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The Relationship between Enzymatic Antioxidant Activities and Reproductive Hormones in Retained Placenta Cows of Basrah Province

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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. Thirty eight pregnant cows have been used in this study. Eight cows from them suffered from RP (RP group), which didn't release the placenta up to 12 hours after birth. The other 30 cows didn't suffer from RP (non RP group) and left as a control. The results revealed that there were significant increase in CAT, GSH-tr and GSH-px enzyme activities after calving in RP animals compared to other group of animals with normal placenta release before and after calving. In addition SOD and GSH showed significant increase in their levels before calving and reduced after birth compared with other antioxidant enzymes levels. Hormonal study revealed a significant decrease in estrogen level in the RP group compared to the non RP group, while the progesterone and cortisol had non-significant levels among the studied groups. In conclusion, there was a strong relationship between retained placenta and the antioxidant enzymatic activity and hormones in RP cow, and suggest to predict the incidence of RP throughout the levels of enzymatic antioxidant before parturition.

Keywords: Enzymatic Antioxidant, Reproductive Hormones, Retained Placenta Cows

Introduction

Retained placenta (RP) is one of the most common periparturient problems that cow face in this period, it can be defined as failure to deliver the fetal membrane within 12 hours after delivery (Blood *et al.*, 2007). The normal release of placenta in cows usually occurs within six hours after birth and the incidence rate ranged from 3% to 12% of dairy cows (Sheldon *et al.*, 2009). RP is

multifactorial problem, although there is no detection factor that provides scientific explanation to the incidence of this syndrome but there are some evidences for various risk factors represented by genetics, environment, age, nutritional status and hormones that may be interfere with the causes of RP (Tucho and Ahmed, 2017). The other pathological condition associated with RP incidence in dairy cattle are abortion, twinning, dystocia, obstetrical complication ,infectious disease and nutritional disorder (Seifi *et al.*,2007).

In the past decades, many researchers that included retained fetal membrane (RFM) in their studies, mentioned alteration in oxidative stress compared to normally released placenta (Elecko *et al.*,1982; Stec *et al.*,1991). An increase in reactive Oxygen species (ROS) might led to damage to cells , tissues and disturbances in metabolic pathways (Sordillo and Aitken,2009) and followed by RP. These may inducte 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 (Trevisi *et al.,* 2016). In this direction kankofer *et al.*,(2005) investigated the imbalance of antioxidant/oxidative activity of carnucles and villi in cows. He with his team (kankofer *et al.,* 2010) recorded high antioxidant and oxidative activity at first and second week after parturition in blood plasma.

This stress can activate the hypothalamic-pituitary-adrenocortical axis (HPA), 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. (Mordak and Stewart, 2015). These disturbances may cause alteration of reproductive hormones concentration, but the relation of antioxidant/oxidative activity and its effect on reproductive hormones in retained placenta of cows have not been investigated. Therefore, the present study was designed to identify the differences in antioxidant enzymes activities and its relation with some reproductive hormones in cows suffering from retained placenta compared to cows with normal delivery and normal placental release .

Material and methods

The study included 38 pregnant cows in the last month of pregnancy. The measurement of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase

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(GSH_Px) and glutathione transferase (GSH-Tr) was done by inspiration 10 ml of blood samples transferred directly into test tube containing Gel/clot Activator (without anti-coagulant) which was immediately centrifuged to collect serum for analysis of enzymes activity as well as hormones concentrations. The activity of CAT enzyme in serum was determined according to method of Beers and Sizer, (1952); modified by Aeba (1984) that depend on monitoring H_2O_2 substrate consumption at 240 nm. Total SOD activity in serum was measured based on (Flobe and Gunzler,1984) by using a special kit (SZA kit, Germany). These methods depend on SOD ability to inhibit epinephrine oxidation to adernochrome. Assay reactions were performed at 37 centigrade in air. The GSH, GSH-px and GSH-Tr were measured according to (Flobe and Gunzler,1984) by using 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 was 0.045 ng/ml for P4,while the sensitivity of the assay for E2 was 9.7pg/ml and 5.5 pg/ml for cortisol.

