d} (6.04 %)	85.76 ± 1.09^{4,e} (10.12 %)	22.46 ± 1.39^{3,e} (39.32 %)	19.53 ± 1.74^{4,d} (21.38 %)

- LSD is 2.91 at P<0.05 and 3.92 at P<0.01 in sperm alive % while, it become 1.59 and 2.15 at P>0.05 and P<0.01 in total abnormalities % respectively.
- Means in the same column with the same superscript letters are not significantly different.
- Means in the same row with the same superscript number are not significantly different. ^{1,2} P<0.05 and ^{3,4} P<0.01.
- Figures in parentheses indicate increase percent in sperm alive % and total abnormalities % in treated and control over the values at time zero.

Table 7: Correlation coefficents between testosterone, (T), testicular size (TS), Scrotal circumferences (SC), body weight (BW), volume of ejaculate (Vol.), individual motility (IM)%, sperm alive (SA) %, sperm abnormalities (Sab) % and sperm concentration (S.conc).

	T	TS	SC	B.W.	Vol.	IM	SA	Sab.
TS	0.98 ***							
SC	0.95 ***	0.99 ***						
B.W	0.95 ***	0.98 ***	0.93 ***					
Vol.	0.93 ***	0.97 ***	0.96 ***	0.99 ***				
IM	0.97 ***	0.98 ***	0.99 ***	0.92 ***	0.96 ***			
SA	0.96 ***	0.99 ***	0.98 ***	0.89 ***	0.85 **	0.94 **		
Sab	0.94 ***	0.98 ***	0.96 ***	0.91 ***	0.87 **	0.96 **	0.97 ***	
S.conc	0.97 ***	0.99 ***	0.99 ***	0.94 ***	0.93 ***	0.91 **	0.78 **	0.84 ***

** = Significant different at 0.01

*** = Significant different at 0.001

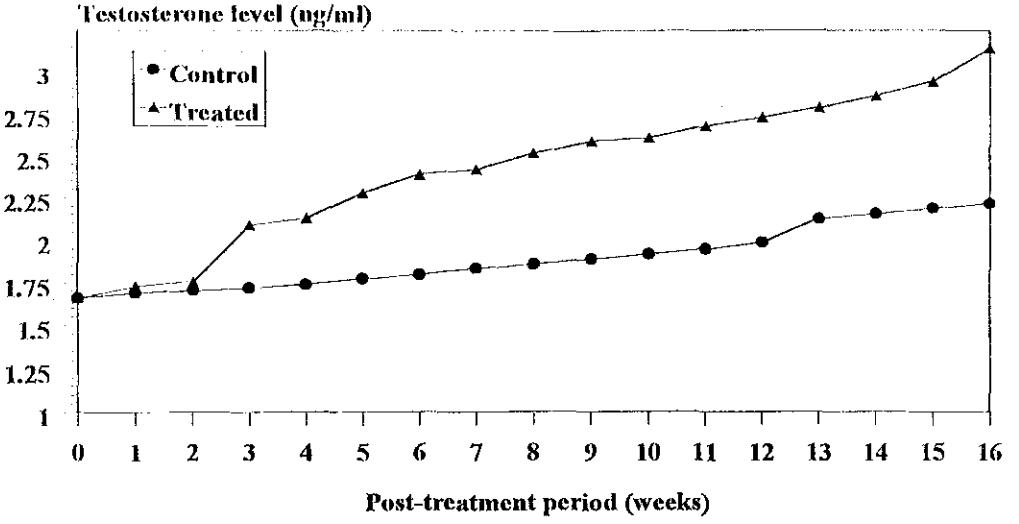

Figure (1) : Effect of antioxidants on testosterone levels (means ± SE) in rams.

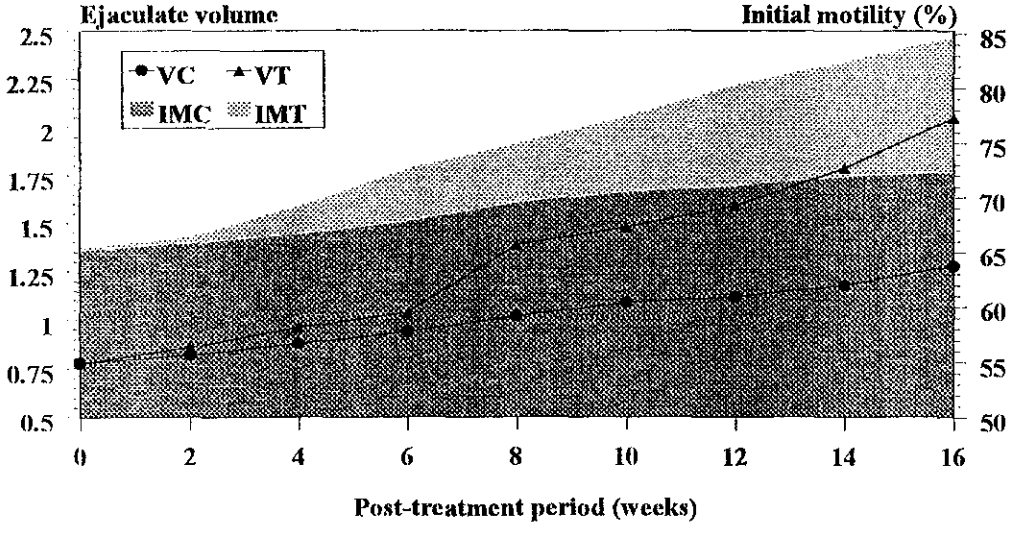

Figure (2) : Effect of antioxidants upon volume of ejaculate (ml) and individual motility (%) of ram sperm.

