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Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep

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ABSTRACT

Thirty male Aohan fine-wool sheep (5 months of age) with similar body weight were procured from the Aohan fine-wool sheep breeding farm of Inner Mongolia Autonomous Region, China. The sheep were divided randomly into five groups, which were labeled as Group 1, 2, 3, 4 and 5 and supplemented respectively with 0, 20, 200, 1000 or 2400 IU sheep^{-1} d^{-1} Vitamin E, for 12 months, respectively. Three Aohan fine-wool sheep in each group were selected randomly for semen collection at the age of 16 months, then slaughtered at 17 months to collect the testis sample for testicular cell membranal and mitochondrial antioxidant abilities analysis. The results showed that supplementing Vitamin E at the concentration of 200 IU sheep^{-1} d^{-1} in diets may have a positive effect in increasing semen quality and quantity ($P < 0.05$), significantly reduce malondialdehyde (MDA) level and improve the activities of superoxide dismutase (SOD)and glutathione peroxidase (GSH-PX) in testicular cell membrane and mitochondria ($P < 0.05$). In conclusion, the present study demonstrated that that supplementing Vitamin E can have a positive role in improving semen quality via protecting testicular cell membrane and mitochondria from antioxidant abilities. However, the optimal level of Vitamin supplement has still to be determined.

1. Introduction

Vitamin E includes a group of lipid soluble compounds, tocopherols and tocotrienols that act as antioxidants defending the organism against oxidative stress. α-Tocopherol, the most abundant and active Vitamin E family member is a chain-breaking antioxidant that prevents the propagation of reactive oxygen species (ROS) reactions (Mardones et al., 2002). Vitamin E is believed to be the primary components of the antioxidant system of the spermatozoa (Surai et al., 1998) and is one of the major membrane protectants against ROS and lipid peroxidation (Akiyama, 1999). Supplemental Vitamin E has been shown to increase total sperm output and sperm concentration in boars (Brzezinska-Slebodzinska et al., 1995), rabbits (Yousef et al., 2003) and rams (Luo et al., 2004). Deficiency of Vitamin E may lead to reproductive organ damage, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Wu et al., 1973; Wilson et al., 2003). Toxicity of accumulating ROS to the membrane structure, leads to a change in permeability and probably to disintegration of cellular organelles (e.g. mitochondria, endoplasmic reticulum, etc.), impairs cellular structure and destroys membranes by interacting with biomolecules that can also induce oxidative stress due to their high reactivity (Muller and Ohnesorge, 1982; Soo et al., 2007; Zhu et al., 2009). Testes, being rich in polyunsaturated fatty acids and having poor antioxidant defences are

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much more vulnerable to peroxidation injury than other tissues (Jones et al., 1979).

As stated above, Vitamin E as natural antioxidants might be helpful in preventing oxidative stress related functional abnormalities in testis. Although many studies have established the effectiveness of Vitamin E against oxidative stress in several animal organs, evidence for effects on the subcellular organelle in the cell is scant. Our previous study (Luo et al., 2004; Liu et al., 2005; Zhu et al., 2009, in press) proved that supplementing Vitamin E in diet improves the qualities of fresh and frozen thawed ram semen in sheep, stimulates significantly the development of reproductive organs in Boer goats, protect testis from damage by preoxidation. The objectives of the present study, as a part of a series of our research about fat-soluble vitamins (Vitamin E), were to investigate the effect of Vitamin E supplementation on semen quality and testicular membranal and mitochondrial oxidative stress of Aohan fine-wool sheep.

2. Materials and methods

2.1. Animals and management

Thirty male Aohan fine-wool sheep (local bred breed) (5 months of age) with similar body weight were procured from the Aohan fine-wool sheep breeding farm of Inner Mongolia Autonomous Region, China. A basic ration was fed in this study with a forage/concentrate ratio of 6/4. The formulation is shown in Table 1 and was developed according to the NRC feeding standard (1985).

Thirty male Aohan fine-wool sheep were divided randomly into five groups ($N = 6$), which are 0, 1, 10, 50 and 120 times of NRC feeding standard (1985). Supplementation was at 0, 20, 200, 1000 or 2400 IU sheep^{-1} d^{-1} for 12 months. These dose levels were based on previous research findings (Luo et al., 2004; Liu et al., 2005; Zhu et al., 2009, in press). Treatments will be referred to as Group 1, 2, 3, 4 and 5 respectively. The control group were receiving 4.3 IU sheep^{-1} d^{-1} Vitamin E which existed in basal diet. Three Aohan fine-wool sheep in each group were randomly selected for semen collection at the age of 16 months and then slaughtered at the age of 17 months to collect the samples for antioxidant abilities analysis.