Results

Throughout monitoring pregnant cows in the present study before and after calving we recorded that eight (21.06%) of cows suffered from retained placenta and spent about 54.62± 9.32 hours to release placenta after calving compared with cows that released placenta normally at 3.35 ± 1.24 hours which represented (78.94%) from the total studied cows (table 1).

Table 1. The incidence of 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 antioxidant enzymes activity in pregnant cows before and after calving relation to the release of placenta are represented by figure (1,2,3,4 and 5) . The activity of catalase enzyme (CAT) showed significant increase ($P<0.05$) in cows retained placenta (11.75± 0.37 U/L) compared with the animals calving normally (8.71±0.61 U/L). While before calving, CAT had less activity (5.60±0.31 U/L) than the other studied group. In contrast, Superoxide dismutase (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 suffered from retained placenta (3.91±0.17 U/L) and with cows normally released placenta after calving (4.68±0.36 U/L).

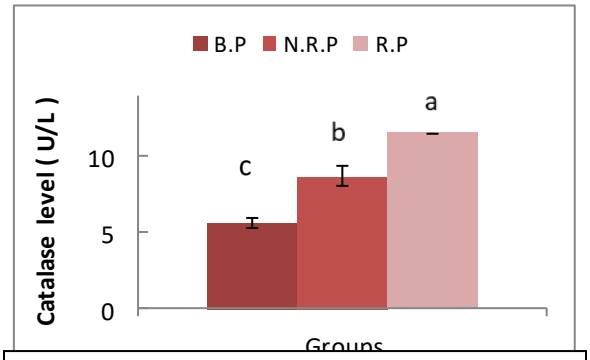


Figure (1) catalase enzymes activity in bovine plasma before calving, normal and retained placenta release significant difference at ($p < 0.05$)

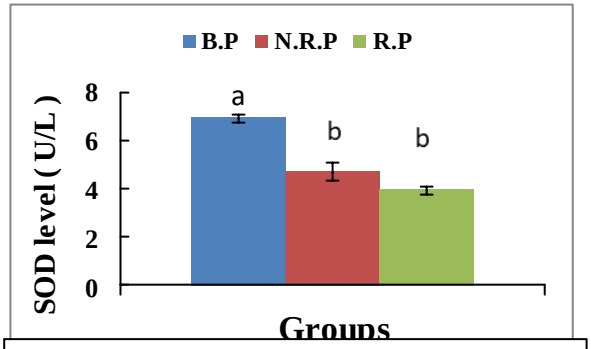
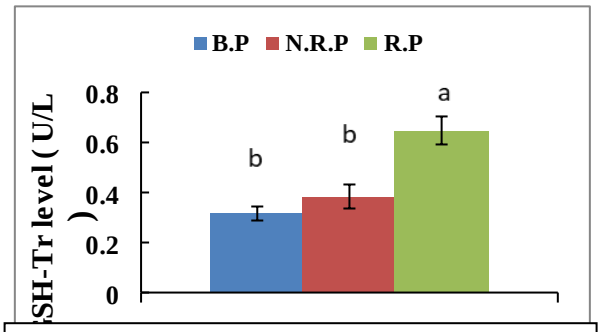


Figure (2) superoxide dismutase (SOD) levels in bovine plasma before calving, normal and retained placenta release. Significant difference at ($p < 0.05$)

In figure (3) , the activity of glutathione transferase (GSH-Tr) showed significant level ($P<0.05$) for the cows retained placenta after parturition (0.65±0.06 U/L) compared with cows before and after calving (0.32±0.03 and 0.39±0.05 U/L), respectively. In addition, there was significant value ($P<0.05$) of Glutathione peroxidase (GSH-Px) in animals retained placenta (135.22±4.44 U/L) ,but the activity of GSH-Px showed a significant increase in animals of normal placental release after calving (84.95±3.77 U/L) compared with the same animals before calving (64.97± 3.81 U/L) as illustrated in figure (4). In contrast, glutathione(GSH) showed significant elevation ($P<0.05$) in non- RP cows(170.98 ±5.78 U/L) compared with RP and cows before calving (113.43±3.98;137.40 ±5.93 U/L) respectively , also GSH of the results revealed significant decrease in RP cows when compared with non- RP cows and before calving (figure 5).



