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ORIGINAL ARTICLE

In vivo adverse effects of alpha-tocopherol on the semen quality of male bucks

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Summary

Oxidative stress has detrimental effects on semen quality during spermatogenesis and semen processing for artificial insemination. This work was conducted to study the effect of different levels of vitamin E on the semen traits, oxidative status and trace minerals in Beetal bucks. Thirty-six bucks of similar body weight and age (1 year) were randomly divided into four groups. One group was kept as control with no supplementation (group 1), and the others were supplemented with 200 (group 2), 400 (group 3) and 800 IU (group 4) vitamin E/animal/day for 2 months. At the end of the experiment, semen samples were collected and evaluated. Seminal plasma was separated to study the concentration of superoxide dismutase (SOD), glutathione peroxidase (GPx), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and trace minerals (Zn, Cu, Mn and Fe). Group 3 showed significantly higher ($p < 0.05$) semen volume and per cent motility and lower dead sperm percentage compared to control group. Superoxide dismutase, GPx, Zn, Cu and Mn were higher in the same group. The level of AST decreased in group 3 without any change on the concentration of ALT. It is suggested that vitamin E at the rate of 400 IU/buck/day supported higher semen volume, per cent motility, per cent live spermatozoa, antioxidants (SOD, GPx) and trace mineral levels (Zn, Cu, Mn) in the seminal plasma. The increased supplementation from 0 to 400 showed a general increasing trend in improving semen quality. However, the dose of 800 IU/kg had no useful effect in further improving the semen quality.

Keywords Beetal bucks, vitamin E, semen traits, stress markers, trace minerals

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Introduction

For the last few decades, study of free radicals and estimation of antioxidants capacity has become an important investigation in animals (Yousef, 2010; Khan, 2011; Panda and Cherian, 2014). Lipids are the major components of the spermatozoa which are involved at various stages of maturation, capacitation and acrosome reaction, a series of biochemical reactions, required for the process of fertilization (Agarwal et al., 2014; Khan, 2011). Extensive lipid peroxidation caused by metabolic, nutritional, environmental, diseases etc., free radicals are constantly produced which are detrimental to the fertilizing potential of sperm (Agarwal et al., 2003; Kothari et al., 2010; Khan, 2011; Rahman et al., 2014). Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major free radicals present in seminal plasma.

Due to increased production of free radicals, oxidative stress occurs which is the imbalance between prooxidants and antioxidants (Agarwal et al., 2003; Hashem et al., 2013). Under normal conditions, the body usually contains sufficient amount of antioxidant to cope with the production of free radicals (Castillo et al., 2001). However, when the generation of free radicals exceeds the body's antioxidant capacity, oxidative stress occurs. Oxidative stress reduces sperm numbers, decreases sperm motility and increases dead sperm (Khan, 2011; Al-Daraji, 2012; Khan et al., 2012a; Hashem et al., 2013).

Vitamin E is believed to be the primary component of the antioxidant defence of spermatozoa (Surai et al., 1998) and a major antioxidant against the free radicals (Khan, 2011). Supplementation of vitamin E has been reported to increase reproductive capacity in chicken (Khan et al., 2012a), boar (Brzezinska-Slebodzinska et al., 1995), rabbit (Yousef et al., 2003) and ram (Luo et al., 2004). Vitamin E is known as an anti-sterility vitamin, and its deficiency leads to degeneration of spermatozoa, testes and seminiferous

tubules (Wilson et al., 2003). Hashem et al. (2013) reported that vitamin E at the dose rate of 150 mg/kg can effectively ameliorate the negative impact of elevated temperature on semen quality and oxidative stress.