Table 1
The composition and nutrient level of the diet and concentrate.

Ingredients	%	Nutrient contents	of DM
Diet			
Forge	60	CP (%)	4.86
Concentrate	40	EE (%)	8.97
		NDF (%)	31.23
		ADF (%)	22.33
		Vitamin E (IU kg^{-1})	3.42
Concentrate			
Corn	62	CP (%)	18.68
Soybean meal	26	EE (%)	14.41
Wheat bran	8	NDF (%)	10.14
$CaHPO_4$	2	ADF (%)	6.52
Salt	1	Vitamin E (IU kg^{-1})	7.56
Additives	1		

CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre.

In this study, all procedures involving animals were conducted under the approval of China Agricultural University Animal Care and Use Committee. Vitamin E powder consisting of Vitamin E acetate (1 mg contains 1 IU Vitamin E) was bought from the Zhejiang Guobang Pharmaceutical Co., Ltd. (China).

2.2. Semen collection and analysis

Semen collection was performed with a standardised procedure by presenting female sheep in oestrus to the rams. Semen was collected by use of a heated artificial vagina and collection vial, twice 1 week, during a 4-week period. Temperature of the water in the lining of the artificial vagina ranged from 39 to 42 °C at the time of seminal collection. The semen was analysed immediately after collection according to Marti et al. (2008).

The volume of each ejaculate was recorded using a disposable syringe of 2 ml. Sperm concentration was determined using semen diluted with 3% NaCl, the diluted semen was placed on a hemocytometer with the sperm counted in five squares of one chamber. Total sperm output was calculated by multiplying semen ejaculate volume and semen concentration.

Sperm motility was identified as those sperm cells that demonstrated progressive motility. Sperm motility was scored from 0 to 100% by a qualified and experienced investigator. Semen was placed on a heated glass slide, and scoring was performed at microscopic magnification of 200×. Each sample was evaluated twice. The mean value was used for data analysis.

Tailless sperm, those with coiled, bent, or shoehook tails, and those with cytoplasmic droplets were counted as abnormal sperm, and the percentage of the abnormal and nomal sperm was calculated. Assessment of abnormal and nomal spermatozoa was performed using an eosin–nigrosin blue staining mixture (Marin et al., 1997).

2.3. Isolation of testicular cell membrane and mitochondria fractions

Testis was dissected out quickly, cleaned in ice-cold normal saline (0.9%, w/v), pat dried and weighed. A 10% (w/v) homogenate of testis was prepared using a Potter-Elvejhem motor driven glass Teflon homogenizer in ice-cold phosphate buffer (50 mM, pH 7.4) containing 0.25 M sucrose. The crude homogenate was filtered through four-layers of absorbent gauze before, and the filtrate was centrifuged at 600 × g for 10 min at 4 °C to precipitate nuclei and cellular debris. The supernatant was centrifuged at 2000 × g for 15 min at 4 °C to collect the cell membrane fraction which is composed of bigger fragments (Dealtry and Rickwood, 1992; Spector et al., 2001).

The supernatant obtained was then centrifuged at 10,000 × g for 15 min at 4 °C to separate the mitochondrial pellet. The cell membrane fraction and mitochondrial pellet were washed three times with phosphate buffer (50 mM, pH 7.4) each time the precipitation was obtained through centrifugation at 2000 × g or 10,000 × g for 5 min at 4 °C (Sahoo et al., 2005). The purity of the cell membrane fraction and mitochondrial fraction obtained by differen-

Table 2
Effect of Vitamin E supplementation on semen quantity and quality.

