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Original Article

Relationship between Enzymatic Antioxidant Activities and Reproductive Hormones in the Cows with Retained Placenta in Basrah Province, Iraq

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Abstract

The current study was conducted to evaluate the activity of enzymatic antioxidants (superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GSH-px], glutathione transferase [GSH-tr], and glutathione [GSH]) in the plasma of cows that suffered from retained placenta (RP), compared to cows without RP. Estrogen, progesterone, and cortisol hormones were also evaluated for the two groups of animals. In total, 38 pregnant cows were investigated in this study, and eight cows were suffering from RP (RP group) and did not release the placenta up to 12 h after giving birth. The other 30 cows did not suffer from RP (non-RP group) and left as a control. The results revealed a significant increase in CAT, GSH-tr, and GSH-px enzyme activities after calving in RP animals, compared to the other group of animals with normal placenta release before and after calving. In addition, SOD and GSH showed a significant increase in their levels before calving that reduced after giving birth, compared to the other antioxidant enzymes levels. The hormonal study also revealed a significant decrease in the estrogen level in the RP group, compared to the non-RP group, while the progesterone and cortisol showed non-significant levels in the studied groups. In conclusion, a strong relationship of RP with the antioxidant enzymatic activity and hormones in RP cow was observed in this study, which can be used to predict the incidence of RP throughout the levels of enzymatic antioxidants before parturition.

Keywords: enzymatic, antioxidant, before parturition

1. Introduction

Retained placenta (RP) is one of the most common periparturient problems that cows face in this period. This complication can be defined as failure to deliver the fetal membrane within 12 hours after delivery (1). The normal release of the placenta in cows usually occurs within six hours after giving birth, and the incidence rate ranged from 3% to 12% in dairy cows (2). The RP is a multifactorial problem, and although there is no detection factor to provide a scientific explanation to the incidence of this syndrome, there is some evidence for various risk factors represented by genetics, environment, age, nutritional status, and hormones that may interfere with the causes of RP (3). The other pathological conditions associated with RP incidence in dairy cattle are abortion, twinning, dystocia, obstetrical complication, infectious diseases, and nutritional disorders (4).

In the past decades, many researchers that included retained fetal membrane in their studies mentioned alteration in oxidative stress, compared to the normally released placenta (5, 6). An increase in reactive oxygen species (ROS) may result in damages to cells and tissues, as well as disturbances in metabolic pathways

(7), followed by RP. These may induce the activity of glutathione peroxidase (GSH-px), glutathione transferase (GSH-Tr), and catalase (CAT) in addition to superoxide dismutase (SOD) to protect the cells and tissues against ROS by neutralizing the production and degradation of ROS (8). In this regard, Kankofer, Lipko (9) investigated the imbalance of antioxidant/oxidative activity of caruncles and villi in cows. Furthermore, they (10) recorded high antioxidant and oxidative activities at first and second weeks after parturition in blood plasma.

This stress can activate the hypothalamic-pituitary-adrenocortical axis, which increases plasma corticosteroids. As a result, the cortisol concentration during the peri-parturient period increases by several folds particularly on the day of calving (11). These disturbances may cause the alteration of reproductive hormones' concentration; however, the relationship of antioxidant/oxidative activity and its effect on reproductive hormones in the RP of cows have not been investigated yet. Therefore, the present study aimed to identify the differences in antioxidant enzyme activities and their relationship with some reproductive hormones in cows suffering from RP, compared to cows with normal delivery and normal placental release.

2. Materials and Methods

This study included 38 pregnant cows in the last month of pregnancy. The measurement of CAT, SOD, glutathione (GSH), GSH-Px, and GSH-Tr was conducted by the inspiration of 10 ml of blood samples transferred directly into the test tube containing Gel/clot Activator (without anticoagulant) which was immediately centrifuged to collect the serum for the analysis of enzymes activity, as well as hormone concentrations. The activity of the CAT enzyme in the serum was determined according to the method of Beers and Sizer (12) that was modified by Aebi (13) depending on monitoring H_2O_2 substrate consumption at 240 nm. The total SOD activity in the serum was measured based on Flohé and Günzler (14) using a special kit (SZA kit, Germany). These methods depend on the SOD ability to inhibit epinephrine oxidation to adrenochrome. Assay reactions were performed at 37°C in air. The GSH, GSH-px, and GSH-Tr were measured according to Flohé and Günzler (14) using a special kit for each one (SZA, Germany) at 340 nm and 37°C. The enzyme assays were determined in a spectrophotometer (Apple, Japan).

