The Indian Journal of Animal Reproduction; 25(1): 19-22; June 2004

Assessment of oxidative stress in dairy cows supplemented prepartum with vitamin E and selenium with reference to retention of fetal membranes*

SANDEEP KUMAR GUPTA[1], HARENDRA KUMAR[2], T. MORE[3]

Division of Animal Reproduction
Indian Veterinary Research Institute, Izatnagar - 243 122 (UP)

Received : September 13, 2002
Accepted : September 23, 2003

ABSTRACT

The occurrence of retention of fetal membranes (RFM), the levels of lipid peroxide (LPO, indices of oxidative stress) and plasma cortisol were studied in cows following prepartum supplementation of 1100 IU Vit. E and 30 mg selenium. The prepartum supplementation of vit. E and selenium alleviated the incidence of RFM from 12 to 0%. The injection of vit. E and selenium significantly ($P < 0.01$) reduced the LPO levels at 0 wk prepartum. A significant ($P < 0.01$) increase in LPO levels (3.67±0.16 Vs 2.46±0.07 nanomoles MDA/mg of Hb) was noticed from 3 to 0 wk inthose cows which experienced RFM. Furthermore, a significant ($P < 0.05$) increase was observed in plasma cortisol level (60.50±4.33 Vs 25.64±6.26 ng/ml) at prepartum period in RFM cows as compared to non-RFM. It can be concluded that the prepartum supplementation of Vit. E and Se reduced the levels of LPO and thereby the incidence of RFM.

Keywords : Cows, retention of fetal membranes, vit. E, selenium, oxidative stress, lipid peroxide and cortisol.

The role of oxidative stress (Miller *et al.*, 1993; Brazezinska *et al.*, 1994), leukocytes (Gunnick, 1984) and some enzymes (Kankofer *et al.*, 1998) have been shown in the etiopathogenesis of retention of fetal membranes (RFM). Antioxidants are agents which break the oxidative chain reaction thereby reduce the oxidative stress (Miller and Madsen, 1994; Nockels, 1996). Oxidative stress results from imbalance between production of oxygen centred free radicals also referred reactive oxygen metabolites (ROM) and their safe disposal (Powell, 1991). Stress elicit increased ROM production which causes extensive damage to the cell membranes (lipids), DNA and enzymes through oxidative chain reaction (Nockels, 1996). Possible relationship among dietary antioxidant, oxidative stress and RFM were investigated in the periparturient dairy cows (Miller *et al.*, 1993). The presence of high levels of unsaturated fatty acids in the diet increases the requirements for Vit. E and with an inadequate levels of selenium in the diet, tissue oxidation occurs, resulting in degeneration and necrotic changes in the cell and finally cell death (Radostits, 1994). Though the pathogenesis of RFM due to Vit. E and selenium deficiency is not clearly understood but the involvement of oxidative stress in the etiology of certain metabolic disorders of dairy cattle is suggested by reduction in the incidence of RFM when the anticxidants, Vit. E and Se are supplemented (Harrison *et al.*, 1984). The present experiment was designed to study effect of pre-partum supplementation of Vit. E and Se on the incidence of RFM and to find out the relation between levels of red blood cell lipid peroxide in RFM Vs non-RFM cows.

MATERIALS AND METHODS

Animals : Friesian x Sahiwal crossbred cows of fourth to sixth parity were maintained under standard schedule of feeding and managemental conditions. Before two months of expected calving date, the cows were separated into a calving shed. Fifty pregnant cows were selected and randomly divided into two groups. Each animal in Group I (n = 25) was injected (i.m.) with Vit. E and Se at the dose rate of 1100 IU and 30 mg, respectively (E-care Se, Vetcare Co.) at 3 wk before the expected date of calving. While cows in Group II (n = 25) served as control and were administered with normal saline only.

Clinical observations : Cows that calved normally and failed to expel fetal membranes spontaneously within 12 hrs were considered as clinical cases of RFM as per the routine practice.

*Part of M.V.Sc. thesis
[1]Ph.D. student
[2]Sr. Scientist
[3]Principal Scientist, Biochmiestry & Food Science

†Corresponding author

The expulsion of fetal membranes in cows before 12 hrs were considered as a case of non-RFM.

Collection of blood : The peripheral blood samples were collected from all the cows by juglar venipuncture in a heparinized glass tubes at 3 wk (before treatment), 0 wk and also at 3, 2.1 and 0 day before expected date of calving. RBC hemolysate from former samples was prepared as per method of Cohn *et al.* (1970). Immediately after collection, blood was centrifuged at 3000 rpm for 10 minutes. the plasma and buffy coat was removed by aspiration. The cells were washed three times by resuspending in isotomic (0.9%) saline followed by recentrifugation and removal of supernatent fluid. The cells were lysed in 10 parts of ice cold distilled water and kept in appendorf. The plasma from later samples was separated by centrifugation (3000 rpm, 15 min) and stored at -20°C until assayed for hormone.