Figure(3) glutathione transferase (GSH-Tr) activity in bovine plasma before and after calving, normal and retained placenta release. Significant difference at ($p < 0.05$)

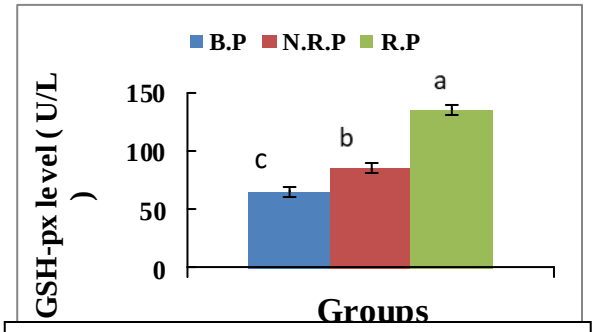


Figure (4) Glutathione peroxidase (GSH-Px) activity in bovine plasma before and after calving in normal and retained placenta release. Significant difference at ($p < 0.05$)

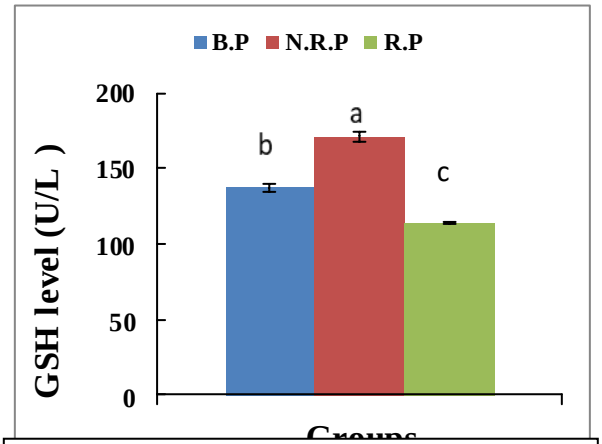
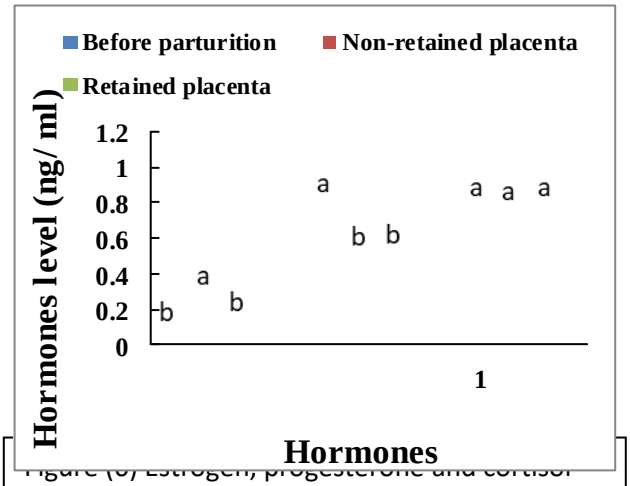


Figure (5)glutathione levels in bovine plasma before and after calving in normal and retained placenta release. Significant difference at ($p < 0.05$)



levels in bovine plasma before and after calving in normal and retained placenta release. Significant difference at ($p < 0.05$)

Figure (6) showed the concentration of hormones (progesterone, estradiol and cortisol) for retained and non-retained placenta cows before and after calving. Significant decrease ($P<0.05$) in progesterone level occurred after parturition for both groups (retained and non-retained) which appear without any difference between them(2.80±0.55 ; 2.91±0.76 ng/ml) respectively, while the elevation was clear before calving (4.91±1.48 ng/ml). 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). While the cortisol didn't show difference before and after calving for the both groups although there was slight rise before calving for all studied animals (4.66±0.88; 4.14±0.85 and4.46±0.9 ng/ml) respectively .