Progesterone (P4),estradiol (E2), and cortisol were estimated using ELISA commercial kits (DRG, Germany). The sensitivity of the assay was0.045 ng/ml for P4, while the sensitivities of the assay for E2 and cortisol were 9.7 and 5.5 pg/ml, respectively.

3. Results

Throughout monitoring pregnant cows in the present study before and after calving, it was found that eight (21.06%) cows were suffering from RP and spent about 54.62±9.32 hours to release placenta after calving, compared to cows that released placenta normally at 3.35±1.24 hours which represented 78.94% of the total studied cows (Table 1).

Table 1. Incidence of the retained placenta from experimental cows and the time for placenta expulsion

Experimental cows	No. (%)	Time to release placenta after calving/h
Cows normally released placenta	30 (78.94%) [a]	3.35±1.24[a]
Cows with retained placenta	8 (21.06%) [b]	54.62±9.32[b]
Total number	38	

Different letters within each column indicate significant difference at ($P<0.05$)

The results of the antioxidant enzyme activity in pregnant cows before and after calving related to the release of the placenta are represented in figures 1, 2, 3, 4, and 5. The activity of CAT showed a significant increase (P<0.05) in cows with RP (11.75±0.37 U/L), compared to the animals calving normally (8.71±0.61 U/L). However, before calving, CAT had less activity (5.60±0.31U/L)than that in the other studied group. In contrast, the SOD activity had the highest significant value (P<0.05) in cows before calving (6.90±0.18 U/L), and this value reduced significantly in animals suffering from RP (3.91±0.17 U/L) and cows with the normally released placenta after calving (4.68±0.36 U/L).

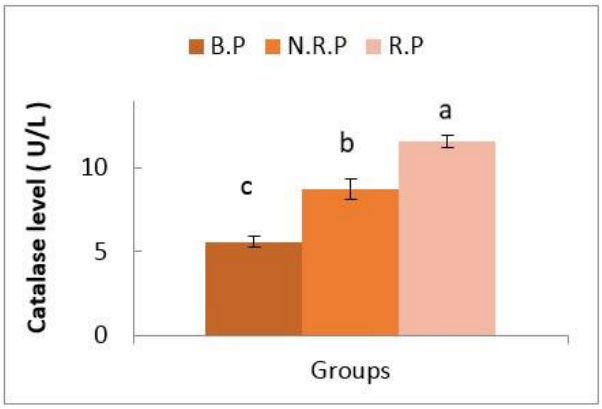

Figure 1. Catalase enzyme activity in bovine plasma before calving in normal and retained placenta release (significant difference at P<0.05)

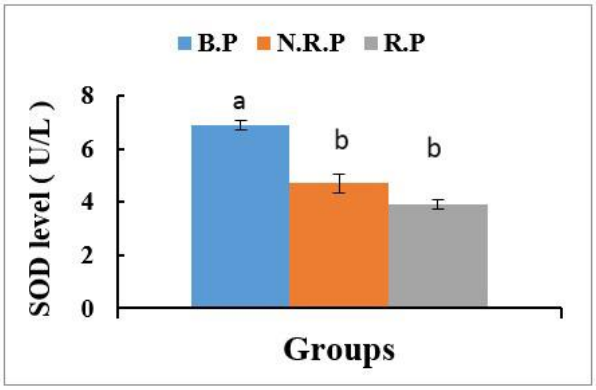

Figure 2. Superoxide dismutase levels in bovine plasma before calving in normal and retained placenta release (significant difference at P<0.05)

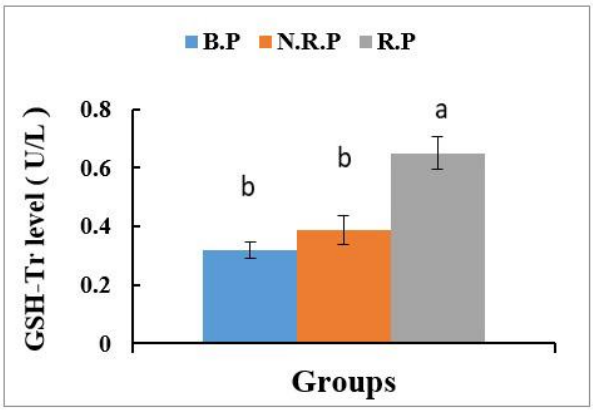

Figure 3. Glutathione transferase activity in bovine plasma before and after calving in normal and retained placenta release (significant difference at P<0.05)