Superoxide dismutase (SOD) is an important component of seminal plasma and plays a key role in striking a balance production of free radicals and destruction by SOD through scavenging process (Rahman et al., 2014). Superoxide dismutase converts superoxide anion, both inside and outside the cell, into hydrogen peroxide, which is ultimately converted into water. Decreased concentration of SOD has been linked to poor sperm quality and reproductive performance (De Lamirande and Gagnon, 1995; Khan et al., 2012b). Glutathion peroxidase (GPx) is an important enzyme which detoxifies lipid peroxide. It is an important antioxidant and converts hydrogen peroxide into less harmful components. This enzyme is considered qualitative superior to catalase and works in synergy with vitamin E (Rahman et al., 2014).

Most of the previous studies have shown improved semen characteristics in domestic species under small doses of vitamin E (Yousef et al., 2003; Yousef, 2010; Hashem et al., 2013) or failed to show the effect under larger doses for prolonged period (Hong et al., 2010). Previously, Yousef (2010) reported that vitamin E at the level of 2 IU/kg ameliorated oxidative stress in seminal plasma induced by pyrethroid- and cyhalothrin-induced toxicity. Hong et al. (2010) found that vitamin E supplementation at higher dose (880 IU/kg) had no useful effect on the antioxidant enzymes in Boar goats. Most of the previous studies have shown the effect of vitamin E on antioxidant contents in blood or tissues homogenates specially testes and liver (Hong et al., 2010; Yue et al., 2010; Mahmoud et al., 2013). Studies are scarce on the seminal plasma antioxidant enzymes such as SOD, GPx and trace minerals in male bucks. Therefore, the present research work was designed to study the effect of different levels of dietary vitamin E on semen traits and seminal plasma antioxidants enzymes and trace minerals in Beetal bucks.

Materials and methods

Animals' feeding and management

A total of 36 healthy Beetal bucks (*Capra aegagrus hircus*) with similar body weight (45 ± 2.34 kg) having average age of 2 year were selected. The bucks were further distributed into four groups. The composition of basal diet is given in Table 1. The bucks were allowed to graze on alfalfa for 3 h in the morning and 2 h in the evening. The concentrate was fed to each buck at the rate of 500 g/buck/day. Vitamin E (alpha-tocopherol) was consisted of vitamin acetate (1 mg contains 1 IU vitamin E) and was obtained from BA Traders, Lahore, Pakistan. The treatments are referred to as group 1, 2, 3 and 4 and supplemented with vitamin E at the level of 0, 200 and 400 and 800 IU/buck/day respectively. The supplementation of vitamin E was continued for 2 months. The average temperature was 31 + 0.5 °C.

Table 1 Composition and chemical analysis of the experimental basal diet of bucks

Ingredients	%
Alfalfa forage	60
Concentrate	40
Total	100
Concentrate (% on DM basis)	
Corn	59
Soya bean meal	29
Wheat bran	8
$CaHPO_4$	2
Salt	1
Additive	1
Chemical composition	
CP (%)	17.65
DE (MJ/kg)	9.32

Semen evaluation

Semen from the experimental bucks was collected early in the morning. For this purpose, female goats were presented as a teaser. Each buck was given enough time (2–3 min) for sexual motivation and one or two false mounts were allowed for sexual preparation before the semen collection. Semen was collected with the help of artificial vagina by maintaining the temperature at 37 °C. Soon after collection, semen was shifted to water bath at 35 °C and subjected to qualitative and quantitative tests for evaluation. At the end of the experimental period, semen samples were obtained from each buck.

Volume of each ejaculate was recorded with the graduated collection tube. Sperm concentration was determined using the improved Neubauer hemocytometer slide (Leica Biosystems Nussloch GmbH, Nussloch, Germany). The percentage of motile sperm was estimated by visual examination under low-power magnification (10×) using a compound microscope. Assessment of dead spermatozoa was performed using an eosin–nigrosin blue staining mixture (Blom, 1950).

Separation of seminal plasma and biochemical analyses

After initial evaluation, the rest of the semen was centrifuged at 700 *g* for 15 min to separate the seminal plasma which was stored at −20 °C for later analyses.

