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Assessment of the Levels of Biomarkers of Oxidative Stress during Peri-parturient Period in Gir Cows

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ABSTRACT

Background: Dairy cows during late pregnancy, parturition or early lactation may be subjected to oxidative stress or the production of reactive oxygen metabolites. An imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to peri-parturient disorders in dairy cows therefore in present investigation, of biomarkers of oxidative stress during peri-parturient period in Gir cows were studied.

Methods: Total 42 peri-parturient Gir cows were included in the present study. Ten apparently healthy Gir cows were also selected (which were outside the peri-parturient period) to have base line data on levels of biomarkers of oxidative stress for the comparison and analysis. The study was conducted in southern part of Rajasthan.

Result: The mean values of malondialdehyde (n mol/g Hb), super oxidase dismutase (U/mg Hb), reduced glutathione (mM) and catalase (µmol of H_2O_2 consumed /min/mg Hb) during pre-partum period and post-partum period in Gir cows was 227.34±9.48 and 255.85±11.15; 72.97±4.96 and 56.13±4.30; 2.72±0.15 and 2.27±0.15 and 139.85±3.81 and 121.57±4.65, respectively. Determination of levels of biomarkers of oxidative stress revealed that level of malondialdehyde (MDA) was significantly ($P<0.05$) increased during peri-parturient period in Gir cows whereas, levels of superoxide dismutase (SOD) and reduced Glutathione (GSH) were significantly ($P<0.05$) decreased. The level of catalase was also significantly ($P<0.01$) decreased during peri-parturient period in Gir cows.

Key words: Biomarkers, oxidative stress, peri-parturient period, Gir cows.

INTRODUCTION

Peri-parturient period is especially critical for health and subsequent productivity (Pathan *et al*., 2013). Peri-parturient period, which is often considered to be between 3 weeks pre-partum to 3 weeks post-partum, is the most stressful phase of transition for dairy cows from pregnant non-lactating state to lactation. During this transition period, the body of cows undergoes a series of physiological adaptations, resulting in marked metabolic profile changes (Puppel and Kuczynska, 2016).

Oxidative stress is a relatively new field of research in Bovine Medicine but it is also thought to be a significant underlying factor in dysfunctional host immune and inflammatory responses, which can increase cows' susceptibility to health disorders. Pregnancy in dairy cows induces oxidative stress that can be a significant underlying factor leading to dysfunctional host immune and inflammatory responses that can increase the incidence and severity of infectious diseases (Sordillo and Mavangira, 2014). In the peri-parturient cows, tissues consume more oxygen through normal cellular respiration during times of increased metabolic demand in order to provide the energy needed for the onset of lactation. This increase in metabolic activity results in enhanced accumulation of reactive oxygen species (ROS) and depletion of important antioxidant defences around the time of calving (Gitto *et al*., 2002).

Free radicals are unstable molecules that have an unpaired electron, which makes them highly energized and reactive. They seek out other electrons, setting off chain reactions that lead to damage to body cells and DNA until they are quenched and return to a stable state (Rajoria *et al.,* 2010). An imbalance between increased production of ROS and reduced availability of antioxidant defenses near the time of parturition increases oxidative stress and may contribute to peri-parturient disorders in dairy cows (Waller, 2000; Gitto *et al*., 2002). Ample evidence suggests that the progressive development of oxidative stress in peri-parturient dairy cows is a significant underlying factor leading to dysfunctional inflammatory responses (Sordillo and Aitken 2009; Osorio *et al*. 2014).

Several parameters can be used to measure the severity of peri-parturient stress and subsequent disease risk in peri-parturient cows. The antioxidant defenses of the body include scavenging enzymes like malondialdehyde

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(MDA), superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GSH) (Sood *et al*., 2019). There is need to focus on assessment of metabolic profiling and biomarkers of oxidative stress that can be used to monitor peri-parturient cows health and productivity. Present investigation was planned to study the levels of biomarkers of oxidative stress during peri-parturient period in Gir cows.

MATERIALS AND METHODS

Present research work was conducted at Veterinary College, Navania, Udaipur in the year 2021.

Animals

Total 42 peri-parturient Gir cows were included in the present study. Ten apparently healthy Gir cows were also selected (which were outside the peri-parturient period) to have base line data on levels of biomarkers of oxidative stress for the comparison and analysis.

Sample collection time

From each animal, blood samples were collected twice during peri-parturient period *i.e*. first time between 21 to 3 days prior to expected date of calving and second time, between 3 to 21 days after calving. Three days before and after parturition were excluded as these days are critical because most of the endocrinological changes occur during this period.