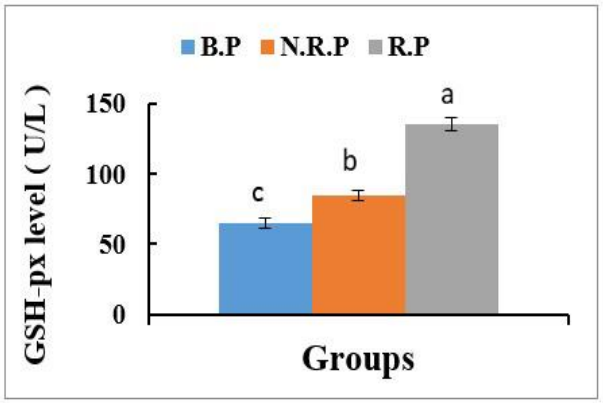

Figure 4. Glutathione peroxidase activity in bovine plasma before and after calving in normal and retained placenta release (significant difference at P<0.05)

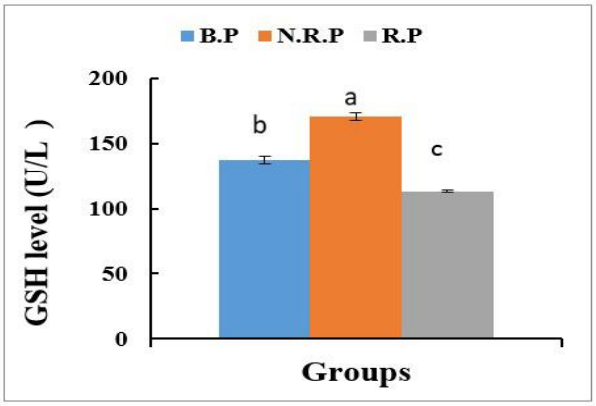

Figure 5. Glutathione levels in bovine plasma before and after calving in normal and retained placenta release (significant difference at P<0.05)

In figure 3, the activity of GSH-Tr showed a significant level (P<0.05) for the cows with RP after parturition (0.65±0.06 U/L), compared to cows before and after calving (0.32±0.03 and 0.39±0.05 U/L, respectively).

In addition, GSH-Px showed a significant value (P<0.05) in animals with RP (135.22±4.44U/L); however, the activity of GSH-Px showed a significant increase in animals with normal placental release after calving (84.95±3.77U/L), compared to the same animals before calving (64.97±3.81U/L) as illustrated in Figure 4. In contrast, GSH revealed a significant elevation (P<0.05) in non-RP cows (170.98±5.78U/L), compared to RP and cows before calving (113.43±3.98 and 37.40±5.93U/L, respectively); moreover, the GSH of the results indicated a significant decrease in RP cows when compared to non-RP cows and before calving (Figure 5).

4. Discussion

Figure 6 illustrates the concentration of hormones (progesterone, estradiol, and cortisol) in cows with retained and non-retained placenta before and after calving. A significant decrease (P<0.05) in the progesterone level occurred after parturition in both groups (retained and non-retained) which showed no difference between them (2.80±0.55 and 2.91±0.76 ng/ml, respectively), while the elevation was clear before calving (4.91±1.48ng/ml). Moreover, estradiol concentration revealed a significant increase (P<0.05) in non-retained placenta cows (1.15±0.34 ng/ml) after calving, compared to retained placenta cows (0.84±0.17 ng/ml). However, the cortisol showed no difference before and after calving in both groups although there was a slight rise before calving in all studied animals (4.66±0.88, 4.14±0.85, and 4.46±0.9 ng/ml, respectively). The RP is one of the common problems that veterinarians face in the field, especially in cattle; additionally, it is very difficult and consumes the time and effort of the clinicians. Many factors may cause RP in animals, such as infection, hormonal disturbance, nutritional deficiency, or immunologic problems (15). There is also evidence that links immunity status with oxidative stress as promoters fo rRP (7, 16); however, the exact mechanism of oxidative stress effect on placental release in cows is still unclear.

