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## Vitamin E and selenium treatment during transition period curtails oxidative stress exacerbated incidence of postpartum diseases

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#### Abstract

The transition period in dairy cattle, spanning from three weeks prepartum to three weeks postpartum, is characterized by intense metabolic and oxidative stress, predisposing animals to postpartum diseases. This study evaluated oxidative stress biomarkers and the incidence of postpartum disorders in 30 dairy cows divided into three groups: control (n = 10), untreated oxidative stress group (n = 10), and treated group (n = 10) administered oral Vitamin E and selenium. Biomarkers assessed included NEFA, MDA, GPX, SOD, and catalase, alongside disease incidence. Results suggest that NEFA levels were significantly higher in the untreated group (mean: 0.659 mmol/L) compared to the treated (0.413 mmol/L,  $p \le 0.05$ ) and control groups (0.280 mmol/L,  $p \le 0.05$ ). MDA concentrations peaked in the untreated group (25.64±18.13 µmol/mL at 2 weeks postpartum), significantly higher than the treated (5.152±2.22 μmol/mL) and control groups (p≤0.05). GPX activity increased markedly in the treated group post-calving (38.91±12.16 IU/mg protein), significantly higher than untreated (17.86±15.93,  $p \le 0.05$ ) and control groups. SOD levels increased significantly in the control group  $(0.575\pm0.28 \text{ to})$ 1.869±0.65 IU/mg protein, p≤0.05), while the treated group showed a significant decline (1.061±0.59 to  $0.109\pm0.03$ ,  $p\le0.05$ ), indicating reduced oxidative burden. Catalase levels increased significantly in untreated animals (1.052 $\pm$ 0.16 to 1.927 $\pm$ 0.29,  $p\leq$ 0.05) but declined in the treated group (1.165 $\pm$ 0.28 to  $0.211\pm0.1$ ,  $p\le0.05$ ). Postpartum disease incidence was 90% in the untreated group, 30% in the treated group, and 20% in the control group. The findings demonstrate that Vitamin E and selenium supplementation significantly alleviated oxidative stress, enhancing antioxidant defence and reducing postpartum disease incidence.

Keywords: Postpartum diseases, oxidative stress, vitamin E, selenium, cow

#### Introduction

Transition period in dairy cattle is the period from three weeks prepartum to three weeks postpartum and is widely acknowledged as the most metabolically and physiologically challenging phase during lactation <sup>[1]</sup>. During this period, dairy cows undergo a sequence of endocrine, metabolic, and immunological adaptations that enable the shift from gestation to lactation <sup>[2]</sup>. These metabolic changes are essential for meeting the increased nutritional demands required for foetal growth, parturition, and the initiation of milk production. However, these rapid variations also render cows highly susceptible to metabolic disorders, oxidative stress, and immune suppression, which mutually contribute to the high incidence of postpartum diseases observed during this period <sup>[3]</sup>.

Concomitant with metabolic stress, oxidative stress becomes a major physiological burden during the transition period. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the animal's antioxidant defence systems <sup>[4]</sup>. ROS are natural by-products of cellular respiration and oxidative phosphorylation in mitochondria <sup>[5]</sup>. Under standard physiological conditions, ROS levels are tightly regulated and can even serve signalling functions. However, during early lactation, the combination of increased oxygen consumption, mitochondrial activity, and inflammatory responses leads to the overproduction of ROS, overwhelming antioxidant defences and resulting in cellular damage <sup>[6,7]</sup> leading to oxidative stress.

Malondialdehyde (MDA), a by-product of lipid peroxidation, is commonly used as a biomarker to assess oxidative damage in dairy cattle [8].

Elevated MDA is an indicator of oxidative stress, which is associated with augmented disease risk and impaired immune function <sup>[9]</sup>. The body's primary enzymatic antioxidant defenses—superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX)—play essential roles in neutralizing ROS and maintaining cellular redox balance. Among these, GPX is particularly important due to its reliance on selenium, a micronutrient that is often marginal or deficient in dairy rations <sup>[10]</sup>. Vitamin E, a fatsoluble antioxidant, works synergistically with selenium and GPX to stabilize cell membranes and reduce oxidative damage <sup>[11, 12]</sup>. Numerous studies have confirmed that supplementing Vitamin E and selenium can efficiently ameliorate oxidative stress markers and improve postpartum health outcomes in dairy cattle <sup>[13, 14]</sup>.

