



Effect of vitamin E and selenium supplementation on oxidative markers and semen quality parameters in breeding bulls

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The reactive oxygen species (ROS) are produced physiologically in various organs including the testis. Overproduction of ROS can be detrimental to sperm leading to male infertility (Mehrotra *et al.* 2013). Antioxidants protect against the damaging effect of reactive oxygen species on spermatozoa. Vitamin E and selenium (Se) are considered to be the primary components of the antioxidant system of the spermatozoa and one of the major membrane protectants against ROS induced lipid peroxidation (Moslemi and Tavanbakhsh 2011). Selenium is an essential dietary trace element required for the maintenance of male fertility by way of testosterone biosynthesis, formation and normal development of spermatozoa. Both testis and epididymis require exogenously supplied Se in order to synthesize a variety of known selenoproteins, essential for spermiogenesis and post testicular sperm maturation (Moslemi and Tavanbakhsh 2011). In males bred on a low selenium diet, male hypogonadism was found with reduced semen production and deteriorated semen quality. In many areas of the world including India, plants do not provide levels of this element adequate to meet dietary requirements. Vitamin E and selenium act synergistically and affect many biological processes including spermatogenesis (Singh *et al.* 2018) by protecting against oxidative stress. Supplementation with vitamin E and selenium has been reported to improve semen quality and reproductive performance in boar, ram and dogs (Marin-Guzman *et al.* 2012, Domoslawska *et al.* 2019, Ozer Kaya *et al.* 2020). However, the information on effect of vitamin E and Se on semen quality in breeding bulls is meager in scientific literature. The aim of the present study was to evaluate the effect of the combination of vitamin E and Se supplementation on oxidative markers and semen quality parameters in breeding bulls.

The study was conducted to evaluate the effect of vitamin E and selenium supplementation on oxidative markers in seminal plasma and semen quality parameters

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in crossbred (Holstein Friesian × Sahiwal) breeding bulls. Eighteen bulls (six good bulls and twelve poor bulls) were divided into three groups consisting 6 bulls in each (G1, G2 and G3). Six good bulls (G1) served as healthy control. G2 poor bulls were administered vitamin E and selenium @ 10ml SC (Vitamin E 50 mg as DL- α -tocopheryl acetate and selenium 15 mg as sodium selenite) once weekly for three weeks while G3 poor bulls were kept as untreated control. Classification of bulls between good and poor categories was based on initial sperm motility (Good bulls- >60% motile, Poor bulls- <60% motile). Other factors like frequency of freezing and pass/fail on post thaw motility examination were also considered. The bulls were kept under loose housing system in individual pens and maintained on standard feeding schedule and management conditions. The semen samples were collected on a regular schedule using an artificial vagina.

Seminal plasma samples were collected and semen quality and biochemical parameters were done at fortnightly interval upto two months. The semen samples were centrifuged (10 min, 8000 × g, 4°C) and seminal plasma was separated and transferred into 1.5 mL tubes and kept frozen (–80°C) until analysis.

The fresh ejaculates were subjected to evaluation for volume, sperm concentration and initial motility as per standard procedures. Semen volume (ml) was measured using a graduated collection tube to the nearest 0.1 ml. The concentration of spermatozoa was measured with Accucell bovine photometer (IMV, France). Sperm motility was directly observed using Olympus BX40 phase contrast microscope (Olympus, Tokyo, Japan). Hypo-Osmotic Swelling test (HOST) and acrosome integrity were done as per standard procedures.

Oxidative stress parameters were measured based on a colorimetric reaction of the target substance and a subsequent UV/VIS spectrophotometric detection at a specific wavelength. The lipid peroxidation (LPO) was determined by measuring the levels of malondialdehyde (MDA) using thiobarbituric acid (TBA) assay. Superoxide dismutase (SOD) was estimated as per the method of Madesh and Balasubramanian (1998). Catalase activity was

Table 1. Effect of vitamin E and selenium supplementation on seminal oxidative stress parameters in breeding bulls