Signalment and clinical examination

Complete history including, age, parity, pregnancy, lactation, milk yield, history of previous illness if any, housing and managemental practices and any other relevant information was collected for each animal. The date of service (natural/ artificial insemination), date of pregnancy diagnosis and calving was also recorded for each Gir cow. Each Gir cows was subjected to thorough physical and clinical examination as per the methods described by Radostitis *et al*. (2007) followed by blood collection for estimation of the biomarkers of oxidative stress.

Biomarkers of oxidative stress

Blood samples were collected in sterilized test tubes keeping all aseptic precautions from the jugular vein using labelled sterile disposable syringes. Heparinised blood samples were centrifuged at 2500 rpm for 10 minutes. Plasma and buffy coat was removed and the erythrocyte pellet was washed and centrifuged thrice with normal saline solution. The haemolysate was prepared by adding double distilled water slowly up to initial marked level with constant stirring. The haemolysate was quickly stored in aliquats at -20ÚC until analyzed for various biomarkers of oxidative stress (enzymatic analysis). Haemoglobin concentration was estimated by cyanomethaemoglobin method (Vankannpep and Zinglstar, 1961). Erythrocytic lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was assayed by the method described by Placer *et al.* (1966). Erythrocytic reduced glutathione (GSH) was estimated by the method described by Hafeman *et al.* (1974). Activity of SOD in erythrocyte lysate was determined by using method described by Marklund and Marklund (1974). The activity of the enzyme was estimated by Spectro-photometerically using the method described by Aebi (1984).

Statistical analysis

The statistical analysis of the data was done using statistical methods described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Mean±SE value of various biomarkers of oxidative stress in control animals and peri-parturient Gir cows is depicted in Table 1.

Malondialdehyde (MDA)

Mean±SE values of malondialdehyde (n mol/g Hb) in control group was 209.26±9.7 whereas, the mean±SE values of malondialdehyde (n mol/g Hb) in Gir cows during pre-partum period and post-partum period was 227.34±9.48 and 255.85±11.15, respectively (Table 1). Mean values of malondialdehyde (MDA) (nmol/g Hb) during post-partum period in Gir cows was significantly ($P<0.05$) higher as compared to control animals and pre-partum period whereas, there was non-significant difference in the mean values malondialdehyde (MDA) in control animals and pre-partum period in Gir cows.

Findings of present study are in agreement with that of Castillo *et al*. (2006), Saleh *et al*. (2007), Sharma *et al*. (2011), Singh *et al*. (2014), Gong and Xiao (2015), Koujalagi *et al.* (2020), Singh *et al*. (2020) in dairy cows and Singh *et al*. (2015) in buffaloes.

Lipid peroxidation is a non-enzymatic chain reaction which based on oxidation of mainly unsaturated fatty acids and is associated with the presence of reactive oxygen species (ROS). It leads to the production of lipid peroxides and other intermediates. These intermediates affect the properties of cell membranes and their physiological functions (Halliwell and Gutteridge, 1985). Higher level of MDA in post-parturient Gir cows might be due to higher levels of glucocorticoids and eicosanoids and adrenaline-induced pathways of aerobic energy production associated with parturition which produces reactive oxygen metabolites and lipid peroxidation. Lipid peroxidation may be used as a biomarker of oxidative stress in peri-parturient cows. Increase in the LPO levels has been observed after calving, beacause lipids are most susceptible to peroxidative damage due to the presence of unsaturated bonds. The significant increase in the lipid peroxidation especially after calving might be due to the increased metabolic demands imposed on the cow by sudden milk production that far exceeded the demands of the foetus. Significantly higher level of malondialdehyde (MDA) during post-partum period was suggestive of increased oxidative stress in post-partum period (Konvicna *et al*., 2015).

Table 1: Mean±SE values of various biomarkers of oxidative stress in control animals and during peri-parturient period in Gir cows.

Biomarkers of oxidative stress	Control group	Peri-parturient period	
		Pre-partum	Post-partum
Malondialdehyde (MDA)*	209.26±9.7[a]	227.34±9.48[a]	255.85±11.15[b]
Superoxide dismutase (SOD)*	81.20±10.79[b]	72.97±4.96[b]	56.13±4.30[a]
GSH*	2.96±0.22[b]	2.72±0.15[b]	2.27±0.15[a]
Catalase**	154.64±5.14[c]	139.85±3.81[b]	121.57±4.65[a]

Means with different superscripts differ significantly.