Determination of seminal plasma antioxidant enzymes

Seminal plasma SOD activity was assayed using an SOD Assay Kit from BioVision (Mountain View, CA, USA). This assay utilizes WST-1 (Water soluble tetrzolium), a tetrazolium salt, which produces a water soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related with the amount to the xanthine oxidase activity and is inhibited by SOD. The SOD activity was expressed as the percentage of inhibition of the WST-1 reduction rate.

Seminal plasma GPx activity was evaluated by spectrophotometry (IRMECO Model U2020) at 340 nm with a commercial kit supplied by BioVision. GPx converts reduced glutathione to oxidized glutathione while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water. The activity of GPx was expressed in mU/ml.

Determination of seminal plasma AST and ALT

Seminal plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured with UV-Vis spectrophotometer (IRMECO Model U2020) using a commercial kit (Randox Laboratories, Crumlin, UK).

Mineral determination

The preparation of seminal samples for mineral analysis was carried out as described by Khan et al. (2012c). Briefly, the sample of 1 ml was taken into a digestion flask and added 10 ml concentrated nitric acid. The mixture was placed on a hot plate until all the fumes were evaporated. The flask was removed and cooled for 5 min. Five millilitre perchloric acid was added into the flask, and the mixture was heated again on a hot plate. When the volume was reduced to 1–2 ml, the flask was removed and the content was cooled again. The contents were diluted with 50 ml deionized water, filtered and kept in a clean bottle until analysis. First standard solutions were run followed by samples. The concentration of minerals in the samples was obtained from the absorbance of standards and their corresponding concentrations (Khan et al., 2012c). Concentration of iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) concentration was measured with the help of atomic absorption spectrophotometer (PRKIN-ELMER, Connecticut, USA). The local committee of Use and Care of Animals at the University approved this study.

Statistical analysis

Data were statistically analysed with the help of statistical software (SPSS, version 12.0 Chicago, IL, USA). One-way analysis of variance was used to test the significance of treatment (four dietary treatments) on the studied traits (Steel et al., 1997). Means of the significantly affected traits were separated by Duncan multiple range test (Duncan, 1955). p-Value <0.05 was considered to be statistically significant.

Results

The result of vitamin E supplementation on sperm parameters is shown in Table 2. Semen volume and sperm motility were significantly higher in group 3 ($p < 0.05$) than the other groups. No significant change was observed in sperm concentration between the control and the treated groups. Similarly, dead sperm percentage also decreased significantly in group 3 bucks.

Seminal plasma enzymes are presented in Table 3. Seminal plasma enzymes revealed that the concentration of GPx and SOD increased significantly in group 3. The AST concentration decreased significantly in group 4. However, no significant change was observed in the concentration of ALT. Seminal plasma trace element concentration of control and treated groups (Table 3) revealed that Zn, Mn and Cu increased significantly in group 3 while Fe concentration did not change significantly between the control and treated groups (Table 4).

Table 2 Mean ± SEM semen characteristics of control and treated male Beetal bucks

Parameters	Group 1*	Group 2	Group 3	Group 4	SEM
Semen volume (ml)	1.49^{b}	1.66^{ab}	1.90^{a}	1.80^{a}	0.05
Sperm concentration (10^9/ml)	1.13^{a}	1.55^{a}	1.53^{a}	1.13^{a}	0.01
Sperm motility (%)	61.66^{c}	68.43^{b}	88.33^{a}	78.73^{b}	2.54
Dead sperm percentage (%)	6.66^{a}	3.46^{b}	2.66^{b}	4.33^{ab}	0.76

Mean value having different superscript within the same row differ significantly ($p < 0.05$).

*Group 1 to 4: Dietary supplementation of Vitamin E at the rate of 0, 200, 400 and 800 IU/buck/day respectively.