Parameter	Group	Before supplementation	Post-supplementation days			
			15	30	45	60
MDA (μM)	G1	2.13 $\pm$ 0.09 ^{Aa}	1.61 $\pm$ 0.18 ^{Aa}	1.84 $\pm$ 0.24 ^{Aa}	1.75 $\pm$ 0.16 ^{Aa}	1.73 $\pm$ 0.26 ^{Aa}
	G2	2.48 $\pm$ 0.18 ^{Ba}	2.51 $\pm$ 0.06 ^{Ba}	1.95 $\pm$ 0.21 ^{Bbc}	1.67 $\pm$ 0.27 ^{Abc}	1.76 $\pm$ 0.19 ^{Ac}
	G3	2.49 $\pm$ 0.18 ^{Ba}	2.62 $\pm$ 0.13 ^{Ba}	2.48 $\pm$ 0.10 ^{Ba}	2.44 $\pm$ 0.17 ^{Ba}	2.39 $\pm$ 0.06 ^{Ba}
SOD (U/mg of protein)	G1	1.74 $\pm$ 0.06 ^{Aa}	1.92 $\pm$ 0.08 ^{Aa}	1.91 $\pm$ 0.05 ^{Aa}	1.77 $\pm$ 0.08 ^{Aa}	1.87 $\pm$ 0.08 ^{Aa}
	G2	0.87 $\pm$ 0.04 ^{Ba}	0.95 $\pm$ 0.04 ^{Ba}	1.96 $\pm$ 0.07 ^{Ab}	1.82 $\pm$ 0.07 ^{Ab}	1.91 $\pm$ 0.06 ^{Ab}
	G3	1.05 $\pm$ 0.11 ^{Ba}	0.92 $\pm$ 0.05 ^{Ba}	0.91 $\pm$ 0.04 ^{Ba}	1.06 $\pm$ 0.16 ^{Ba}	0.92 $\pm$ 0.04 ^{Ba}
Catalase (U/mg of protein)	G1	2.20 $\pm$ 0.08 ^{Aa}	2.19 $\pm$ 0.11 ^{Aa}	2.34 $\pm$ 0.11 ^{Aa}	2.25 $\pm$ 0.10 ^{Aa}	2.29 $\pm$ 0.10 ^{Aa}
	G2	1.40 $\pm$ 0.07 ^{Ba}	1.60 $\pm$ 0.20 ^{Ba}	1.77 $\pm$ 0.10 ^{Bab}	2.17 $\pm$ 0.11 ^{Ac}	1.96 $\pm$ 0.14 ^{Aab}
	G3	1.34 $\pm$ 0.08 ^{Ba}	1.38 $\pm$ 0.10 ^{Ba}	1.50 $\pm$ 0.14 ^{Ba}	1.40 $\pm$ 0.04 ^{Ba}	1.45 $\pm$ 0.17 ^{Ba}

Observations having different capital letters in a column and small letters in a row as superscripts differ significantly ($p < 0.05$). G1, Healthy control; G2, treatment group (supplemented with vitamin E and selenium injection @ 10 ml SC weekly for three weeks); G3, untreated control.

determined using method of Bergmeyer and Grable (1983). The data were analyzed by applying ANOVA to find out the statistical difference between the mean values of various parameters of different groups using SPSS 16 for Windows software (SPSS Incorporated Chicago, IL, USA).

The bulls of supplemented group (G2) had significantly ($p < 0.05$) low levels of MDA in post supplementation period from second fortnight onwards as compared to pre-supplementation period and also as compared to bulls of group 3 (Table 1). The activities of SOD significantly ($p < 0.05$) improved during second fortnight onwards in bulls of group 2 as compared to their pre-supplementation values and became at par as with values as in good bulls of group 1. The catalase activity was significantly higher in bulls of supplemented group (G2) during third fortnight onwards as compared to their pre-supplementation values and group 3 animals (Table 1). The ameliorative effects of selenium and vitamin E on oxidative stress markers observed in the present study can be attributed to their antioxidant effects by scavenging oxygen free radicals which are toxic byproducts of many metabolic processes (Mittal *et al.* 2014, Singh *et al.* 2018). Free radicals are highly reactive oxygen species that can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired. Spermatozoa exhibit a capacity to generate ROS and initiate peroxidation of the unsaturated fatty acids in the sperm membrane, which plays a key role in the etiology of male infertility (Mehrotra *et al.* 2013). The intrinsic reactivity of these metabolites in peroxidative damage induced by ROS, particularly hydrogen peroxide (H_2O_2) and the superoxide anion has been proposed as a major cause of defective sperm function in cases of male infertility. Generation of ROS and peroxidation of sperm membrane can have negative effects on semen quality. Similar to results of present study, Akiyama (1999) reported that vitamin E and Se supplementation reduced ROS generation, increased antioxidant profile and improved semen quality. Brzezinska-Slebodzinska *et al.* (1995) observed that seminal plasma superoxide dismutase (SOD) increased with the time of

vitamin E administration in boar. Massaeli *et al.* (1999) also reported that vitamin E was effective in inhibiting lipid peroxidation. The beneficial effect of vitamin E and Se combination observed in present study was possibly due to a reduction in lipid peroxidation potential and increased antioxidant enzyme activity in the seminal plasma of breeding bulls of the supplemented group. Selenium and vitamin E act as a potent antioxidant and protect sperm membrane from oxidative damage caused by reactive oxygen species (Moslemi and Tavanbakhsh 2011).