However, reports suggest that approximately 75% of disease incidences in dairy herds, including mastitis, metritis, retained foetal membranes (RFM), ketosis, and displaced abomasum, occur within the initial month following parturition [15]. The most critical period of vulnerability is the initial 10 days postpartum, which coincides with the most intense metabolic activity and the sudden rise in milk

synthesis [16].

Considering the complex interplay of oxidative and metabolic stressors during the transition period, it is critical to monitor and manage these changes to enhance animal health and productivity. Laboratory-based metabolic profiling, including the assessment of NEFA, MDA, SOD, catalase, GPX, has arose as a valuable approach to assess health status at both the individual and herd levels [17, 18]. These biomarkers not only help in the initial detection of subclinical diseases but also guide nutritional and therapeutic interventions. Therefore, the objectives of the current study are aimed at studying oxidative stress during transition period and associated incidence of the postpartum diseases in those animals and antioxidants Vit E and selenium role in alleviating this incidence

#### **Materials and Methods**

Thirty cows which were in the transition period were taken up for the study and were divided into three groups, as follow which were screened for any incidence of clinical and subclinical manifestations of different most common diseases that are encountered during Transition period.

S No	Group	Animal				
1	Normal control group	10 Cattle that were not exposed to any experimental condition.				
2	2 Untreated group 10 Cattle that were under oxidative stress but did not receive treatment.					
3	Treated group	10 Cattle that were under oxidative stress and received treatment with oral Vit E and Selenium (E CARE SE) for postpartum diseases.				

#### **Preparation of Samples**

Hemolysate was prepared by blood collected in EDTA vials. Blood samples were centrifuged at 3000 rpm for 15 min for separation of RBC pellet. The plasma and the buffy coat separated, were aspirated and removed. The resulting erythrocyte sediment was done washing thrice with 0.9% w/v NaCl solution, each time by mixing and centrifuging the suspension at 2500 rpm for ten minutes and discarded the supernatant. The washed red cells were lysed by addition of 3 parts of cooled distilled water slowly to erythrocyte pellet with constant mixing up to marked level to give stock hemolysate solution (20% V/V) which was quickly frozen at-20 °C. The hemolysate was used immediately to estimate the OS and antioxidant status.

#### **Estimation of Oxidative Stress Parameters**

Non Esterified Free Fatty Acid Assay (NEFA) milli moles/Litre: It was estimated using a colorimetric kit M/S Elab Science was bought from Clementia Bioteh, New Delhi.

**Malondialdehyde (MDA) micro mol/ml:** It was estimated by Niehaus and Samuelsson's method [19] for hemolysate was used to measure the MDA concentration to estimate the lipid peroxidation.

Serum Total Protein estimation was done by using bovine serum albumin (BSA) as a standard by Lowry method [20]. Glutathione Peroxidase (GPX) IU/milligram Protein was estimated by Rotruck *et al.* (1973) method for hemolysate [21], Superoxide Dismutase (SOD) IU/milligram Proteinwas measured according to the method of Misra and Fridovich (1972) for hemolysate [22] and Catalase IU/milligram Protein activity was measured by the method of Beers and Sizer (1952) for hemolysate [23].

The observed data of the present study was analyzed by two-way Anova multiple comparison using Graph Pad Prism version 8.4.3 to study the significant difference in the means of all parameters in between the groups and in across the weeks under study and the results were interpreted.