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Discussion

Retained placenta is one of the common problems that veterinarian face in the field, especially in cattle, it is very difficult and consume time and effort of the clinician. There are many factors that may causes retained placenta in animal such as infection, hormonal disturbance, nutritional deficiency, or immunologic problem and others (Boro *et al.*,2014). There are evidence that link immunity status and oxidative stress as promoters for retained placenta (Sordillo *et al.*,2009: Waldron,2010), but still the exact mechanism of oxidative stress effect on placental release in cow is unclear.

The present study tested the antioxidant enzymes activity in pregnant cows before and after calving and monitored animals suffered from retained placenta. The results revealed that there were significant value for CAT,GSH-tr, GSH-px in cows retained placenta compared with cows released placenta normally. While SOD showed opposite behavior ,it showed significant increase in cows plasma before calving compared with cows after birth.

The antioxidative defense mechanisms against oxidative stress depends on the ability of antioxidative enzymes to neutralize substrates that work to create hydroxyl radicals or superoxide anions radical and then lipid peroxidation (Kankofer, 2001).

There were evidences from some researchers about the level of SOD activity in peri-parturient cows status, such as increase in SOD level in case of heat stress (Bernabucci *et al.*, 2002) and decrease in case of mastitis infection (Machado *et al.*, 2014). Wischral *et al.* (2001) found that ther was no change in concentration of SOD in cows with or without retained placenta. Whereas, Kankofer, (2001) reported significant increase in SOD activities in RP cows at pre and at term. This increase in SOD level may be attributed to inflammation through transition period to lactation (Bradford *et al.*, 2015) , preterm hyperinsulinemia and metabolic disorder (Mordak *et al.*, 2015) , and change in blood pressure on the placental side (Hartmann *et al.*, 2013).

The levels of others antioxidant enzymes activities(GSH-tr, GSH-px and GSH) before calving showed reduction in activity than after parturition and this may be due to imbalance between production and neutralization of ROS appears closely to, or at parturition. The highest concentration of SOD at preterm also may effect on other antioxidative enzymes to nutrilaized the ROS and competitive to protect the fetal placental tissues (Kono and Fridovich, 1982).

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These results agreed with (Brzezin´ska-Sulebodzin´ska *et al.*, 1994) when recorded higher GSH-Px activity in red blood cells at week 0 compared to week 2 of delivery.

The GSH-Px and GSH-Tr activity presented in figure 3,4 are related with GSH level, which may depend on -glutamyltranspeptidase which transports -glutamyl residues on amino acids or small peptides. The activity of this enzyme increases in cases of retained placenta in comparison with animals calving normally . This may suggest an increase in GSH turnover and activation of defense mechanisms against ROS (Kankofer and Maj, 1997).

The activation of defense mechanisms induced due to increase in ROS production to regulate this process, lack of this process control lead to inconsistent defense systems . Furthermore, as mentioned previously by (Kono and Fridovich, 1982) that SOD activities may cause an effect on CAT activity, because the substrate for SOD may inhibit CAT activity and vice versa. This exchange in roles of enzymes may lead to uncontrolled ROS condition and induction of less efiecient antioxidant defense mechanisms with inefficiency to control the increased ROS in animal tissues (Pigeolet *et al.*,1990).

After parturition, 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 production of estrogen and decrease progesterone levels (Yusuf,2016) .Therefore, reduction in estrogen level is a marker for RP due to disturbance in the hormonal levels through increase cortisol and progesterone levels and reduced estrogen in cow plasma to indicate RP(Michal *et al.*,2006)

Previous studies showed a clear decrease in the level of estrogen in cows which suffered from fetal membrane retention (Tsumagari *et al.*, 1993; Wischral *et al.*, 2001). These results agreed with the present study results. Adequate level of estrogen plays a very important role during parturition as well as the repulsion of fetal membrane (Rasmussen *et al.*, 1996; Gross *et al.*, 1986). Lower levels of estrogen may coincide with an apparent decrease in the activity of antioxidant enzyme in the placentae (Kankofer and Schmerold, 2002; Kankofer *et al.*, 2005; Kankofer *et al.*, 2010). This explains the close relationship between estrogen and the activity of antioxidant enzymes which can predict the incidence of retained placenta according to the activity of antioxidant enzymes in the blood.

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