Items	Group 1	Group 2	Group 3	Group 4	Group 5
EV (ml)	0.85 ± 0.05^{a}	1.08 ± 0.06^{b}	1.21 ± 0.11^{b}	1.09 ± 0.07^{b}	1.03 ± 0.06^{ab}
Sp.Conc. ($\times 10^8$ ml^{-1})	27.5 ± 2.1^{a}	30.1 ± 4.5^{ab}	38.9 ± 3.1^{b}	27.0 ± 2.8^{a}	25.2 ± 3.2^{a}
T.Sp.O. ($\times 10^8$)	24.7 ± 2.8^{a}	31.3 ± 4.1^{a}	43.6 ± 3.9^{b}	31.2 ± 5.3^{a}	27.5 ± 3.6^{a}
SM (%)	52.9 ± 6.6^{a}	67.5 ± 6.7^{ab}	73.7 ± 2.4^{b}	59.7 ± 3.7^{ab}	49.2 ± 5.7^{a}
NM Sp. (%)	85.9 ± 1.3^{ab}	85.9 ± 1.2^{ab}	90.2 ± 0.9^{b}	85.2 ± 2.1^{ab}	82.7 ± 2.4^{a}
Ab.Sp. (%)	14.1 ± 1.3^{ab}	14.0 ± 1.2^{ab}	9.8 ± 0.9^{b}	14.8 ± 2.1^{ab}	17.3 ± 2.4^{a}

Values in same row with different superscripts differ significantly ($P < 0.05$). EV: ejaculate volume; Sp.Conc.: sperm concentration; T.Sp.O.: total sperm output; SM: sperm motility; NM: normal sperm; Ab.Sp.: abnormal sperm.

tial centrifugation, was assessed by means of the specific activity of 5′-nucleotidase and succinate dehydrogenase enzyme. The cell membrane fraction and mitochondrial fraction were used immediately for various biochemical analyses. Protein content of samples was estimated using the method of Bradford (1976).

2.4. Antioxidant enzyme activity

The antioxidant enzyme activity assay, such as SOD (superoxide dismutase), MDA (malondialdehyde) and GSH-PX (glutathione peroxidase), were tested by means of commercially available assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

2.5. Statistical analyses

Data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's new multiple range tests with the SPSS 13.0 software program to determine the level of significance among mean values. Results are expressed as a mean and standard error. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Effect of Vitamin E supplementation on semen quantity and quality

The male Aohan fine-wool sheep quickly adapted to the semen collection procedure. The majority of the treated rams, showed indication of increased libido, especially those rams in Group 3. Data on the changes of the ejaculate volume, sperm concentration, total sperm output, sperm motility and the percentage of the normal and abnormal sperm of rams treated with Vitamin E are presented in Table 2.

The ejaculate volume was significantly increased in Groups 2, 3 and 4 supplemented with Vitamin E compared with the control (Group 1) ($P < 0.05$). Sperm concentration was significantly lower in Groups 1, 4 and 5 than in Group 3 ($P < 0.05$), however, the effect was not significant between Group 2 and Group 3 ($P > 0.05$). Total sperm output in Group 3 was significantly higher compared with other groups ($P < 0.05$). Sperm motility in Group 3 was significantly higher than in Group 1 and Group 5 ($P < 0.05$). The percentage of normal sperm was lower in Group 5 compared to other groups and significantly lower than group 3 ($P < 0.05$). The percentage of abnormal sperm was significantly lower in Group 3 than that in Group 5 ($P < 0.05$), but the difference was not significant among other groups ($P > 0.05$).

3.2. Effect of Vitamin E supplementation on testicular cell membranal antioxidant abilities

The testicular cell membranal MDA, SOD and GSH-PX activities are shown in Table 3. Results showed the testicular cell membranal MDA level in groups supplemented with Vitamin E was significantly lower compared with the controls ($P < 0.05$), and significant changes were observed among Group 3 and other Vitamin E treated groups ($P < 0.05$). As showed in Table 3, the activity of SOD in testicular cell membrane was significantly higher in Group 3 than in other groups ($P < 0.05$), but the effect was not significant among other groups ($P > 0.05$). The testicular cell membranal GSH-PX activity in Group 3 and Group 4 was significantly higher than in Group 1, 2 and 5 ($P < 0.05$), respectively, and significant difference was observed between Group 3 and Group 4 ($P < 0.05$).

3.3. Effect of Vitamin E supplementation on testicular mitochondrial antioxidant abilities

Results in Table 4 showed the testicular mitochondrial MDA level was significantly lower in Group 3 than in Groups 1 and 4 ($P < 0.05$). The activity of SOD of testicular mitochondria in Group 3 was significantly higher than in other group ($P < 0.05$). The testicular mitochondrial GSH-PX activity was also significantly higher in Group 3 than in other groups ($P < 0.05$), however, significant decrease was observed in Group 5 compared with other groups ($P < 0.05$).

