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Effect of Vitamin-E and Selenium Supplementation on Oxidative Stress Parameters in Postpartum Anestrus Buffaloes

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ABSTRACT

The present experiment was conducted to evaluate the effect of vitamin-E-Selenium on stress parameters in anestrus Murrah buffaloes located at R.S. Pura, Jammu. Twelve animals with similar age and reproductive status were selected for experiment. The selected animals were divided in two groups, each comprising six animals. Group I was given used CIDR protocol while in group II animals along with used CIDR protocol, were administered two injections of vitamin-E-care-Se (50 mg α-tocopheryl acetate and 1.5 mg selenium per ml) at the dose rate of 1ml/50 kg b. wt. on day 0 and day 7 through intramuscular route. The blood samples were collected on day 0, day 7 and day 9 during the experiment. The collected samples were stored at -20^{0}C and later evaluated for oxidative stress parameters. A significant decrease ($P<0.05$) in lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activity along with non-significant increase in glutathione peroxidase (GPx) level was observed when postpartum anestrus buffaloes were treated with used CIDR protocol either alone or in combination with vitamin-E and Selenium. The with used CIDR protocol in combination with vitamin-E and Selenium resulted in significant reduction in oxidative stress parameters (except MDA) at day 7 and day 9. However, with respect to MDA, there was no significant difference between two groups at start of treatment and after treatment.

Keywords: Catalase, Glutathione peroxidase, Lipid peroxidation, Superoxide dismutase

Postpartum anestrus is a serious problem causing reproductive inefficiency in buffaloes. Free radicals and reactive oxygen species (ROS) which disrupt the reproductive biology in animal (Riley and Behrman, 1991) are considered as important factor that induce post partum anestrus. Vitamins and trace minerals control metabolic pathways or gene expression and enhance ROS trapping activity. Vitamin-E-selenium injections prior to mating have shown significant increase in terms of oestrus response, fecundity rates and lamb body weight (Koyuncu and Yerlikaya, 2007). Vitamin-E and selenium supplementation is beneficial in reducing oxidative stress in dystocia-affected buffaloes in the immediate post-partum period (Sathya *et al.,* 2007). Their deficiency induces high rate of ROS production resulting in various disorders of gestation (Aurousseau *et al.,* 2006).

Vitamin-E prevents oxidative damage to membrane lipids by destroying hydro peroxide formation (Putman and Comben, 1987). Oxidative stress results in poor embryo competence, DNA fragmentation and poor pregnancy rates (Agarwal *et al.,* 2005). Vitamin-E is a potent chain breaking antioxidant, which inhibits propagation of peroxidation reactions by scavenging oxygen radicals and terminating free radical chain reaction (Burton and Traber, 1990). Selenium (as selenoprotein) a component of enzyme glutathione peroxidase (GPx) in combination with vitamin-E serves as a biological antioxidant to maintain cellular integrity. The action of vitamin-E and selenium appears to be synergistic (Papas *et al.,* 1990). Keeping in view all above facts the present study was conducted to study the effect of Vitamin-E and Selenium on oxidative stress parameters.

MATERIALS AND METHODS

Experimental location

The present study was conducted at Division of Animal Reproduction, Gynaecology and obstetrics, Faculty of Veterinary Sciences and Animal Husbandry R.S. Pura, Jammu.

Selection of the Animals

Twelve non-cyclic Murrah buffaloes aged between 4.5-8 years, with parity 1-6, average body condition score of 3.5 (Edmonson *et al.*, 1989) and 90 or more than 90 days postpartum were selected from R.S. Pura. The average body condition score of animals were 3.5 (1 = thin, 5=fat) with parity 1-6 at estrus. Selected animals were gynaeco-clinically examined twice at an interval of 10 days using per-rectal technique for confirmation of anestrus ovaries.

Managemental Practices

The buffaloes were kept under semi-intensive housing system. They were fed ration consisting of concentrates (groundnut cakes, mustard cake, maize grain and wheat bran), roughages (either berseem and maize or oat fodder), mineral mixture and salt. Fresh tap water was available *ad-libitum.* The selected animals were divided into two groups (n-6) *viz.* **group I and group II.** The animals in group I were administered used- CIDR (controlled intravaginal progesterone-releasing) implant on day 0, followed by i/m injection of $PGF_2\alpha$ (Clostenol) 500µg on day 6, 24 h before used CIDR removal, artificial insemination (A.I.) was done on day 9. In addition to above treatment, the animals in group II were administered inj. Vit. E and Se (E-care Se) @ 1 mg/kg body weight on day 0 and day 7. Each ml contains α Tocopheryl Acetate equivalent to Tocopherol 50 mg, Sodium Selenite 1.5 mg in a vail of 10 ml and is manufactured by VETCARE.