#### Results

#### Incidence of post-partum diseases in cattle

The incidence of postpartum diseases in cattle across the study groups is presented in Table1. Each group consists of 10 animals, and the table shows the number of affected animals (with percentages) for different postpartum diseases. Hypocalcaemia/Milk Fever is not observed in the control group, but its incidence was noticed more in untreated group (2/10) than in the treated group. Mastitis incidence was comparatively less (1/10) in the control and treated groups than in the untreated group (2/10). Metritis was found only in the untreated group (1/10) and absent in others. Less incidence of Retained Fetal Membranes was seen (1/10) in both control and treated groups, than (2/10) in the untreated group. Pre/Postpartum Uterine Prolapse was not reported in any group. Ketosis incidence was observed (1/10) only in the untreated group. Downer's Cow Syndrome incidence was also seen (1/10) only in the untreated group. In Normal control group (apparently healthy), no incidence of any postpartum diseases was seen highest in control group (8/10), followed by the treated group and lowest in the untreated group (1/10).

#### **Oxidative stress Parameters**

Non-Esterified Fatty Acids (NEFA) and Malondialdehyde (MDA) estimations during the Transition Period in Cross Bred Dairy Cattle (n = 10)

In cross bred dairy cattle, the levels of Non-Esterified Fatty Acids (NEFA) and Malondialdehyde (MDA) were estimated during the transition period in three groups and are presented in terms of mean values along with standard deviation values in Table 2 and in figures Fig 1 and 2 for

NEFA and MDA respectively.

#### Non-Esterified Fatty Acids (NEFA) Levels

NEFA levels were lowest in the control group (0.255-0.475 mmol/L), moderate in the treated group (0.376-0.521), and highest in the untreated group (0.426-0.867) during the periparturient period. In the control group, NEFA peaked at 1week post-calving then significantly declined ( $p \le 0.05$ ). The untreated group showed a continuous and significant rise ( $p \le 0.05$ ), peaking at 2 weeks post-calving (0.899±0.453). Treated animals had a significant precalving rise ( $p \le 0.05$ ) followed by post-calving decline. Across all stages, untreated animals consistently had significantly higher NEFA than treated and control groups ( $p \le 0.05$ ).

#### Malondialdehyde (MDA) Levels

MDA levels increased significantly ( $p \le 0.05$ ) in the control group before calving (from  $12.342\pm1.276$  to  $16.877\pm3.917$ ), then declined steadily postpartum. In the untreated group, MDA remained high throughout, peaking post-calving at  $25.64\pm18.13$  (2 weeks), significantly higher than control and treated groups ( $p \le 0.05$ ). The treated group showed a non-significant rise pre-calving and a decline post-calving (down to  $5.152\pm2.221$  by 3 weeks). Between groups, MDA was significantly higher ( $p \le 0.05$ ) in untreated animals during all postpartum weeks. Treated animals maintained the lowest MDA levels after calving, indicating reduced oxidative stress.

#### Antioxidant enzymes

The estimated levels of Glutathione Peroxidase (GPX), Super Oxide Dismutase (SOD) and Catalase are presented in terms of mean values along with standard deviation values in Table 3 and in figures Fig 1, 2, and 3 for GPX, SOD and Catalase respectively.

#### Glutathione Peroxidase (GPX) Levels

GPX levels remained stable in the control group (range:  $12.175\pm2.77$  to  $23.124\pm2.84$ ) with no significant changes. The untreated group showed fluctuating but non-significant values throughout (e.g.,  $14.918\pm5.69$  to  $17.856\pm15.93$ ). In contrast, the treated group had a significant rise ( $p\le0.05$ ) from pre-calving ( $17.162\pm5.03$ ) to a peak at 1-week post-calving ( $38.91\pm12.16$ ), remaining high up to 2 weeks. Before calving, treated GPX was significantly higher than untreated at one week ( $28.82\pm8.53$  vs.  $12.175\pm8.82$ ,  $p\le0.05$ ). Post-calving, treated animals consistently showed significantly higher GPX activity than both control and untreated groups ( $p\le0.05$ ).

#### **SOD** Levels

SOD levels in the control group increased significantly from 0.575 $\pm$ 0.28 (3 weeks pre-calving) to 1.869 $\pm$ 0.653 (2 weeks post-calving,  $p\leq$ 0.05). The untreated group showed low, stable SOD levels (around 0.6-0.98) with no significant changes. The treated group exhibited a significant gradual decline from 1.061 $\pm$ 0.59 (3 weeks pre-calving) to 0.109 $\pm$ 0.03 (3 weeks post-calving,  $p\leq$ 0.05). Before calving, no significant differences existed between groups, but post-calving, untreated and treated groups had significantly lower SOD than control ( $p\leq$ 0.05). By 3 weeks post-calving, all groups differed significantly from each other ( $p\leq$ 0.05).