- The data are presented in means ± SE.
- VC = volume of ejaculate in control rams.
- VT = volume of ejaculate in treated rams.
- IMC = individual motility of sperm in control rams.
- IMT = individual motility of sperm in treated rams.

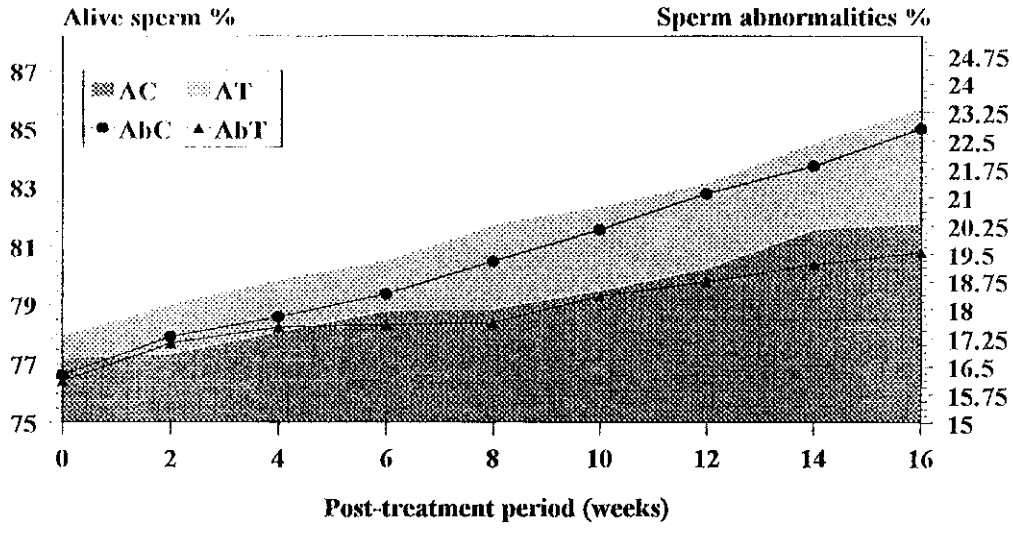

Figure (3) : Effect of antioxidants upon sperm alive % and sperm abnormalities % in ejaculate of rams.

- The data are presented in means ± SE
- AC = alive sperm % in control rams.
- AT = alive sperm % in treated rams.
- AbC = sperm abnormalities % in control rams.
- AbT = sperm abnormalities % in treated rams.

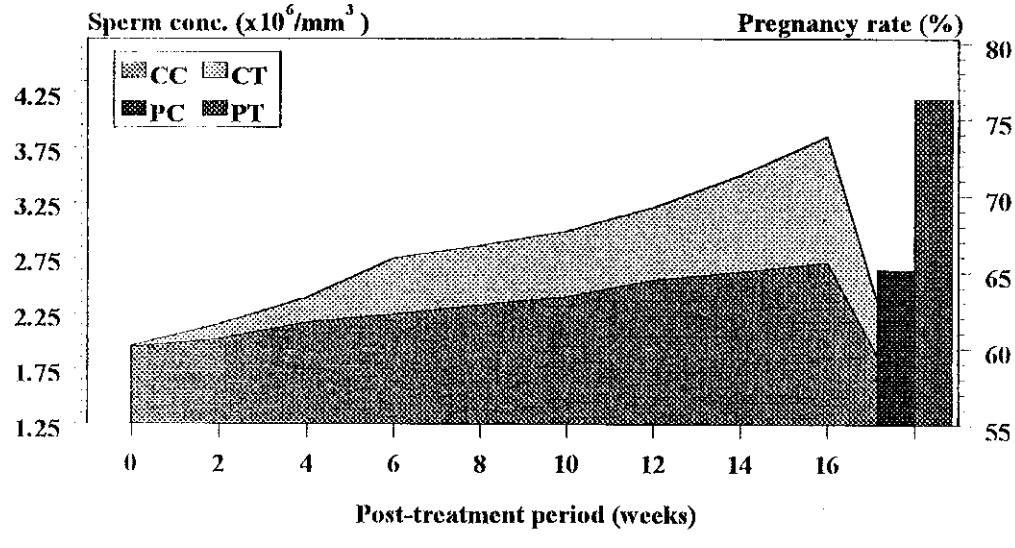

Figure (4) : Effect of antioxidants upon sperm concentrations and pregnancy rate of rams.

- The data are presented in means ±SE.
- CC = sperm concentrations in control rams.
- CT = sperm concentrations in treated rams.
- PC = pregnancy rate in control rams.
- PT = pregnancy rate in treated rams.