Table 3 Mean ± SEM seminal plasma enzymes concentration of control and treated male Beetal bucks

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
SOD (per cent inhibition)	6.37[c]	7.28[b]	10.66[a]	7.45[b]	0.19
GPx (mU/ml)	10.86[c]	13.29[b]	23.55[a]	20.26[b]	1.03
AST (U/l)	69.44[a]	69.63[b]	62.68[c]	68.96[b]	0.05
ALT (U/l)	63.68	61.63	62.44	64.42	0.05

SOD, superoxide dismutase; GPx, glutathione peroxidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
Mean value having different superscript within the same row differ significantly ($p < 0.05$).
Group 1 to 4: Dietary supplementation of Vitamin E at the rate of 0, 200, 400 and 800 IU/buck/day respectively.

Table 4 Mean ± SEM seminal plasma trace minerals (mg/kg) of control and treated male Beetal bucks

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Zn	5.52[b]	7.33[a]	8.12[a]	6.53[b]	0.59
Mn	12.35[c]	14.05[b]	20.85[a]	18.26[b]	0.34
Fe	2.59[a]	4.13[a]	3.26[a]	1.84[b]	0.12
Cu	0.21[c]	0.48[ab]	0.69[a]	0.34[b]	0.04

Zn, zinc; Mn, manganese; Fe, iron; Cu, copper.
Mean value having different superscript within the same row differ significantly ($p < 0.05$).
Group 1 to 4: Dietary supplementation of Vitamin E at the rate of 0, 200, 400 and 800 IU/buck/day respectively.

Discussion

The present study indicates that supplementing vitamin E may ameliorate the semen quality especially at the dose rate of 400 IU/animal/day. Yue et al. (2010) concluded that vitamin E had a positive effect on improving semen volume (1.21 ± 0.11 ml), sperm concentration (38.9 ± 3.1 × 10^8 ml), total sperm output (43.6 × 10^8 ml) and sperm motility (73.7 ± 2.4%) in male Aohan fine-wool sheep. These results are almost close to our findings. Yousef (2010) found that vitamin E at the dose rate of 2 IU/kg body weight increased semen volume (0.86 ± 0.02 ml), sperm concentration (322 ± 7.4 × 10^6 ml), sperm motility (80 ± 0.77%) and decreased dead sperm percentage (18 ± 0.46%) in male rabbits. These results are little lower than our findings in the current study which may be due to the difference in species, dose and duration of vitamin E and other differences in experimental protocol. In another study, Yousef et al. (2003) reported that vitamin E improved semen traits and seminal plasma biochemical parameters in rabbit.

Under normal conditions, spermatozoa produce reactive oxygen species (ROS) which are required for the processes of acrosome reaction, capacitation and fusion of oocyte and spermatozoa as reviewed by Khan (2011). Excessive production of ROS causes destruction of antioxidant capacity and results in the lipid peroxidation of the sperm plasma membrane (Khan, 2011; Khan et al., 2012c). In the present study, the beneficial effect on semen parameters could be attributed to the antioxidant effect of vitamin E. This vitamin is a lipid soluble antioxidant which plays a major role in the attenuation of ROS which are by-products of biological membrane (Khan, 2011). In addition, vitamin E protects testis, epidermis and accessory glands which are critical in the process of spermatogenesis. Khan et al. (2012a) suggested that vitamin E protects the spermatozoa in several ways. First, it protects the biological membrane from lipid peroxidation. Second, vitamin E reduces the lipid peroxidation in seminal plasma to support the viability of the sperm. Third, dietary vitamin E enhances sperm number and quality.