Table 3
Effect of Vitamin E supplementation on testicular cell membranal antioxidant abilities of Aohan fine-wool sheep.

Items	Group 1	Group 2	Group 3	Group 4	Group 5
MDA (nmol/mg prot)	8.68 ± 0.14^{a}	7.13 ± 0.20^{b}	4.47 ± 0.41^{c}	6.19 ± 0.44^{b}	6.07 ± 0.72^{b}
SOD (U/mg prot)	52.33 ± 1.24^{a}	50.80 ± 3.70^{a}	77.98 ± 6.24^{b}	62.40 ± 6.01^{a}	58.72 ± 4.12^{a}
GSH-PX (activity unit)	28.69 ± 0.32^{a}	29.39 ± 3.41^{a}	62.03 ± 4.86^{c}	52.65 ± 2.88^{b}	31.56 ± 0.38^{a}

Values in same row with different superscripts differ significantly ($P < 0.05$).

Table 4
Effect of Vitamin E supplementation on antioxidant abilities in testicular mitochondria of Aohan fine-wool sheep.

Items	Group 1	Group 2	Group 3	Group 4	Group 5
MDA (nmol/mg prot)	5.42 ± 0.35^{a}	4.78 ± 0.76^{ab}	3.41 ± 0.27^{b}	5.07 ± 0.39^{a}	4.36 ± 0.25^{ab}
SOD (U/mg prot)	28.33 ± 1.19^{a}	28.76 ± 1.10^{a}	43.45 ± 3.48^{b}	31.23 ± 0.50^{a}	31.19 ± 1.15^{a}
GSH-PX (activity unit)	28.17 ± 3.18^{a}	33.11 ± 4.27^{a}	48.83 ± 2.47^{b}	32.16 ± 5.54^{a}	14.66 ± 1.40^{c}

Values in same row with different superscripts differ significantly ($P < 0.05$).

4. Discussion

4.1. Effect of Vitamin E supplementation on semen quantity and quality of Aohan fine-wool sheep

The results of the present study suggest that supplementing Vitamin E could ameliorate semen quality and quantity, especially in the group of supplementation with Vitamin E at the concentration of 200 IU sheep^{-1} d^{-1}. Similar results are also found in boars (Brzezinska-Slebodzinska et al., 1995), humans (Akiyama, 1999), rams (Luo et al., 2004; Liu et al., 2005), rats (Sönmez et al., 2005) and chickens (Cerolini et al., 2006) where supplementation of food rich in Vitamin E improved semen quality and quantity. Marin et al. (1997) reported Vitamin E deficiency in boars adversely affected sperm motility compared to animals given a 220 IU kg^{-1} supplemental Vitamin E.

Oxidative stress affects the organism when the generation of ROS products exceeds the capacity of the cells to protect or repair themselves (Venditti and Meo, 2006). Free radicals are highly ROS that have been implicated in the pathogenesis of many diseases including degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Wu et al., 1973; Wilson et al., 2003; Zhu et al., 2009, in press). ROS can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired (Devi et al., 2000; Yousef et al., 2003). In normal circumstances, male reproductive organs and spermaromaza exhibit a capacity to genetate ROS, but there is an equilibrium between the generation of ROS and antioxidant strategies, leaving only a critical amount of ROS required for normal sperm functions, as capacitation, acrosome reaction and fusion with the oocyte membrane. Excessive production of ROS, however, results in destruction of the antioxidant capacity of tissues which initiates peroxidation of the unsaturated fatty acids in the sperm plasma membrane therefore resulting in defective sperm function (Yousef et al., 2003).

The beneficial effects of Vitamin E noted in the present study can be attributed to the antioxidant effects similar to those reported by Sen et al. (2004), Vitamin E as a lipid soluble antioxidant plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals (particularly strong scavenger of hydroxyl radicals) which are toxic byproducts of many metabolic processes in biological membranes (Akiyama, 1999; Takanami et al., 2000; Verma and Nair, 2001; Sundararaian et al., 2006). In addition, Vitamin E is important in maintaining the physiological integrity of testis, epididymis and accessory glands (Sönmez et al., 2005; Cerolini et al., 2006), which is critical in spermatogenesis and sperm maturation thus improving sperm quality and quantity. Luo et al. (2004) reported that Vitamin E may have effect on sexual function by regulating the secretion of gonadotropin in anterior pituitary, then playing a positive role in promotion of spermatogenesis and semen motility.