Blood sampling schedule

Blood samples were collected from postpartum anestrus buffaloes for oxidative stress parameters from jugular vein. One sample was taken immediately before treatment and the second sample on the day of CIDR removal. About 5 ml blood was collected early in the morning in clean dry sterilized glass vial containing heparin as anticoagulant for oxidative stress parameters. Immediately after collection samples were taken to laboratory, centrifuged at 3000 rpm for 15 minutes, supernatant were discarded and RBC sediment obtained were stored at -20ºC. Prior to preparation of RBC lysate, red blood cells were washed thrice with normal saline solution. RBC sediment obtained after harvesting of plasma was diluted with normal saline solution in the ratio of 1:1 (v/v basis). The diluted erythrocytes were centrifuged at 3000 rpm for 10 minutes. The supernatant was discarded along with buffy coat and the process was repeated 3 times. After final washing, 1% hemolysate which was prepared by adding 100µl washed RBC and 9.9 ml PBS. This 1% hemolysate was used for the estimation of catalase (CAT), SOD, GPx and 33 per cent hemolysate which was prepared by adding 330µl washed RBC and 670µl phosphate buffer solution (PBS) at pH 7.4 was used for estimation of lipid peroxidation.

Determination of oxidative stress parameters

The activity of lipid peroxidation in erythrocytes and tissues was determined according to method described by Shafiq-ur-Rehman (1984). Membrane peroxidative damage in erythrocytes or tissue was determined in terms of MDA production, determined by TBA (thiobarbituric acid).

The activity of SOD in erythrocyte lysate and tissue homogenates was determined by method of Marklund and Marklund (1974).

The ac Activity of GPx in erythrocytes and tissues was assayed by the method of Hafeman *et al.* (1974).

The activity of catalase in erythrocyte lysate and tissue samples was determined by method described by Aebi (1983).

STATISTICAL ANALYSIS

For evaluating the effect of antioxidant supplement, data analysis was done by using two way ANOVA (using SPSS-16.0 Inc).

RESULTS AND DISCUSSION

Nutritional imbalance, environmental insults, inflammatory responses, parturition, heavy milk yield in

animals stimulate the production of compounds called reactive oxygen species (ROS) or free radicals (Jain *et al.,* 2013). These radicals damage ovarian steroidogenic tissues and represent a source of infertility or anestrus (Carlson *et al.,* 1993; Maas, 1993). During the present experiment, oxidative stress parameters in blood *viz.* lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were assessed on day 0 (pre treatment), day 7 (post treatment) and day 9 at the time of (A.I.)

In group I, a non-significantly decrease in mean (±SE) values of MDA was observed on day 0 and day 7 which further decreased significantly (P<0.05) on day 9. In group II, the mean value of MDA level decreased significant (P<0.05) on day 7 and day 9 as compared to day 0. On day 0, no significant change was observed between group I and II. On day 7, MDA level in group II decreased significantly (P<0.05) from group I. On day 9, non significant comparable values of MDA concentration was observed between group I and II. The present findings are in agreement with the earlier findings of Kahlon *et al.* (2006) in anestrus buffaloes and Sharma *et al.* (2011) in early lactating cows who also reported similar trend of MDA levels. This significant decrease in MDA level can be explained in the light that vitamin-E is an internal component of all lipid membranes and serves to protect lipid membranes from attack by ROS (Rice and Kennedy, 1988). Polyunsaturated fatty acids of membranes are particularly vulnerable to attack by ROS which can initiate a chain reaction of lipid peroxidation that destroys the membrane of the cell. Vitamin-E can quench peroxidation reactions in membranes and is probably the most important antioxidant located in cell membrane (Putman and Comben, 1987). Vitamin-E and GPx function at two different locations within the cell, GPx functions in the cytosol and vitamin-E within lipid membrane. An important function of both systems is the final protection of membrane PUFA (Jain *et al.,* 2013). This may explain the role of Se, the component of GPx used in the present study in reducing MDA level along with Vitamin-E. Staats *et al.* (1988) showed that vitamin-E protected steroidogenic enzymes from oxidative degeneration and Rapoport *et al.* (1998) found that concentration of alpha tocopherol in ovarian tissue was related to animals with consumption of vitamin-E during the period of maximal progesterone production. Barnes and Smith (1975) suggested that vitamin-E promoted the release of FSH, ACTH and LH by protection of pathway from archidionic acid to prostaglandins which gets inhibited in oxidative stress. UCIDR treated animals also showed significant reduction of MDA on day 9 as compared to day 0. This could possibly be the protective effect of UCIDR containing progesterone in reducing the free radical generation.