#### **Catalase Levels**

Catalase levels in the control group increased significantly from  $1.170\pm0.39$  (3 weeks pre-calving) to  $1.743\pm0.71$  (3 weeks post-calving,  $p\le0.05$ ). The untreated group showed a similar significant rise from  $1.052\pm0.16$  to  $1.927\pm0.29$  post-calving ( $p\le0.05$ ). In contrast, the treated group experienced a significant decline from  $1.165\pm0.28$  pre-calving to  $0.211\pm0.1$  at 3 weeks post-calving ( $p\le0.05$ ). Between groups, the treated group had significantly lower Catalase levels than control and untreated groups before 1 week pre-calving and at 2-and 3-weeks post-calving ( $p\le0.05$ ). This indicates reduced antioxidant enzyme activity in the treated group postpartum.

#### Discussion

Transition period in cattle is a critical period in dairy animals. During this stage, dairy animals must adopt and transform physiologically to meet the needs of growing foetus, parturition, lactation, and resumption of reproductive cycle [24]. Further due to metabolic and nutritional demands during this period it is common to experience metabolic and oxidative stress. However, some animals can get through these conditions unaffected and can tolerate the stress due to their better genetical makeup and some animals may accede for oxidative stress and may suffer with postpartum diseases. The increased incidence of postpartum diseases during this period is majorly because of stress, oxidative stress in particular and managemental practices being other reasons [25]. Therefore, present study is designed to study an effective treatment protocol that can normalize the stress during transition period and could decrease the incidence of postpartum diseases.

Current study has chosen Vitamin E and Selenium treatment for studying its effect on controlling the incidence of postpartum diseases by alleviating the oxidative stress, because it has proven to be a better treatment strategy in various other similar conditions [12, 13].

#### **NEFA** (Non esterified fatty acids)

NEFA an Indicator of metabolic stress was found to be normal three weeks prior to partum later it started to increase in all the groups as it approached parturition owing to the metabolic demands around parturition and postpartum. The levels of this indicator were found to revert to normal levels in normal control and treated group, but not to a similar extent in untreated group, which indicates vitamin E plus selenium treatment was moderately effective in ameliorating the metabolic stress caused by metabolic demands during postpartum. Similar findings are also observed in other studies where in NEFA levels before 1 to 2 weeks of calving was proposed as a predictive indicator of postpartum disease incidence [26, 27]. In current study the incidence of post-partum diseases was also found to be in parlance with the average NEFA value during transition period, where in average NEFA of 0.659 in untreated group has an incidence of 90% of postpartum diseases, unlike treated and normal control group where the average NEFA values are 0.413 and 0.2803 and the incidence of postpartum diseases was 30% and 20% respectively. Further, the postpartum disease incidence was more of infectious diseases (Metritis, mastitis) indicating the compromised immune function with elevated NEFA [28].

#### Malondialdehyde

The MDA levels of oxidative stress groups were significantly (p<0.05) higher than the levels of MDA in normal control group 3 weeks pre-partum. Based on this, initially animals under study were grouped into normal control group (Group I) and oxidative stress group (Group II & Group III). Further, in treated group (Group III) MDA levels has decreased gradually as it approached parturition and same trend has followed till 3 weeks post-partum and were on par with normal control group. Whereas, in untreated group the MDA levels has gradually increased throughout the week's understudy and has shown significantly (p<0.05) high levels of MDA when compared to Group III and Group I, which clearly indicates that the oxidative stress in untreated animals has intensified. This intensified oxidative stress postpartum might be the reason for increased incidence of postpartum diseases observed in this group. Similar findings of increased MDA levels as it approaches partum were reported by previous studies [29]. This increased MDA levels indirectly indicates increased ROS levels and their associated damage [9]. However, treatment with vitamin E and selenium in group III could alleviate the oxidative stress and in turn postpartum diseases incidence which is comparable with normal control group. This decrease in MDA levels in treatment group can be attributed to the better antioxidant capacity in the animals in the form of increased glutathione peroxidase levels observed in the treatment group when compared to untreated and normal control group, which was also asserted by various previous studies indicating the importance of vitamin E and selenium in contending oxidative stress [12, 30].