*Significant at 5% level (P<0.05) ** Significant at 1% level (P<0.01).

Superoxidase dismutase (SOD)

Mean±SE values of super oxidase dismutase (U/mg Hb) in control animals, pre-partum and post-partum period in Gir cows were 81.20±10.79, 72.97±4.96 and 56.13±4.30, respectively (Table 1). Mean values of superoxidase dismutase (U/ mg Hb) in Gir cows during post-partum period was significantly (P<0.05) lower as compared to control group and pre-partum period whereas, there was non-significant difference in the mean values of superoxidase dismutase (U/ mg Hb) in control animals and pre-partum period in Gir cows.

Similar findings were also reported by Bernabucci *et al.* (2005), Singh *et al*. (2014), Koujalagi *et al*. (2020), Singh *et al*. (2020) in dairy cows and Singh *et al*. (2015) in buffaloes. Researchers have also reported a decrease in superoxidase dismutase levels with the advancement of lactation and observed lowest levels during the early lactation period. On contrary Bernabucci *et al*. (2005) reported a progressively increasing in blood superoxidase dismutase values from three weeks pre-partum to four days post-partum.

The superoxidase dismutase is a major intracellular enzymatic antioxidant and known as the first defense against pro-oxidants that convert the superoxide (O_2-) to hydrogen peroxide (H_2O_2), which is further converted into less dangerous forms by other antioxidants (Halliwell *et al*., 1993). Lower values of superoxidase dismutase during peri-parturient period in Gir cows might be due to oxidative stress.

Reduced Glutathione (GSH)

Mean±SE values of reduced glutathione (mM) in control animals was 2.96±0.22 whereas, the mean values of reduced glutathione (mM) in pre-partum and post-partum period in Gir cows were, 2.72±0.15 and 2.27±0.15, respectively (Table 1). Mean values of reduced glutathione (mM) in Gir cows during post-partum period was significantly (P<0.05) lower as compared to control group and pre-partum period whereas, there was non-significant difference in the mean values of reduced glutathione (mM) in control group and pre-partum period.

Findings of present study are in agreement with that of Sharma *et al*. (2011), Singh *et al*. (2014), Koujalagi *et al*. (2020), Singh *et al*. (2020) in dairy cows and Singh *et al*. (2015) in buffaloes.

Reduced Glutathione is a major endogenous antioxidant produced by the cells. It has important role in the neutralization of free radicals and reactive oxygen species, as well as maintaining exogenous antioxidants such as vitamin C and vitamin E in its reduced (active) forms (Scholz *et al*., 1989). Significant decrease in the reduced glutathione levels during the peri-parturient period in Gir cows might be due to the increased production of reactive oxygen metabolites (ROM).

Catalase (CAT)

Mean±SE values of catalase (µmol of H_2O_2 consumed / min/mg Hb) in control animals was 154.64±5.14 whereas, the mean values of catalase (µmol of H_2O_2 consumed / min/mg Hb) in Gir cows during pre-partum and post-partum period were 139.85±3.81 and 121.57±4.65, respectively (Table 1). There was highly significant difference in the mean value of catalase in Gir cows of control group and during peri-parturient period. Mean values of catalase were significantly (P<0.01) lower during peri-parturient (pre-partum and post-partum period both) in Gir cows as compared to control animals.

Similar findings were also reported by Sathya *et al*. (2007), Mishra (2011) and Beigh, (2014) in cows. Lower values of catalase in Gir cows might be due to the stressful condition of cows during peri-parturient period. Catalase is one of the important anti-oxidant enzymes.

Determination of levels of biomarkers of oxidative stress revealed that level of malondialdehyde (MDA) was significantly (P<0.05) increased during peri-parturient period in Gir cows whereas, levels of superoxide dismutase (SOD) and reduced Glutathione (GSH) were significantly (P<0.05) decreased. The level of catalase was also significantly (P<0.01) decreased during peri-parturient period in Gir cows.

CONCLUSION

It was concluded that there was variations in levels of biomarkers of oxidative stress during peri-parturient period in Gir cows. Alterations in the level of biomarkers of oxidative stress indicate an imbalance between increased production of reactive oxygen species (ROS) and reduced availability of antioxidant defence near parturation and early lactation. Further, peri-parturient period is the most stressful phase for the dairy cows. During peri-parturient period, the body of cows undergoes a series of physiological adaptations, resulting in marked oxidative stress. Most of these physiological adaptations occur in a very short period of time and are major contributors to most of the health problems in dairy cows, including productions diseases.

Conflict of interest: None.

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