In the present study, SOD and GPx increased significantly when the level of vitamin E was increased to 400 IU/animal/day. Yue et al. (2010) concluded that vitamin E supplementation at the level of 200 IU increased the SOD (77.98 ± 6.24 U/mg protein) and GPx (62.03 ± 4.86 unit activity) concentration in testicular mitochonderia of Aohan fine-wool sheep and suggested that vitamin E has an effect on antioxidant enzymes which act as a primary defence mechanism in testes. In the study of Hong et al. (2010), increased activity of SOD (218.33 ± 35.54 U/mg) and GPx (90.67 ± 10.91 activity unit) were reported in testes in response to vitamin E dose at the rate of 320 IU/kid/day vitamin E Boer goats. Superoxide dismutase is an important antioxidant enzyme which protects the body against the free radicals. Decreased SOD activity may be linked with increased peroxide, destruction of nuclear materials and damage the protein and enzymes (Hong et al., 2010; Khan, 2011). GPx mainly exists in the mitochondria and scavenges lipid and hydrogen peroxides (Khan, 2011). Hong et al. (2010) reported that vitamin E at the level of 80 and 320 IU per buck per day protected the testis from damage of peroxidation. Further, SOD can catalyse superoxide into hydrogen peroxide which is a safer molecule. In the present study, relatively low activity of SOD and GPx were observed compared to the previous reports. The difference may be due to the experimental protocol, dose and duration of the treatment of vitamin E and the tissue homogenate (testes).

The transaminase activity in seminal plasma is the index of sperm membrane stabilizing capacity. When sperm cell membrane damages, AST and ALT are

released into the extracellular medium (Hong et al., 2010; Khan et al., 2012c). In the present study, AST activity was significantly lower in response to vitamin E (400 IU) treatment. Al-Daraji et al. (2011) concluded that acrosomal damage results in the leakage of enzymes from the spermatozoa. Yousef et al. (2003) found that 12 weeks treatment of vitamin E significantly decreased AST (31.8 ± 1.88 IU/l) in seminal plasma of male rabbits. Hashem et al. (2013) reported no significant effect on the concentration of seminal plasma AST and ALT under the dose rate of 150 IU/kg in rabbits during the hot season. The reason could be due to the lower level of the dose of the vitamin E used in the study. In the same study, a negative correlation was found between AST, ALT and ejaculated volume and sperm concentration. Therefore, the activities of these enzymes could be used as indicator of sperm integrity (Khan et al., 2013).

Trace elements are considered very beneficial to farm animals. Trace elements act as a functional and structural co-factor in metal containing enzymes and their deficiency may cause impaired reproductive performance (Yue et al., 2010). In our study, mean Zn, Cu and Mn concentration increased significantly in bucks in group 3. The literature dealing with the effect of vitamin E on the seminal plasma concentration of trace elements is scarce. Cu is a part of SOD and thus involved in scavenging free radicals (Khan, 2011). Zn deficiency may cause gonadal dysfunction, low testicular weight and impaired growth of seminiferous tubules (Chan et al., 1998). It has been known that Mn deficiency may cause impaired or depressed reproductive efficiency. Mn is an essential part of SOD (Khan, 2011), and its deficiency may adversely affect the seminal plasma SOD. The literature review is scarce on the seminal plasma trace minerals concentration under the influence of vitamin E in bucks. The increased availability of trace minerals in the seminal plasma due to the supplementation of vitamin E in broiler breeder have been previously documented (Khan et al., 2012c). The increased seminal plasma trace elements may be due to the special characteristics of the vitamin E to absorb these minerals.

In the present study, we observed that 800 IU/animal/day had no beneficial impact on the sperm parameters, antioxidant enzymes and trace minerals concentrations. Our results are similar to the findings of Hong et al. (2010) who reported that when Boer kids were supplemented with 0, 80, 320 and 880 IU/kid/day vitamin E for 5 months, the higher level (880 IU/kid/day) had no effect on the antioxidant ability of testis. In line with our results, Yue et al. (2010) found that 1000 and 2400 IU/sheep/day vitamin E had no beneficial effect on the semen quality and antioxidant capacity of Aohan fine-wool sheep. The pro-oxidant behaviour of vitamin E under high doses has previously been reported (Bowry et al., 1995; Santanam and Parthasarathy, 1995; Pearson et al., 2006; Winterbone et al., 2007). Explaining the mechanism of pro-oxidant effect of vitamin E, Winterbone et al. (2007) suggested that encountering with a ROS, the molecule of vitamin E becomes oxidized which require another antioxidant (e.g. Ascorbic acid) to become regenerate. If the tocopherol radical is not neutralized, there is an increase in lipid peroxidation which is also called tocopherol-mediated peroxidation (Stocker, 1999). This process causes production of large number of ROS which result in pro-oxidant effect of vitamin E.