### Super Oxide Dismutase, Catalase and Glutathione Peroxidase

The levels of SOD and catalase in all the three groups under study has increased non significantly form three weeks prepartum towards calving, which indicates the increased requirement of these enzymes during peripartum period to contend the ongoing release of ROS. Further, due to continued metabolic stress and oxidation stress (as indicated by increasing NEFA and MDA levels) even after calving in normal control group and untreated group, the levels of SOD and the catalase was also observed to increase in these groups. This increase was significantly higher by three weeks postpartum compared to three weeks prepartum. In contrast, the levels of these enzymes in treated group have significantly decreased from one week prior to calving and continued to decrease by 3 weeks after calving. This indicates that vitamin E & selenium treatment has compromised the need of these enzymes in mitigating oxidative stress postpartum. This trend of decrease in the levels of SOD and catalase during postpartum were also observed in other similar studies [12]. Further the decrease in SOD and catalase levels postpartum would be because of change in kind of ROS molecules from superoxide's to peroxides and hydroxyl ions [31].

In addition, GPX levels before 3 weeks of parturition was initially observed to be moderately elevated in all the three groups under study. Later, the levels of GPX have decreased in subsequent weeks under study in normal control and untreated group and has stayed equal and showed nonsignificant decrease or increase, when compared to their levels during three weeks prepartum. However, in treated group the GPX levels has increased significantly by first week of postpartum when compared to their levels three weeks before calving and continued to increase by three weeks post calving. This increase in GPX could be the reason for better antioxidative capacity observed in treated group when compared to other two groups. Vitamin E and selenium treatment could be able to deal with the oxidative stress in group II both by directly acting as membrane antioxidants [9, 32] and indirectly by enhancing the levels of GPX as it is a selenoprotein [33].

		Normal Control Group	Untreated Group	Treated Group	
S. No	Name of the Disease	Number of animals	Number of animals	Number of animals	
		Affected (Percentage)	Affected (Percentage)	Affected (Percentage)	
1	Hypocalcaemia/Milk Fever	0	2 (20)	1 (10)	
2	Mastitis	1 (10)	2 (20)	1 (10)	
3	Metritis	0	1 (10)	0	
4	Retained Foetal Membranes	1 (10)	2 (20)	1 (10)	
5	Pre/Post partum Uterine Prolapse	0	0	0	
6	Ketosis	0	1 (10)	0	
7	Downer's cow Syndrome	0	1 (10)	0	
8	Normal (apparently Healthy)	8 (80)	1 (10)	7 (70)	

**Table 1:** Incidence of Post-Partum Diseases in Cattle (n = 10 animals in each Group)

Table 2: Non-Esterified Fatty Acids (NEFA) and Malondialdehyde (MDA) estimations during the Transition Period in Cross Bred Dairy Cattle (n=10) (Mean ± SD)