The supplementation of vitamin E and selenium found to have improved the semen quality in poor bulls (G2) in terms of volume, motility, sperm concentration and HOST. The semen volume was improved significantly ($p < 0.05$) during fourth fortnight in supplemented group (G2) bulls as compared to their pre-supplementation values which was significantly higher than G3 bulls during post supplementation third and fourth fortnights (Table 2). Sperm concentration did not show significant variation within group at different time intervals of trial in the supplemented bulls (G2). However, significantly ($p < 0.05$) higher sperm concentration in bulls of G2 was found as compared to G3 bulls during third and fourth fortnights after supplementation (Table 2). Initial sperm motility was significantly higher in G2 bulls during third and fourth fortnight post-supplementation period than their pre-supplementation values and G3 animals. The values of HOST were significantly improved in G2 animals during post supplementation third fortnight onwards than their pre-supplementation and values of G3 animals (Table 2). The acrosome integrity did not show significant variation in the bulls of different groups on within and between group comparisons during whole study period. The percentage of live sperm cells was significantly ($p < 0.05$) higher during post supplementation fourth fortnight in the bulls of vitamin E and Se supplemented group (G2) as compared to G3 animals. However, within group comparison did not show any significant variation in the percentage of live sperms in G2 animals at different time intervals of study (Table 2). The improvement in semen quality and quantity

Table 2. Effect of vitamin E and selenium supplementation on semen quality parameters of breeding bulls

Parameter	Group	Before supplementation	Post-supplementation days			
			15	30	45	60
Volume (ml)	G1	3.71±0.23 ^{Aa}	4.40±0.53 ^{Aa}	3.56±0.64 ^{Aa}	3.93±0.66 ^{Aa}	3.58±0.73 ^{Aa}
	G2	2.67±0.39 ^{Aa}	4.20±1.07 ^{Aab}	4.13±0.99 ^{Aab}	4.80±0.87 ^{Aab}	6.03±1.06 ^{Ab}
	G3	3.46±0.39 ^{Aa}	4.00±0.65 ^{Aa}	3.43±0.78 ^{Aa}	2.46±0.31 ^{Ba}	4.71±0.72 ^{Ba}
Sperm conc (million/ml)	G1	1015.70±20.29 ^{Aa}	840.54±15.13 ^{Aa}	793.83±13.07 ^{Aa}	999.67±13.83 ^{ABa}	862.17±22.48 ^{ABa}
	G2	979.33±21.72 ^{Aa}	957.50±10.40 ^{Aa}	993.00±19.05 ^{Aa}	1261.80±18.74 ^{Aa}	1309.0±15.38 ^{Aa}
	G3	750.50±18.75 ^{Aa}	809.17±17.35 ^{Aa}	903.33±21.41 ^{Aa}	735.37±12.66 ^{Ba}	776.33±14.87 ^{Ba}
Motility (%)	G1	66.26±9.88 ^{Aa}	60.61±7.92 ^{Aa}	61.66±3.07 ^{Aa}	68.33±3.07 ^{Aa}	65.00±3.41 ^{Aa}
	G2	51.66±6.01 ^{Aa}	55.00±10.56 ^{Aab}	61.67±1.67 ^{Aabc}	70.00±0.00 ^{Abc}	68.33±4.01 ^{Ac}
	G3	46.66±4.21 ^{Aa}	42.50±9.81 ^{Aa}	46.66±9.18 ^{Aa}	50.00±8.56 ^{Ba}	45.00±7.63 ^{Ba}
HOST (%)	G1	58.41±3.85 ^{Aa}	52.10±3.73 ^{Aa}	50.88±1.02 ^{Aa}	56.78±3.71 ^{Aa}	56.91±2.51 ^{Aa}
	G2	46.36±3.23 ^{Ba}	44.16±1.92 ^{Ba}	50.22±3.75 ^{Aab}	57.70±2.65 ^{Ab}	55.76±3.98 ^{Ab}
	G3	41.20±1.35 ^B	42.27±1.96 ^B	44.95±2.98 ^B	41.85±3.06 ^B	40.58±2.07 ^B
Acrosome (%)	G1	86.65±2.03 ^{Aa}	74.58±2.81 ^{Ab}	73.36±1.68 ^{Ab}	79.06±1.68 ^{Ab}	81.28±1.84 ^{Aa}
	G2	71.00±2.94 ^{Aa}	71.85±2.40 ^{Aa}	68.05±4.77 ^{Aa}	72.48±3.28 ^{Aa}	74.80±3.12 ^{Aa}
	G3	67.10±4.77 ^{Aa}	72.55±2.78 ^{Aa}	63.03±6.22 ^{Aa}	63.33±4.23 ^{Ba}	68.01±3.01 ^{Ba}
Live (%)	G1	74.13±4.09 ^{Aa}	71.95±3.31 ^{Aa}	75.73±2.14 ^{Aa}	72.54±3.72 ^{Aa}	70.24±2.74 ^{Aa}
	G2	59.65±3.69 ^{Bab}	60.36±2.62 ^{Bab}	58.93±1.83 ^{Ba}	64.85±2.95 ^{ABab}	68.01±3.57 ^{Ab}
	G3	61.19±2.60 ^{Ba}	58.66±4.00 ^{Ba}	51.66±4.58 ^{Ba}	57.43±2.66 ^{Ba}	60.26±1.31 ^{Ba}