4.2. Effect of Vitamin E supplementation on testicular cell membranal and mitochondrial antioxidant abilities of Aohan fine-wool sheep

4.2.1. Effect of Vitamin E supplementation on testicular cell membranal antioxidant abilities

Direct detection of ROS and other free radicals is difficult, because these molecules are shortlived and highly reactive in a nonspecific manner. Oxidative damage is generally analysed by measurement of secondary products, including derivatives of amino acids, nucleic acids, and lipid peroxidation in the pro-oxidant/antioxidant status (Kohen and Nyska, 2002). MDA is a stable end product of lipid peroxidation and therefore can be used as an indirect measure of the cumulative lipid peroxidation.

Results presented in Table 3 suggest that supplementing Vitamin E in diets could reduce the oxidative damage in testicular cell membrane and mitochondria caused by lipid peroxidation, especially at the concentration of 200 IU sheep^{-1} d^{-1}. In normal circumstances, cell membrane and mitochodria are the main target of ROS due to the high content of polyunsaturated fatty acids (Doran and Halestrap, 2000). Vitamin E may play a majoy protective role against oxidative stress and prevent the production of lipid peroxides by scanvenge ROS in biological membranes, moreover, Vitamin E has effect on activities of antioxidant enzymes which are known to act as primary defence against oxidative stress in testicular cell membrane and mitochondria in this study.

A cell membrane which is a selectively permeable lipid bilayer found in all cells is an important subcellular organelle. It contains a wide variety of biological molecules, primarily proteins and lipids, which are involved in a vast array of cellular processes such as cell adhesion, ion channel conductance and cell signaling. A cell membrane, being very rich in polyunsaturated fatty acids and having poor antioxidant defense system is much more susceptible to oxidative damage. Research findings suggest that oxidative damage to the human testicular cell membrane is an important pathophysiological mechanism in male infertility (Koksal et al., 2003).

SOD dismutates superoxide radicals which produce hydroxyl radicals and subsequently initiate the process of lipid peroxidation to hydrogen peroxidates, which is further neutralized by catelase and GSH-PX (Sahoo et al., 2008). Emanuela et al. (1997) reported that the decreased

SOD activity could be related to the metabolic disorder of some enzymes (i.e. glutathione peroxidase, etc.) and Vitamin E in the blood and tissues caused by certain chemical substances. Thus, in present study the variation of SOD activity may be due to the change of some correlated enzymes and content of Vitamin E in testicular cell membrane, in addition, supplementing Vitamin E may change the content of testicular cell membranal superoxide radicals, futher affect the SOD activity.

GSH-PX, a tripeptide, is an important endogenous intracellular antioxidant. GSH-PX is an enzyme which can oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG) and whose biochemical function of GSH-PX is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Sahoo et al., 2008). In this study, the variation of GSH-PX activity may be in response to the change of SOD activity and lipid hydroperoxides level in testicular cell membrane.

4.2.2. Effect of Vitamin E supplementation on testicular mitochondria antioxidant abilities

Mitochondrial function is also crucial for germ cells during the course of spermatogenesis (Meinhardt et al., 1999), and it has been proposed that ROS are a common mediator of several pathologies that are currently thought to affect the reproductive function (Agarwal et al., 2003) and that mitochondrial function is impaired in infertility (Nakada et al., 2006; Amaral et al., 2008). The mitochondrial electron transport chain is the main source of ROS during normal metabolism. The rate of ROS production from mitochondria is increased in a variety of pathological condition such as hypoxia (Becker et al., 1999), ischemia (Kevin et al., 2003), aging (Moghaddas et al., 2003). Mitochondria are also the main target of ROS and an increase in ROS production results in enhanced mitochondrial component damage. It has already been demonstrated that spermatozoa containing defective mitochondria not only produce ATP in a less efficient way, but also generate higher oxidative stress, leading to a decline in fertility. In addition, mitochondria are also involved in apoptosis (Crompton, 1999; Doran and Halestrap, 2000).

As showed in Table 4, Vitamin E supplementation decreased testicular mitochondrial MDA level compared to the control animals. This was most apparent for sheep supplemented with 200 IU sheep^{-1} d^{-1}.