Transition	Non-Esterified Fa	atty Acid (NEFA)	(m mol/L)	Malondialdehyde (μ mol/ml Haemolysate)			
Period	Normal Control Group	<b>Untreated Group</b>	<b>Treated Group</b>	Normal Control Group	<b>Untreated Group</b>	<b>Treated Group</b>	
Before 3 Weeks	0.2552±0.075 a,pqr	0.426±0.075 a,p	0.376±0.152 a,pqrs	2.342±1.276 a,p	11.585±5.046 b,pq	12.973±5.31 b,pq	
Before 2 Weeks	0.304±0.107 a,pqr	0.395±0.126 a,p	0.529±0.248 a,pqr	12.31±2.942 a,pq	13.495±4.64 a,q	16.14±5.28 a,q	
Before 1 Week	0.405±0.109 a,q	0.772±0.332 b,qr	0.567±0.248 ab,qr	16.877±3.917 a,qr	14.999±5.819 a,qs	10.132±7.502 a,pq	
After 1 Week	0.475±0.131 a,q	0.867±0.374 b,qr	0.521±0.187 a,pqr	12.002±5.321 a,pq	25.15±16.795 b,rs	6.554±3.331 a,pq	
After 2 Weeks	0.148±0.061 a,rt	0.899±0.453 b,q	0.273±0.156 a,ps	10.615±4.714 a,pq	25.64±18.13 b,r	5.742±2.824 a,pr	
After 3 Weeks	0.095±0.056 a,psr	0.595±0.277 b,pr	0.216±0.157 a,st	9.406±5.456 a,pq	24.64±15.221 b,rs	5.152±2.221 a,pr	

**Table 3:** Glutathione Peroxidase (GPX), Super Oxide Dismutase (SOD) and Catalase estimations during the Transition Period in Cross Bred Dairy Cattle (n=10) (Mean ± SD)

Transitio	Glutathione Peroxidase (GPX) (IU/mg Protein)			Super Oxide Desmutase (SOD) (IU/mg Protein)			Catalase (IU/mg Protein)		
n Period	Normal Control Group	Untreated Group	Treated Group	Normal Control Group	Untreated Group	Treated Group	Normal Control Group	Untreated Group	Treated Group
Before 3 Weeks	20.17±4.28 <sup>a,pr</sup>	14.918±5.69 <sup>a,p</sup>	17.162±5.03 <sup>a,p</sup>	0.5748±0.28 <sup>a,p</sup>	$0.657 \pm 0.6^{ab,p}$	1.061±0.59 <sup>b</sup> ,	1.170±0.39 <sup>a,p</sup>	1.052±0.16 <sup>a,p</sup>	1.165±0.28 <sup>a,p</sup>
Before 2 Weeks	23.124±2.84 <sup>a</sup> ,	17.674±7.62 <sup>a,p</sup>	20.38±3.36 <sup>a,pq</sup>	0.74±0.295 <sup>a,pq</sup>	0.985±0.45 <sup>ab,</sup>	1.237±0.59 <sup>b</sup> ,	1.137±0.13 <sup>a,pq</sup>	1.173±0.39 <sup>a,p</sup>	1.051±0.13 <sup>a,p</sup>
Before 1 Week	21.96±2.77 <sup>a,pq</sup>	12.175±8.82 <sup>b</sup> ,	28.82±8.53 <sup>a,qrs</sup>	1.209±0.39a,qr	0.817±0.21 <sup>a,p</sup>	0.956±0.37 <sup>a,p</sup>	1.374±0.3 <sup>a,pqr</sup>	1.439±0.16 <sup>a,pq</sup>	0.534±0.24 <sup>b</sup> ,
After 1 Week	19.463±3.2 <sup>a,pq</sup>	17.86±15.93 <sup>a,p</sup>	38.91±12.16 <sup>b,s</sup>	1.512±0.415 <sup>a,r</sup>	0.602±0.19 <sup>b,p</sup>	0259±0.13 <sup>b,q</sup>			
After 2 Weeks	14.88±9.45 <sup>a,pq</sup>	14.882±9.45 <sup>a,p</sup>	38.6±11.64 <sup>b,rt</sup>	1.869±0.653a,s	0.611±0.29 <sup>b,p</sup>	0.28±0.27 <sup>b,rs</sup>	$1.549\pm0.46^{a,pq}$	1.886±0.29a,rs	0.268±0.11 <sup>b,</sup>
After 3 Weeks	12.175±2.7a,qr	14.276±8.34 <sup>a,p</sup>	25.122±7.2 <sup>b,pq</sup>	1.594±0.6a,rs	0.602±0.36 <sup>b,p</sup>	0.11±0.03c,st	1.743±0.71 <sup>a,r</sup>	1.927±0.29 <sup>a,s</sup>	0.211±0.1 <sup>b,q</sup>