Estimation of lipid peroxide levels : Lipid peroxide was estimated in erythrocyte and expressed as nanomoles malonyl dialdehyde (MDA)/mg of hemoglobin as per method of Placer *et al.* (1966).

Estimation of hemoglobin : Hemoglobin in 10% erythrocyte hemolysate was estimated by cyanomethemoglobin method (Van Kampen and Ziglastra, 1961). The value was expressed in mg/ml of hemolysate.

Estimation of plasma cortisol : The concentration of plasma cortisol in antioxidant-treated (n = 10) and control cows (10) was estimated using solid phase ^{125}I Radio Immuno Assay technique with the help of standard diagnostic kit (Coat-A - Count, Diagnostic Products Corporation, Los Angeles, USA). The radioactivity was measured in Gamma counter and sensitivity of assay was 1.5 ng/ml.

Statistical analysis : Standard statistical methods of Snedecor and Cochran (1989) were followed to analyse the data. The data of LPO levels were analysed by 't' test where as the plasma cortisol values by ANOVA.

RESULTS AND DISCUSSION

The present finding that administration of fast acting antioxidants (Vit. E and selenium) 3 wk prior to calving reduced the incidence of RFM in cattle is similar to the results of Harrison *et al.* (1984) and Ivandija (1987). Apart from the injection schedules employed, there was no changes in nutritional or herd health management programme that would have accounted for the sudden, significant reduction in RFM incidence. Injection of Vit. E and Se increase the levels of α-tocopherol in RBC, neutrophil and plasma and increase the biochemical activity of glutathione peroxidase (GSH-Px) (Finch and Turner, 1996). Vit. E which is considered to be that of an

Table 1. Effect of prepartum supplementation of Vit. E and selenium on the occurrence of RFM in crossbred cows

	Treated group I (n = 25)		Control group II (n = 25)	
	No.	(%)	No.	(%)
Cows without RFM	25	100	22	88.0
Cows with RFM	0	0	3	12.0
Total	25	100	25	100.0

Group I : Animals were administered with 1100 IU Vit. E and 30 mg selenium at 3 wk before expected date of calving
Group II : Saline treated control

Table 2. Mean (±SE) lipid peroxide levels (nanomoles MDA/mg of Hb) in erythrocytes at 3 and 0 wk prepartum period in RFM and non-RFM cows

Group	Animal No.	Period of observations before calving	
		3 week	0 week
I (Treated) non-RFM	25	2.69 ± 0.06^{1}	2.45 ± 0.05^{2a}
II (Control) non-RFM	22	2.78 ± 0.06	2.76 ± 0.08^{b}
RFM	3	2.46 ± 0.07^{1}	3.67 ± 0.1^{2c}

Values within same column (^{a,b,c}) and within same rows (^{1,2}) having different superscripts differ significantly ($P < 0.01$).
Group I : Animals were administered with 1100 IU Vit. E and 30 mg selenium at 3 wk before expected date of calving
Group II : Saline treated control.

Table 3. Mean (±SE) plasma cortisol concentration (ng/ml) at days 3, 2, 1 and 0 before calving in RFM and non-RFM crossbred cows

Days before calving	Plasma Cortisol Concentration		
	Group I Treated non-RFM (n=10)	non-RFM (n = 7)	Group II Control RFM (n = 3)
3	2.52±0.33[a]	8.10±2.10[ab]	10.43±4.07[b]
2	4.20±1.36[a]	7.33±1.74[a]	29.73±6.49[b]
1	7.08±1.22[a]	12.20±4.12[a]	40.66±5.34[b]
0	12.46±2.36[a]	25.46±6.26[b]	60.50±4.33[c]

Values within same rows, having different superscripts (a, b and c) differ significantly ($P < 0.05$)

antioxidant, preventing oxidative damage to sensitive membranes lipids by destroying hydroperoxide formation (Putnam and Comben, 1987), acting in conunction with Se which is a part of GSH-Px, protects cellular membrane and lipid containing organelles from peroxidative damage by inhibition and destruction of endogenous peroxides, thus maintains the integrity of membrane and reduce the oxidative stress (Hogan *et al.*, 1993). Therefore, it is reasonable to suggest the supplementation of Vit. E along with Se reduced the oxidative stress and alleviated the incidence of RFM.

