



Supplementation of vitamin E, selenium and increased energy allowance mitigates the transition stress and improves postpartum reproductive performance in the crossbred cow

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ABSTRACT

Dairy cow undergoes tremendous physiological challenges during the transition period leading to negative energy balance (NEBAL), impaired immunity and oxidative stress that ultimately compromises the postpartum fertility. Accordingly, we investigated the effects of antioxidant supplementation and increased energy allowance on transition stress and fertility of crossbred cow. Advanced pregnant crossbred cows ($n = 26$) of 2–4 parity and lactation potential of > 10 L/day were divided into two equal groups ($n = 13$ cows/group). Cows were fed diets either (i) supplemented with vitamin E (80 IU/kg DM), Selenium (Se; 0.3 mg/kg DM) and increased energy allowance in the form of 20% additional concentrate (TRT) or (ii) basal diet without any supplementation as control (CON). Vitamin E and Se were supplemented with wheat flour bolus from –4 to 8 week of calving whereas energy allowance was increased from 2 to 8 week of calving on daily basis to individual animal (where 0 is day of calving). Blood samples were collected on weekly interval from –4 to 8 week of calving. Oxidative stress was assessed by estimation of malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD) and catalase (CAT). Immunity level was assessed via estimation of phagocytic activity (PA) of granulocytes and lymphocyte proliferation assay (LPA). Postpartum fertility was assessed by interval to first postpartum estrus (day) and pregnancy rate. Cows in TRT had a significantly lower MDA, higher TAC and decreased activity of SOD and CAT than that of CON ($P < 0.05$). Phagocytic activity increased at –1, 0 and 3–8 weeks postpartum ($P < 0.05$) while LPA showed difference ($P < 0.05$) at parturition, week 4 and 8 postpartum. Marked improvement in the fertility was recorded in terms of early resumption of postpartum estrus ($P < 0.001$) and higher pregnancy rate ($P < 0.05$).

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1. Introduction

Genetic selection of dairy cows for milk yield shows a decline in the fertility over the last five decades [1] at the expense of reproductive performance [2]. The stress encountered during the transition from pregnant-nonlactating state to the nonpregnant-

lactating state is one of the major reasons for the postpartum infertility. Transition or periparturient period, which falls in the window of three or four weeks around calving, predisposes the cow to metabolic, infectious and reproductive diseases [3,4].

In transition period, a marked increase in the cellular metabolism of body tissues including the reproductive organs results in the generation of oxidative free radicals (ROS) that is beyond the antioxidant capacity of cells. Oxidative stress affects the reproductive processes like oocyte maturation, fertilization [5], ovarian steroidogenesis [6], preimplantation embryos [7] and sperm viability [8]. Pathological effects of oxidative stress are mediated

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through lipid peroxidation, inhibition of protein synthesis, and depletion of ATP [9]. In addition, periparturient cows undergo a period of immunosuppression due to compromised leukocyte function, bactericidal efficiency [10,11], phagocytic activity of the neutrophils and proliferation of lymphocytes [12] thereby increasing the risk of uterine infections and endometritis [13]. Vitamin E and Selenium (Se) are potent antioxidants and free radical scavengers. Supplementation of antioxidants compensate free radical burden [14], increased the neutrophil activity, uterine contractions [15], uterine involution [16] and reduced the incidence of retention of fetal membranes (RFM). In addition, it increased the fertilization rate in the bovine [17] and embryonic survival in the ovine [18].

During late gestation and early lactation, the nutrient requirements for the fetal growth and milk synthesis increase dramatically concurrently with a 30% decrease in the feed intake [19]. Consequently, most cows enter a period of negative energy balance (NEBAL) from which it may take many weeks to recover [20]. Increased metabolites (NEFA and ketone bodies) lead to interdependent changes in the GH-insulin-IGF-I-glucose signaling pathway [21], resulting in impaired follicular development and postpartum anestrus [22]. Feed restriction studies in the sheep adversely affected the estrus behavior through the downregulation of estrogen receptors in the brain [23]. Nutritional manipulation during the transition period improves the reproductive performance in the cow [24,25] through the somatotrophic axis [21]. Positive energy balance resulted in greater intrafollicular IGF-I, plasma progesterone levels and produced good quality oocytes in the Holstein cows [26]. It also improved the pregnancy rate [27] and immune cell functions [28]. Supplementation of carbohydrate and amino acids to the culture improved the fertilization rate and blastocyst development [29]. Increasing the energy content of the ration improved embryonic development [30,31], embryo hatching, embryo elongation and expression of interferon-tau [32].