Table 1: Effect of UCIDR alone and in combination with vitamin-E and Se on oxidative stress parameters

	Day	Group I	Group II
MDA (nmol/gHb/hr)	0	17.79±0.55^{a}	19.00±0.83^{a}
	7	16.83±0.98^{a}	15.70±0.81^{b}
	9	14.23±0.49^{b}	13.15±0.85^{c}
SOD (U/mg Hb)	0	50.78±1.72^{a}	49.80±1.86^{a}
	7	47.66±1.78^{ab}*	40.66±1.16^{b}
	9	44.96±1.87^{b}*	31.62±1.19^{c}
CAT (µmol/mg Hb/ min)	0	51.50±1.58^{a}	49.76±1.98^{a}
	7	48.86±1.47^{ab}*	43.15±1.89^{b}
	9	46.30±1.50^{b}*	39.40±1.62^{b}
GPx (U/mg Hb)	0	15.12±0.57^{a}	14.43±0.90^{a}
	7	13.84±0.68^{a}	13.51±0.50^{b}
	9	11.98±0.55^{b}*	15.20±0.31^{a}

Values having different superscripts within a column for each parameter differ significantly (P<0.05).

*Significant difference between two groups at P<0.05

The mean (±SE) values SOD level in Group I decreased significantly (P<0.05) on day 9 while non significant decrease was observed on day 7 as compared to day 0. The mean (±SE) values of SOD activity in group II decreased significantly (P<0.05) on day 7 and day 9 post treatment as compared to day 0. On day 0 non significant alteration in the activities of SOD was observed between group I and II. On day 7, significant (P<0.05) decrease in SOD concentration was observed in group II as compared to group I. On day 9, significant (P<0.05) decrease in SOD activity was observed in group II as compared to group I. Increased erythrocytic SOD activity was observed in pre supplemented anestrus animals which possibly were response to the increased generation of free radicals in them and an attempt to ward off superoxide radicals. Supplementation of UCIDR with vitamin-E and Se reduced

the activity of SOD which could possibly be because of their quenching action on lipid peroxidation and reduction of oxidative stress. Supplementation of vitamin-E and Se might have relieved oxidative stress in anestrus buffaloes; (Putman and Comben, 1987) thus lowered the erythrocytic SOD in treated buffaloes. These findings are in agreement with those reported by Kahlon and Singh (2003) in alpha-tocopherol supplemented anestrus buffalo heifers and Anita *et al.* (2004) in anestrus buffaloes supplemented with vitamin-E and Se.

The mean (±SE) values of catalase concentration in group I decreased non-significant from day 0 to day 7 while a significant ($P<0.05$) decrease was observed on day 9. In group II, a significant decrease ($P<0.05$) was observed in mean (±SE) values of catalase concentration on day 7 and day 9 compared to day 0. On day 0, non significant alteration in catalase concentration was observed between group I and II. On day 7, significant ($P<0.05$) decrease in catalase was observed in group II as compared to group I and on day 9, catalase activity in group II decreased significantly ($P<0.05$) from group I which suggests more activity of SOD which normally produces less effective H_2O_2 to be converted to H_2O and O_2 (Zakaryan *et al.,* 2002). Supplementation of UCIRD, vitamin-E and Se decreased SOD activity and this might be responsible for decreasing activity of CAT in UCIDR, vitamin-E and Se supplemented animals.

This may be due to production of progesterone produced by CL and Se contributes in production of progesterone by CL (Mavi *et al.,* 2006). Decreased SOD activity might be responsible for decreasing activity of CAT. This effect could have reflected through the action of vitamin-E and Se at cellular level to regulate free radical generation in ovaries (Harrison and Conrad, 1984).