The high production of LPO from 3 to 0 wk before calving in cows which failed to expel their fetal membranes is in accordance with the earlier reports of Brazezinska *et al.* (1994). The supplementation of Vit. E and Se at prepartum stage reduced erythrocyte substances reactive to thiobarbituric acid. This observation is in close agreement with Miller *et al.* (1993) and Brazezinska *et al.* (1994). The combined administration of α-tocopherol acetate and sodium selenite at 3 wk prior to calving brought about normalization of qualitative and quantitative content of phospholipids by a simultaneous limitation of lipid peroxidation activity and thus might have reduced the level of lipid peroxidation at 0 wk prior to calving. It is interesting to note from the present study that at 0 wk of prepartum, when the clinical feature of RFM was not exhibited, the MDA (end product of lipid peroxidation) in RBC was increased significantly. The results suggested that the biochemical changes leading to elevated levels of LPO could be detected much earlier before the clinical manifestation of the inflammatory changes were perceptible in the infected tissues. The indication of RFM based on the elevated levels of LPO in RBC hemolysate before parturition could be of great value in the prediction of complications like RFM. A significantly higher concentration of plasma cortisol at prepartum period in RFM cows as compared to non-RFM was observed in this study and coincides with the findings of Peter and Bosu (1987) and Ras *et al.* (1996). It can be concluded that prepartum supplementation of vit. E and SE reduced levels of LPO and thereby incidence of RFM.

REFERENCES

Brazezinska-Slebodzinska, E., Miller, J.K., Quigley, J.D., Moore, J.R. and Madsen, F.C. (1994). Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. J. Dairy Sci., **17**: 3087-3095.

Cohn, G., Dembiec, D. and Marcus, J. (1970). Measurement of catalase activity in tissue extract. Anal. Biochem., **34**: 30-38.

Finch, J.M. and Turner, R.J. (1996). Effect of selenium and vitamin E on the immune responses of domestic animals. Res. Vet. Sci., **60**: 97-106.

Gunnick, J.W. (1984). Retained placenta and leukocytic activity. Vet. Quart., **6**: 49-51.

Harrison, J.P., Hancock, D.D. and Conrad, H.R. (1984). Vitamin E and selenium for reproduction of the dairy cow. J. Dairy Sci., **67**: 123-32.

Hogan, J.S., Weiss, W.P. and Smith, K.L. (1993). Role of vitamin A and selenium in host defense against mastitis. J. Dairy Sci., **76**: 2795-2803.

Ivandija, J. (1987). Interrelationship between adequate intake of vit. E and selenium and prevalence of reproudctive disorders and intramammary infections of dairy cows. Krmiva, **29**: 175-180.

Kankofer, M., Wiercinski, J. and Fidecki, M. (1998). Activity of hyaluronidase in placental tissues from cows with and without retained fetal membranes. J. Vet. Med., **45**: 337-341.

Miller, J.K. and Madsen, F.C. (1994). Transition metals, oxidative status and animal health : do alterations in plasma fast acting, antioxidants lead to disease in livestock ? Proceedings of Altheck's 10th Annual Symposium. In: Lyons, T.B., Jeques, K.A. (Eds.), *Biotechnology in the Feed Industry*, Nottingham University Press, Loughborough, U.K., pp 283-301.

Miller, J.K., Brazezinska-Slebodzinska, E. and Madson, F.C. (1993). Oxidative stress, antioxidants and animal function. J. Dairy Sci., **76**: 2812-23.

Nockels, C.F. (1996). Antioxidants improve cattle immunity following stress. Anim. Feed Sci. Tech., **62**: 59-68.

Peter, A.T. and Bosu, W.T.K. (1987). Peripartal endocrine changes associated with retained placenta in dairy cows. Theriogenology, **28**: 383-394.

Placer, Z.A., Cushman, L.L. and Johnson, B.C. (1996). Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. Anal. Biochem., **16** : 359-366.

Powell, D.W. (1991). Immune physiology of intestinal electrolyte transport. In: *Handbook of Physiology.* VI. The Gastrointestinal system IV. Intestinal absorption and secretion. American Physiology Society, Bethesda, MD, pp 591.

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

Ras, A., Janowski, T., Zdunczyk, S. (1996). Effect of subclinical and acute acidosis in pregnant cows on the course of pregnancy and the steroid hormone profile. Tieraztliche Praxis, **24**: 347-52.

Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods.* 8th edn., Iowa State University Press, Ames, Iowa, USA.

Vankampen, E.J. and Ziglstra, W.B. (1961). Colorimetric determination of hemoglobin. Clin. Chem. Acta, **6**: 5388-5392.