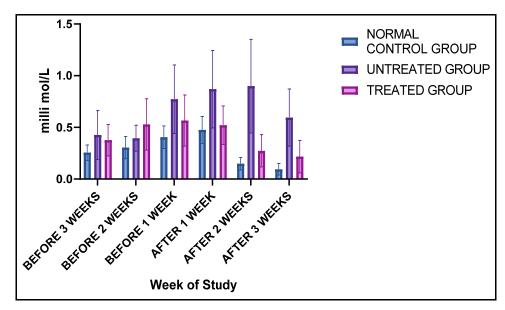
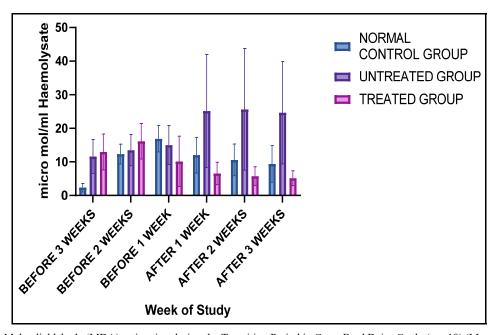


Fig 1: Non-Esterified Fatty Acids (NEFA) estimation during the transition period in Cross Bred dairy cattle (n = 10). (Mean  $\pm$  SD)



 $\textbf{Fig 2:} \ \ \text{Malondialdehyde (MDA) estimation during the Transition Period in Cross Bred Dairy Cattle (n = 10) (Mean \pm SD)$ 

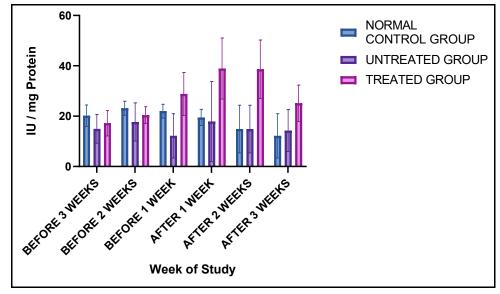


Fig 3: Glutathione Peroxidase (GPX) estimation during the Transition Period in Cross Bred Dairy Cattle (n = 10). (Mean  $\pm$  SD)

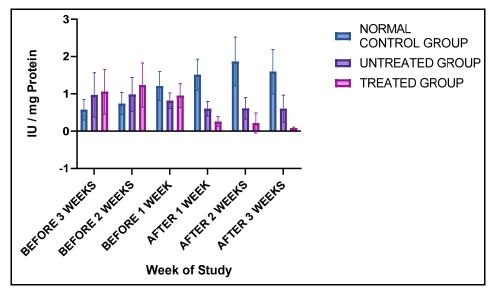


Fig 4: Superoxide Dismutase (SOD) estimation during the Transition Period in Cross Bred Dairy Cattle (n = 10). (Mean ± SD)

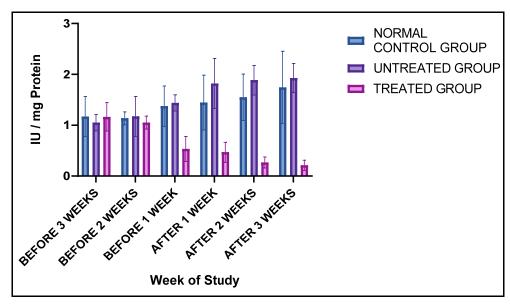


Fig 5: Catalase estimation during the transition period in crossbred dairy cattle (n = 10). (Mean  $\pm$  SD)

#### Conclusion

From present study it is obvious that both oxidative stress and metabolic stress during transition has caused increased incidence of postpartum diseases. Among the observed postpartum diseases, infectious diseases (30%) and RFM (20%) incidence was more and it may be due to compromised immune status instigated by increased oxidative stress during this period. Further, this has been obviated in the treatment group. Even though there was oxidative stress 3 weeks prepartum in the treated group (Group III) the treatment with Vit E and Selenium consequently could alleviate the stress thereafter and the would-be reason for low incidence of postpartum diseases comparable to normal control group.

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#### **Conflict of Interest**

The authors declare no conflict of interest.

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