Crossbred cows are distinctly different from the highyielding purebred HF or Jersey for many reasons: Their genetic makeup and exotic inheritance are not homogenous. They are spread around different agro-ecological niche in the tropical region. In India, crossbred cows are predominantly maintained under semi-intensive production system and they contribute significantly to the total milk production [33]. In India, the scale to define a high yielding crossbred cow is different from other developed countries. Thus, dietary manipulation of transition period in the high yielding crossbred cows is required to improve the fertility [34]. Accordingly, we studied the effect of vitamin E and Se supplementation along with increasing energy content of diet during the transition period on oxidative stress, immune cell functions and postpartum reproductive performance in the crossbred cows.

2. Materials and methods

All the experiments, procedures and protocols on animals were conducted following the approval of the Ethics Committee, Indian Veterinary Research Institute.

2.1. Experimental animals

The experiment involved twenty six apparently healthy advanced pregnant crossbred cattle (Haryana × Holstein Friesian/Brown Swiss/Jersey) maintained at cattle and buffalo farm of Livestock Production and Management Section, Indian Veterinary Research Institute, Izatnagar. The institute is located at an altitude of 564 feet above the mean sea level, at latitude of 28° N and a longitude of 79° E. The experimental cows were in second to fourth parity with milk yield more than 10 L/day in their last lactation.

Animals were maintained under isomanagerial conditions of housing, feeding and milking.

2.2. Experimental design

The experimental crossbred cows were divided into two groups ($n = 13$ cows/group), viz., treated (TRT) and control (CON). Mean body weight (Kg) of TRT and CON group was 418 ± 21.65 and 406.5 ± 14.20 kg, respectively. Independent *t*-test revealed that the mean difference in the body weight between the groups did not differ significantly. Each cow was given access to fodder and water *ad-libitum*. Animals of TRT group were supplemented with vitamin E (DL- α -tocopherol acetate, CDH, India) and Se (Sodium selenite, CDH, India) per oral at the dose rate of 80 IU/kg DM and 0.3 mg/kg DM (NRC 2001), respectively, from –4 to 8 weeks of calving (where 0 is day of anticipated date of calving). The dose of Vitamin E and Se was based on the fact that supplementation of high concentration for a shorter period [86] or low concentration for a longer duration [87,88] did not result in adverse effect or toxicity. Further, experimental hypervitaminosis E is demonstrated in the rat, chick and human, but not in the dairy cow (NRC, 2001). It has been reported that the ruminants can tolerate intakes of about 40,000 IU/day of supplemental Vitamin E for several months without adverse effects (NRC, 1987). The cost of Vitamin E and Se administration was ₹ 14–15 INR per day per cow that summed up to ₹ 1176–1260 INR for the period of supplementation.

Cows in the CON group were given wheat flour bolus, which was used as vehicle to deliver Vitamin E and Se in the TRT. Cows in the TRT received increased energy in the form of 20% additional concentrate mixture from 2 to 8 week postpartum. However, CON group was given the basal diet. According to the farm policy, the experimental herd was examined twice a month by a team of specialists in veterinary medicine, surgery and theriogenology to record the occurrence of acidosis and/or laminitis due to possible carbohydrate overload in the TRT. Cost of concentrate was ₹ 22.47 INR per kg which was supplemented in the range of 1–2.5 kg per cow based on the milk yield.

2.3. Blood sampling

Blood samples from experimental animals were collected by jugular venipuncture aseptically using 18-G needle in sterilized vacutainers (heparinized or clot activators) on weekly interval from –4 to 8 weeks of calving, on the basis of expected date of calving. Heparinized vacutainers were used for isolation of peripheral blood mononuclear cells (PBMC) and granulocyte, while clot activator vacutainers were used for serum separation. Serum was separated by centrifugation at $800 \times g$ for 10 min and stored at -80°C until analysis.

2.4. Assessment of oxidative stress

Oxidative stress was determined by estimating the concentration of serum malondialdehyde (MDA) and total antioxidant capacity (TAC). In addition, the enzymatic activity of total superoxide dismutase (SOD) and catalase (CAT) were assayed. Serum MDA was measured by the double heating method as described by Draper and Hadley [35]. The method is based on spectrophotometric measurements of the purple colour generated by the reaction of thiobarbituric acid (TBA) with MDA. Serum TAC was estimated by the method described by Koracevic et al. [36]. It measures the capacity of the biological fluids to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. Estimation of serum enzymatic antioxidant SOD and

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