Conclusion

It is suggested that vitamin E at the rate of 400 IU/buck/day supported higher semen volume, per cent motility, per cent live spermatozoa, antioxidants (SOD, GPx) and trace minerals (Zn, Cu, Mn) in the seminal plasma and lowered AST; however, the dose of 800 IU/buck/day did not improve the semen quality showing the adverse effect.

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References

Agarwal, A.; Virk, G.; Ong, C.; du Plessis, S. S., 2014: Effect of oxidative stress on male reproduction. *The World Journal of Men's Health* **32**, 1–17.

Agarwal, A.; Saleh, R. A.; Bedaiwy, M. A., 2003: Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertility and Sterility* **79**, 829–843.

Al-Daraji, H. J., 2012: Effect of adding orange juice into semen diluents on quality and storage ability of cocks' semen. *Research Opinions in Animal & Veterinary Sciences* **2**, 485–489.

Al-Daraji, H. J.; Al-Mashadani, H. A.; Mirza, H. A.; Al-Hayani, W. K.; Al-Hassani, A. S., 2011: Effect of dietary parsley (*Petroselinium crispum*) supplementation on semen quality of local Iraqi ganders. *Research Opinions in Animal & Veterinary Sciences* **1**, 525–529.

Blom, E., 1950: A one-minute live-dead sperm stain by means of eosin–nigrosin. *Fertility and Sterility* **1**, 176–177.

Bowry, V. M.; Igold, S.; Stocker, R., 1995: Vitamin E in human low-density lipoprotein. When and how this antioxidant becomes a pro-oxidant. *The Biochemical Journal* **288**, 341–344.

Brzezinska-Slebodzinska, E.; Slebodzinski, A. B.; Pietras, B.; Wieczorek, G., 1995: Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. *Biological Trace Element Research* **47**, 69–74.

Castillo, C.; Benedito, J. L.; Lopez-alonso, M.; Miranda, M.; Hernandez, J., 2001: Importancia del estrés oxidativo en ganado vacuno: en relación con el estado fisiológico (preñez y parto) y la nutrición. *Archivos de medicina veterinaria* **33**, 5–20.

Chan, S.; Gerson, B.; Subramaniam, S., 1998: The role of copper, molybdenum, selenium, and zinc in nutrition and health. *Clinics in Laboratory Medicine* **18**, 673–685.

De Lamirande, E.; Gagnon, C., 1995: Human sperm hyperactivation in whole semen and its association with low superoxide scavenging capacity in seminal plasma. *Fertility and Sterility* **59**, 1291–1295.

Duncan, D. B., 1955: Multiple range and multiple F-test. *Biometrics* **11**, 1–42.

Hashem, N. M.; El-Hady, A. A.; Hassan, O., 2013: Effect of vitamin E or propolis supplementation on semen quality, oxidative status and hemato-biochemical changes of rabbit bucks during hot season. *Livestock Science* **157**, 520–526.

Hong, Z.; Hailing, L.; Hui, M.; Guijie, Z.; Leyan, Y.; Dubing, Y., 2010: Effect of Vitamin E supplement in diet on antioxidant ability of testis in Boer goat. *Animal Reproduction Science* **117**, 90–94.

Khan, R. U., 2011: Antioxidants and poultry semen quality. *World' Poultry Science Journal* **67**, 297–308.

Khan, R. U.; Rahman, Z. U.; Javed, I.; Muhammad, F., 2012a: Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders. *Animal Reproduction Science* **135**, 85–90.

Khan, R. U.; Laudadio, V.; Tufarelli, V., 2012b: Semen traits and seminal plasma biochemical parameters in White Leghorn layer breeder. *Reproduction in Domestic Animals* **47**, 190–195.