Subudhi et al. (2008) studied the effect of Vitamin E and curcumin on l-thyroxine-induced rat liver oxidative stress and showed that Vitamin E and curcumin are efficient in protecting liver mitochondria from oxidative stress generated by l-thyroxine. However, Sahoo et al. (2008) reported that both Vitamin E and curcumin significantly increased SOD activity but decreased GSH-PX activity in mitochondria of testis of T4-treated rats. These results suggested that the mechanism of Vitamin E on the oxidative stress of the testicular mitochondria was very complex and further research should be done.

5. Conclusions

The present study demonstrated that supplementing Vitamin E in concentrate can improve semen quality and ameliorate testicular cell membranal and mitochondrial antioxidant abilities of Aohan fine-wool sheep, especially at 200 IU sheep^{-1} d^{-1} supplement concentration. These results indicate that supplementing Vitamin E has a positive role in improving semen quality via protecting testicular cell membrane and mitochondria from oxidative stress. However, the responses to Vitamin E in diets were not proportional to dose concentrations.

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References

Agarwal, A., Saleh, R.A., Bedaiwy, M.A., 2003. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil. Steril. 79, 829–843.

Akiyama, M., 1999. In vivo scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nippon Hinyokika Gakkai Zasshi 90, 421–428.

Amaral, S., Oliveira, P.J., Ramalho-Santos, J., 2008. Diabetes and impairment of reproductive function: possible role of mitochondria and reactive oxygen species. Curr. Diabetes Rev. 4, 46–54.

Becker, L.B., vanden Hoek, T.L., Shao, Z.H., Li, C.Q., Schumacker, P.T., 1999. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. Am. J. Phys. 46, 2240–2246.

Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

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. Biol. Trace Elem. Res. 47, 69–74.

Cerolini, S., Zaniboni, L., Maldjian, A., Gliozzi, T., 2006. Effect of docosahexaenoic acid and α-tocopherol enrichment in chicken sperm on semen quality, sperm lipid composition and susceptibility to peroxidation. Theriogenology 66, 877–886.

Crompton, M., 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem. J. 341, 233–249.

Dealtry, G.B., Rickwood, D., 1992. Cell Biology Labfax. BIOS Scientific Publisher oxford, United Kingdom.

Devi, G.S., Prasad, M.H., Saraswathi, I., Raghu, D., Rao, D.N., Reddy, P.P., 2000. Free radicals antioxidant enzymes and lipid peroxidation in different types of leukemias. Clin. Chim. Acta 293, 53–62.

Doran, E., Halestrap, A.P., 2000. Cytochrome c release from isolated rat liver mitochondria can occur independently of outer-membrane rupture: possible role of contact sites. Biochem. J. 348, 343–350.

Emanuela, C., Katalin, B., Arthruh, T., 1997. Comparative analysis of antibody and cell-mediated autoimmunity to transaldolase and myelin basic protein in patients with multiple sclerosis. J. Clin. Invest. 99, 1238–1245.

Jones, R., Mann, T., Sherins, R.J., 1979. Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal properties of fatty acid peroxides and protective action of seminal plasma. Fertil. Steril. 31, 531–537.

Kevin, L.G., Camara, A.K., Riess, M.L., Novalija, E., Stowe, D.F., 2003. Ischemic preconditioning alters real time measure of O_2 radicals in intact hearts with ischemia and reperfusion. Am. J. Physiol. Heart. Circ. Physiol. 539, 566–574.

Kohen, R., Nyska, A., 2002. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol. Pathol. 30, 620–650.

Koksal, I.T., Usta, M., Orhan, I., Abbasoglu, S., Kadioglu, A., 2003. Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J. Androl. 5, 95–99.

Liu, H.B., Luo, H.L., Liu, Z.H., Shi, Z.H., Zhao, H.B., 2005. Effect of dietary vitamin E concentration on semen quality of goat. Chin. J. Anim. Sci. 10, 12–14 (in Chinese).