Observations having different capital letters in a column and small letters in a row as superscripts differ significantly ($p < 0.05$). G1, Healthy control; G2, treatment group (supplemented with vitamin E and selenium injection @ 10 ml SC weekly for three weeks); G3, untreated control.

following supplementation of vitamin E and selenium might be due to improvement in the testes function through their beneficial effects on germ cells, sertoli cells, interstitial cells of testes and secretions of accessory sex glands. Vitamin E and selenium are necessary for the development of germ cells in testes during development of spermatozoa and has positive effects on sperm motility and function as well as spermatogenesis (Moslemi and Tavanbakhsh 2011). Furthermore, Se is essential for sertoli cell development and their number in the testes. It affects the interstitial cells of testes through effect on the anterior pituitary hormones secretion and thus enhances testosterone hormone synthesis and secretion in the testes. Fructose synthesis and secretion by the accessory glands is dependent upon the secretion of testosterone hormone by the testes. Thus, the observed increase in sperm motility in bulls of present study could be attributed to the concomitant increase in semen fructose following Se and vitamin E treatment. Motility is critical in enabling the sperm to ascend the female reproductive tract for successful fertilization. Previous studies also showed that vitamin E and selenium improved semen quality and quantity in different animal species. Shi *et al.* (2010) found increased semen volume and sperm motility in goats by adding short-term selenium to their ration. Mahmoud *et al.* (2013) found increased semen volume, sperm motility and sperm concentration in the rams administered with vitamin E and selenium compared with the control groups. Safa *et al.* (2016) found increased progressive sperm motility when they applied 5 g/ml vitamin E and 1% nano-selenium to the rooster. Brzezinska-Slebodzinska *et al.* (1995) found that vitamin E supplementation significantly increased the number of

spermatozoa in boars. In addition, vitamin E deficiency in boars adversely affected sperm motility compared to animals given supplemental vitamin E (Marin-Guzman *et al.* 2012). Dietary deficiencies of vitamin E in growing males cause degenerative spermatogonium resulting in a lower sperm concentration (Marin-Guzman *et al.* 2012). On the contrary, some studies have reported that there were no positive effects of vitamin E and selenium supplementation on sperm quality in humans and boars (Hawkes and Turek 2001, Audit *et al.* 2004). This discrepancy may probably be due to differences among animal species or treatments.

SUMMARY

The objective of the study was to evaluate the effect of vitamin E and Se supplementation on oxidative markers and semen quality parameters in breeding bulls. The study was conducted at ICAR-Central Institute for Research on Cattle, Meerut (subtropical climate), Uttar Pradesh during 2019. Eighteen bulls (six good bulls and twelve poor bulls) were divided into three groups consisting 6 bulls in each. Six good bulls of group 1 served as healthy control. Group 2 poor bulls were administered Vitamin E and Selenium @ 10 ml SC (Vitamin E 50 mg as DL- α -tocopheryl acetate and Selenium 15 mg as sodium selenite) once weekly for three weeks while group 3 poor bulls were kept as untreated control. Semen samples were collected and semen quality and biochemical parameters were done using standard procedures at fortnightly interval upto two months. Significantly low MDA and higher SOD and catalase were observed in bulls of supplemented group. Semen volume, sperm concentration, initial motility, percentage of live

sperm and hypo-osmotic swelling test improved significantly in supplemented group as compared to non-supplemented bulls. The study concludes that supplementation of vitamin E and Se causes significant improvement in oxidative stress markers and semen quality parameters in breeding bulls.