In group I, a non significant decrease in mean (±SE) values of GPx was observed on day 0 and day 7 while further decreased significant ($P<0.05$) on day 9. In group II, the mean (±SE) values GPx activity decreased significantly ($P<0.05$) from day 0 to 7, while a significant increase was observed from day 7 to day 9. On day 0, non significant variation in GPx was observed in group I and II. On day 7, significant decrease in GPx was observed in both groups. On day 9, significant increase in GPx concentration was observed in group II as compared to group I. Glutathione peroxidase is primary antioxidant metalloenzyme containing four atoms of Se per molecule of enzyme and catalyzes reduction of H_2O_2 and organic peroxides (ROOH) (Kahlon and Singh, 2003).

In the present study oxidative stress in anestrus buffaloes accounted for the accumulation of H_2O_2 and ROOH as evident from increased erythrocytic SOD activity and MDA levels. Therefore, elevated erythrocytic GPx activities 15.12±0.57 U/mg Hb during anestrus as compared to 13.84±0.68 U/mg Hb on day 7 was observed, however GPx level 11.98±0.55 U/mg Hb on day 9 significantly decreased ($P<0.05$) as compared to day 0. This suggests up regulation of this enzyme for effective removal of H_2O_2 and ROOH along with CAT. UCIDR has reduced GPx significantly on 9 day in treatment which might be due to protective action of progesterone on free radical generation. On day 7 in UCIDR, Vitamin-E and Se treated group, reduction was non-significant as compared to day 0 pretreated anestrus buffaloes. This might be due to leading role of vitamin-E in lowering GPx activity because of its location in cytosol while vitamin-E preventing oxidative stress in plasma membranes (Bourre *et al.,* 2000). On day 9 statistically non-significant increase in GPx could be due to Se supplementation. Similar findings are reported by Kahlon and Singh (2003) in alpha tocopherol supplemented anestrus buffalo and Anita *et al.* (2004) in vitamin-E and Se treated postpartum anestrus buffaloes and observed no increase in Se level in cow injected with combination of vitamin-E and Se. Animals contain Se containing enzyme, GPx, which reduces hydrogen peroxide forming glutathione disulphide (GSSG) and thereby serves as an alternative means of detoxifying activated oxygen.

CONCLUSION

Supplementation of vitamin-E and selenium to progesterone based estrus induction protocol can be employed successfully in postpartum anestrus buffaloes to induce estrus and to have better fertility response. However, generalization of including vitamin-E and selenium injection along with CIDR protocol could be made after instituting the same treatment in larger group of buffaloes.

Antioxidants play an important role in female reproduction. Addition of vitamin-E and selenium helps in mitigating oxidative stress in postpartum anestrus buffaloes and improving reproductive efficiency.

REFRENCES

Aebi, H.E. 1983. Catalase. In Methods Enzymatic analysis, ed. H.U. Bergmeyer, New York: Academic Press.

Agarwal, A., Gupta, S and Sharma, R.K. 2005. Role of oxidative stress in female reproduction. *Reprod. Biol. Endocrinol.*, **3**: 28.

Anita.,Singha, S.P.S., Dhillon, K.S. and Nayyar, S. 2004. Antioxidant enzymes in Ppostpartum anoestrus buffaloes supplemented with vitamin-E and selenium. *Asian-Australian J. Anim. Sci.*, **17**(5): 608-611.

Aurousseau, B., Gruffat, D. and Durand, D. 2006. Gestation linked radical oxygen species fluxes and vitamins and trace mineral deficiencies in the ruminant. *Reprod. Nutr. Dev.*, **46**: 601-620.

Barnes, M.M.C. and Smith, A.J. 1975. The effects of vitamin-E deficiency on some enzymes of steroid hormone biosynthesis. *Int. J. Vitamin Nutr. Res.*, **45**: 396-403.

Bourre, J., Dumonto, O., Clement, M., Dinh, L., Droy-Lefaix, M. and Christeny, Y. 2000. Vitamin-E deficiency has different effects on brain and liver phospholipid hydroperoxide glutathione peroxidase activities in the rat. *Neurosci. Lett.*, **2**(286): 87-90.

Burton, G.W. and Traber, M.G. 1990. Vitamin-E : antioxidant activity, biokinetics and bioavailability. *Ann. Rev. Nutr.*, **10**: 357-382.