Khan, R. U.; Rahman, Z. U.; Javed, I.; Muhammad, F., 2012c: Effects of vitamins, probiotics and protein level on semen traits and some seminal plasma macro and micro minerals of male broiler breeders after zinc-induced molting. *Biological Trace Element Research* **148**, 44–52.

Khan, R. U.; Rahman, Z. U.; Javed, I.; Muhammad, F., 2013: Effect of vitamins, probiotics and protein level on semen traits and seminal plasma biochemical parameters of post-moult male broiler breeders. *British Poultry Science* **54**, 120–129.

Kothari, S.; Thompson, A.; Agarwal, A.; de Pessis, S. S., 2010: Free radicals: their beneficial and detrimental effects on sperm function. *Indian Journal of Experimental Biology* **48**, 425–435.

Luo, H. L.; Jia, Z. H.; Zhu, S. E.; Ding, J. Z., 2004: Effect of vitamin E on the qualities of fresh and frozen thawed ram semen. *China Herbivores* **24**, 14–16 (in Chinese).

Mahmood, G. B., Abdel-Raheem, S. M., Hussain, H. A., 2013: Effect of combination of vitamin E and selenium injections on reproductive performance and blood parameters of Ossimi rams. *Small Ruminant Research* **113**, 103–108.

Panda, A. K.; Cherian, G., 2014: Role of vitamin E in counteracting oxidative stress in poultry. *The Journal of Poultry Science* **51**, 109–117.

Pearson, P.; Lewis, S. A.; Britton, J.; Young, I. S.; Fogarty, A., 2006: The pro-oxidant activity of high-dose vitamin E supplements *in vivo*. *BioDrugs: Clinical Immunotherapeutics, Biopharmaceuticals and Gene Therapy* **20**, 271–273.

Rahman, H. U.; Qureshi, M. S.; Khan, R. U., 2014: Influence of dietary zinc on semen traits and seminal plasma antioxidant enzymes and trace minerals of Beetal bucks. *Reproduction in Domestic Animals* **49**, 1004–1007.

Santanam, N.; Parthasarathy, S., 1995: Paradoxical actions of antioxidants in the oxidation of low density lipoprotein by peroxidases. *The Journal of Clinical Investigation* **95**, 2594–2600.

Steel, R. G. D.; Torrie, J. H.; Dieky, D. A., 1997: *Principles and Procedures of Statistics*, 3rd edn. McGraw Hill Book, New York.

Stocker, R., 1999: The ambivalence of vitamin E in atherogenesis. *Trends in Biochemical Sciences* **24**, 219–223.

Surai, P. F.; Kostjuk, I.; Wishart, G.; Pherson, A. M. A. C.; Speake, B. K.; Noble, R. C.; Ionov, I.; Kutz, E., 1998: Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes, and liver. *Biological Trace Element Research* **64**, 119–132.

Wilson, M. J.; Kaye, D.; Edward Smith, W.; Quach, H. T.; Sinha, A. A.; Vatassery, G. T., 2003: Effect of vitamin E deficiency on the growth and secretory function of the rat prostatic complex. *Experimental and Molecular Pathology* **74**, 267–275.

Winterbone, M. S.; Sampson, M. J.; Saha, S.; Hughes, J. C.; Hughes, D. A., 2007: Pro-oxidant effect of α-tocopherol in patients with Type 2 Diabetes after an oral glucose tolerance test – a randomized controlled trial. *Cardiovascular Diabetology* **6**, 8.

Yousef, M. I., 2010: Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits. *Food and Chemical Toxicology* **48**, 1152–1159.

Yousef, M. I.; Abdallah, G. A.; Kamel, K. I., 2003: Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. *Animal Reproduction Science* **76**, 99–111.

Yue, D.; Yan, L.; Luo, H.; Xu, X.; Jin, X., 2010: Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. *Animal Reproduction Science* **118**, 217–222.