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).
Mardones, P., Strobel, P., Miranda, S., Leighton, F., Quinones, V., Amigo, L., Rozowski, J., Krieger, M., Rigotti, A., 2002. α-Tocopherol metabolism is abnormal in scavenger receptor class B type I (SR-BI)-deficient mice. J. Nutr. 132, 443–449.
Marin, G.J., Mahan, D.C., Chung, Y.K., Pate, J.L., Pope, W.F., 1997. Effects of dietary selenium and vitamin E on boar performance and tissue responses, semen quality and subsequent fertilization rates in mature gilts. J. Anim. Sci. 75, 2994–3003.
Marti, E., Marti, J.I., Muiño-Blanco, T., Cebrián-Préz, J.A., 2008. Effect of the cryopreservation process on the activity and immunolocalization of antioxidant enzymes in ram spermatozoa. J. Androl. 29, 459–467.
Meinhardt, A., Wilhelm, B., Seitz, J., 1999. Expression of mitochondrial marker proteins during spermatogenesis. Hum. Reprod. Update 5, 108–119.
Moghaddas, S., Hoppel, C.L., Lesnefsky, E.J., 2003. Aging defect at the QO site of complex III augments oxyradical production in rat heart interfibrillar mitochondria. Arch. Biochem. Biophys. 414, 59–66.
Muller, L., Ohnesorge, F.K., 1982. Different response of liver parenchymal cells from starved and fed rats to cadmium. Toxicology 25, 141–150.
Nakada, K., Sato, A., Yoshida, K., Morita, T., Tanaka, H., Inoue, S., Yonekawa, H., Hayashi, J., 2006. Mitochondria-related male infertility. Proc. Natl. Acad. Sci. U.S.A. 103, 15148–15153.
NRC, 1985. Nutrient Requirements of Sheep. Natl. Acad. Sci., Washington, DC.
Sahoo, D.K., Roy, A., Bhanja, S., Chainy, G.B.N., 2005. Experimental hyperthyroidism induced oxidative stress and impairment of antioxidant defence system in rat testis. Ind. J. Exp. Biol. 43, 1058–1067.
Sahoo, D.K., Roy, A., Bhanja, S., Chainy, G.B.N., 2008. Hypothyroidism impairs antioxidant defence system and testicular physiology during development and maturation. Gen. Comp. Endocrinol. 156, 63–70.
Sen, G.R., Sen, G.E., Dhakal, B.K., Thakur, A.R., Ahnn, J., 2004. Vitamin C and Vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol. Cells 17, 132–139.
Sönmez, M., Türk, G., Yüce, A., 2005. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of Wistar rats. Theriogenology 63, 2063–2072.
Soo, I.C., Bohwan, J., Pilju, Y., Changbo, P., Jung, D.P., Doug, Y.R., 2007. Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis. Toxicol. Appl. Pharmacol. 218, 196–203.
Spector, D.L., Goldman, R.D., Leinward, L.A., 2001. Cells: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, America.
Sundararaian, R., Haja, N.A., Venkatesan, K., Mukherjee, K., Saha, B.P., Bandyopadhyay, A., Mukherjee, P.K., 2006. Cytisus scoparius link a natural antioxidant. BMC Complement. Alter. Med. 6, 8.
Subudhi, U., Das, k., Paital, B., Bhanja, S., Chainy, G.B.N., 2008. Alleviation of enhanced oxidative stress and oxygen consumption of L-thyroxine induced hyperthyroid rat liver mitochondria by vitamin E and curcumin. Chem. Biol. Interact. 173, 105–114.
Surai, P., Kostjuk, I., Wishart, G., Macpherson, A., Speake, B., Noble, R., 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. Biol. Trace Elem. Res. 64, 119–132.
Takanami, Y., Iwane, H., Kawai, Y., Shimomitsu, T., 2000. Vitamin E supplementation and endurance exercise: are there benefits. Sports Med. 29, 73–83.
Venditti, P., Meo, S.D., 2006. Thyroid hormone-induced oxidative stress. Cell. Mol. Life Sci. 63, 414–434.
Verma, R.J., Nair, A., 2001. Ameliorative effect of vitamin E on aflatoxin-induced lipid peroxidation in the testis of mice. Asian J. Androl. 3, 217–221.
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. Exp. Mol. Pathol. 74, 267–275.
Wu, S.H., Oldfield, J.E., Whanger, P.D., Weswig, P.H., 1973. Effect of selenium, vitamin E, and antioxidants on testicular function in rats. Biol. Reprod. 8, 625–629.
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. Anim. Reprod. Sci. 76, 99–111.
Zhu, H., Luo, H.L., Meng, H., Zhang, G.J., 2009. Effect of vitamin E supplementation on development of reproductive organs in Boer goat. Anim. Reprod. Sci. 113, 93–101.
Zhu, H., Luo, H.L., Meng, H., Zhang, G.J., Yan, L.Y., Yue, D.B., in press. Effect of Vitamin E supplement in diet on antioxidant ability of testis in Boer Goat. Anim. Reprod. Sci., doi:10.1016/j.anireprosci.2009.03.016.