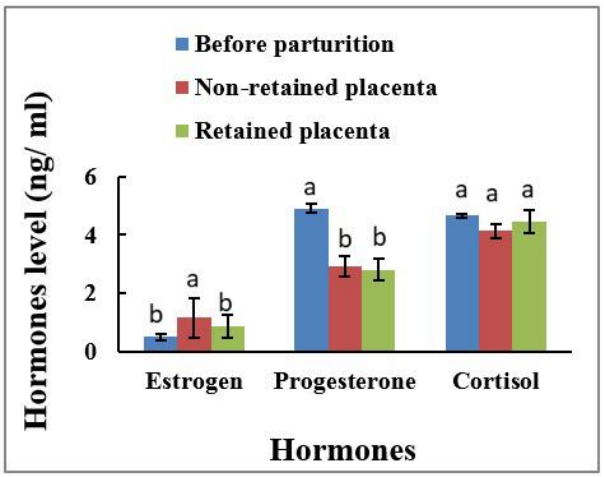

Figure 6. Estrogen, progesterone, and cortisol levels in bovine plasma before and after calving in normal and retained placenta release (significant difference at P<0.05)

The present study investigated the antioxidant enzyme activity in pregnant cows before and after calving; moreover, it monitored animals suffering from RP. The results revealed significant values for CAT, GSH-tr, and GSH-px in cows with RP, compared to cows that released placenta normally. Although SOD showed opposite behaviors, it revealed a significant increase in cows' plasma before calving, compared to cows after birth.

The antioxidative defense mechanisms against oxidative stress depend on the ability of antioxidative enzymes to neutralize substrates that work to createhydroxyl radicals or superoxide anions radical and then lipid peroxidation (17). There are pieces of evidence from some researchers about the level of SOD activity in peri-parturient cow statuses, such as an increase in SOD level in case of heat stress (18) and a decrease in case of mastitis infection (19). Wischral, Nishiyama-Naruke (20) found no changes in the concentration of SOD in cows with or without RP. However, Kankofer (17) reported a significant increase in the SOD activities in RP cows at pre and at term. This increase in SOD level may be attributed to the

inflammation through a transition period to lactation (21), preterm hyper insulinemia, metabolic disorders (11), and changes in blood pressure on the placental side (22).

The levels of other antioxidant enzyme activities (GSH-tr, GSH-px, and GSH) before calving showed a reduction in the activity, compared to the time after parturition. This may be due to an imbalance between production and neutralization of ROS that appears close to or at parturition. The highest concentration of the SOD at preterm may also affect other antioxidative enzymes to neutralize the ROS and is competitive to protect the fetal placental tissues (23).

These results are in line with the findings of a study conducted by Brzezinska-Slebodzinska, Miller (24) who recorded higher GSH-Px activity in red blood cells at week 0, compared to week 2 of delivery. The GSH-Px and GSH-Tr activities are presented in Figures 3 and 4. In addition, they are related to the GSH level, which may depend on glutamyl transpeptidase which transports glutamyl residues on amino acids or small peptides. The activity of this enzyme increases in cases of RP in comparison with animals calving normally. This may suggest an increase in the GSH turnover and activation of the defense mechanisms against ROS (25).

The activation of the defense mechanisms is induced due to an increase in the ROS production to regulate this process. Lack of this process control leads to inconsistent defense systems. Furthermore, as mentioned previously byKono and Fridovich (23), the SOD activities may cause an effect on the CAT activity since the substrate for SOD may inhibit the CAT activity and vice versa. This exchange in the roles of enzymes may lead to uncontrolled ROS condition and induction of less efficient antioxidant defense mechanisms with inefficiency to control the increased ROS in animal tissues (26).

After parturition, the placenta release is influenced by the action of enzymes, 1 7-hydroxylase, and aromatase which are induced by fetal cortisol in the placenta. These, in turn, favor the production of estrogen and decrease progesterone levels (27). Therefore, a reduction in the estrogen level is a marker for RP due to a disturbance in the hormonal levels through an increase in the cortisol and progesterone levels and reduced estrogen in cow plasma to indicate RP (28). Previous studies showed a clear decrease in the level of estrogen in cows that suffered from fetal membrane retention (20, 29). These results are consistent with the findings of the present study. Adequate levels of estrogen playa very important role during parturition, as well as the repulsion of the fetal membrane (30, 31). Lower levels of estrogen may coincide with an apparent decrease in the activity of antioxidant enzyme in the placenta (9, 10, 32). This explains the close relationship between estrogen and the activity of the antioxidant enzymes which can predict the incidence of retained placenta according to the activity of antioxidant enzymes in the blood.

Authors' Contribution

Study concept and design: N. A. K.
Acquisition of data: H. R. A.
Analysis and interpretation of data: H. A. A.
Drafting of the manuscript: H. R. A.
Critical revision of the manuscript for important intellectual content: N. A. K.
Statistical analysis: H. A. A.
Administrative, technical, and material support: N. A. K.

Ethics

All the procedures were approved by the Ethics Committee at the University of Basrah, Basrah, Iraq under the project number of 2020-5478-2542.

Conflict of Interest

The authors declare that they have no conflict of interest.

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