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REFERENCES

- Akiyama M. 1999. *In vivo* scavenging effect of ethylcysteine on reactive oxygen species in human semen. *Nippon Hinyokika Gakkai Zasshi* **90**: 421–28.
- Audit I, Laforst J P, Martineau G P and Matte J J. 2004. Effect of vitamin supplements on some aspect of performance, vitamin status, and semen quality in boars. *Journal of Animal Sciences* **82**: 626–33.
- Bergmeyer H U J and Grabl M. 1983. *Methods of Enzymatic Analysis*. 3rd edn. Weinheim Verlagchemie. pp. 273–302
- Brzezinska-Slebodzinska E, Slebodzinska A B, Pietras B and Wiczorek G. 1995. Antioxidant effect of vitamin E and glutathione on lipid peroxidation in boar semen plasma. *Biological Trace Element Research* **47**: 69–74.
- Domoslawska A, Zduńczyk S and Janowski T. 2019. Improvement of sperm motility within one month under selenium and vitamin E supplementation in four infertile dogs with low selenium status. *Journal of Veterinary Research* **63**(2): 293–97.
- Hawkes W C and Turek P. 2001. Effects of dietary selenium on sperm motility in healthy men. *Journal of Andrology* **22**(5): 764–72.
- Madesh M and Balasubramanian A K. 1998. Microtiter plate assay for SOD using MTT reduction by superoxide. *Indian Journal of Biochemistry and Biophysics* **35**: 184–88.
- Mahmoud G B, Abdel-Raheem S M and Hussein 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**: 103–08.
- Marin-Guzman J, Mahan D C and Whitmoyer R. 2012. Effect of dietary selenium and vitamin E on the ultrastructure and ATP concentration of boar spermatozoa, and the efficacy of added sodium selenite in extended semen on sperm motility. *Journal of Animal Sciences* **78**: 1544–50.
- Massaeli H, Sobrattee S and Pierce G N. 1999. The importance of lipid solubility in antioxidants and free radical generating systems for determining lipoprotein peroxidation. *Free Radical Biology and Medicine* **26**: 1524–30.
- Mehrotra A, Katiyar D K, Agarwal A, Das V and Pant K K. 2013. Role of total antioxidant capacity and lipid peroxidation in fertile and infertile men. *Biomedical Research* **24**(3): 347–52.
- Mittal P K, Anand M, Madan A K, Yadav S and Kumar J. 2014. Antioxidative capacity of vitamin E, vitamin C and their combination in cryopreserved Bhadavari bull semen. *Veterinary World* **7**(12): 1127–31.
- Moslemi M K and Tavanbakhsh S. 2011. Selenium–vitamin E supplementation in infertile men: Effects on semen parameters and pregnancy rate. *International Journal of General Medicine* **4**: 99–104.
- Ozer Kaya S, Gur S, Erisir M, Kandemir F M, Benzer F, Kaya E, Turk G and Sonmez M. 2020. Influence of vitamin E and vitamin E-selenium combination on arginase activity, nitric oxide level and some spermatological properties in ram semen. *Reproduction in Domestic Animals* **55**: 162–69.
- Safa S, Moghaddam G, Jozani R J, Kia H D and Janmohammadi H. 2016. Effect of vitamin E and selenium nanoparticles on postthaw variables and oxidative status of rooster semen. *Animal Reproduction Science* **174**: 100–06.
- Shi L G, Zhang C, Yue W, Shi L, Zhu X and Lei F L. 2010. Short-term effect of dietary selenium-enriched yeast on semen parameters, antioxidant status and Se concentration in goat seminal plasma. *Animal Feed Science and Technology* **157**(1–2): 104–08.
- Singh A K, Rajak S K, Kumar P, Kerketta S and Yog R K. 2018. Nutrition and bull fertility: A review. *Journal of Entomology and Zoology Studies* **6**(6): 635–43.