Carlson, J.C., Wu, X.M., Sawada, M. 1993. Oxygen radicals and the control of ovarian corpus luteum function. *Free Radical Biol. Med.*, **14**: 79-84.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G. 1989. A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.*, **72**: 68-78.

Hafeman, D.G., Sunde, R.A. and Hoekstra, W.G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rats. *J. Nutr.*, **104**: 580-87.

Harrison, J.H. and Conrad, H.R. 1984. Selenium content and glutathione peroxidase activity in tissues of the dairy cow after short term feeding. *J. Dairy Sc.*, **67**: 2426-70.

Jain, A., Jadhav, A.A. and Verma, M. 2013. Relation of oxidative stress, zinc and alkaline phosphatase in protein energy malnutrition. *Archives Physiol. Biochem.*, **119**(1): 15-21.

Kahlon, R.S. and Singh, R. 2003. Status of antioxidant enzymes in normal cycling and α-tocopherol supplemented anestrus buffalo heifers (*Bubalusbubalis*). *Asian-Australasian J. Anim. Sci.*, **16**(2): 217-221.

Kahlon, R.S., Sodhi, S.P.S. and Singh, R. 2006. Status of lipid peroxidation in normal cycling and α-tocopherol supplemented anestrus buffalo heifers (*Bubalusbubalis*). *Asian-Australasian J. Anim. Sci.*, **19**(9): 1262-1265.

Koyuncu, M. and Yerlikaya, H. 2007. Effect of selenium-vitamin-E injections of ewes on reproduction and growth of their lambs. *South African J. Anim. Sci.*, **37**: 233-236.

Maas, J. 1993. Studies on selenium metabolism in cattle: Deficiency, supplementation and environmental fate of supplemented selenium in: Selenium Tellurium Development Association, 6th International symposium, May 10-12 Scottsdale, AZ.

Marklund, S. and Marklund, G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European J Biochem.*, **47**: 469-476.

Mavi, P.S., Pangaonkar, G.R. and Sharma, R.K. 2006. Effect of vitamin-E and selenium on postpartum reproductive performance of buffaloes. *Indian J. Anim. Sci.* **76**(4): 308-310.

Papas, A.M., Cambre, R.C. and Citino, S.B. 1990. Species differences in the utilization of various forms of vitamin-E. *Proceeding of the American Association of Zoo Veterinarians Annual General Meet.*, 186-190.

Putman, M.E. and Comben, N. 1987. Vitamin-E. *Vet. Rec.*, **121**: 541-545.

Rapoport, R., Sklan, D., Wolfenson, D., Shaham-Albalancy, A., Hanukoglu, I. 1998. Antioxidant capacity is correlated with steroidogenic status of the corpus luteum during the bovine estrous cycle. *Biochimica Biophysica Act*., **1380**: 133-140.

Rice, D.A. and Kennedy, S. 1998. Assessment of vitamin-E, selenium and poly unsaturated fatty acid interactions in the aetiology of diseases in the bovine. *Proceedings of Nutritional Society,* **47**: 177.

Riley, J.C. and Behrman, H.R. 1991. Oxygen radicals and reactive oxygen species in reproduction. *Proc. Soc. Exp. Biol. Med.*, **198**: 781-791.

Sathya, A., Prabbhakar, S., Sangha, S.P. and Ghuman, S.P. 2007. Vitamin-E and Selenium supplementation reduces plasma cortisol and oxidative stress in dystocia affected buffaloes. *Vet. Res. Commu.*, **31**: 809-818.

Shafiq-ur-rehman. 1984. Lead-induced regional lipid peroxidation in brain. *Toxicol. Lett.*, **21**(3): 333-337.

Sharma, N., Singh, K., Singh, O.P., Pandey, V. and Verma, P.K. 2011. Oxidative stress and antioxidant status during transition

period in dairy cows. *Asian-Australian J. Anim. Sci.*, **24**(4): 479-484.

Staats, D.A., Lohr, D.P. and Colby, H.D. 1988. Effects of tocopherol depletion on the regional differences in adrenal microsomal lipid peroxidation and steroid metabolism. *Endocrinology.*, **123**: 975-980.

Zakaryan, A.E., Aivazyan, N.M. and Karagezyan, K.G. 2002. Comperative analysis of the superoxide dismutase activity in tissues of higher vertebrates. *Doklady Biochem. Biophy.*, **382** (1): 13-15.