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Evaluation of enzymatic antioxidant activity and reproductive hormones near and after lambing in pregnant ewes

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ABSTRACT

The current study examined the levels of antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), and Malondialdehyde (MDA) and reproductive hormones (progesterone, estrogen, and cortisol) in pregnant local breed ewes and after lambing in the Basrah governorate. Twenty ewes were used in this study from several fields in the Basrah governorate. Blood sample collection was done in three different periods, pre-lambing, immediately after lambing, and two weeks after lambing. The result showed a significant decrease ($p<0.05$) in SOD, CAT, and GSH-px activity before and during lambing compared to after lambing. The results also confirmed a significant increase ($p<0.05$) in the level of GSH pre and during lambing compared to after lambing. While evaluating, the level of MDA revealed a significant increase ($p<0.05$) in its level during lambing compared to two weeks after lambing. Hormonal analysis showed a significant increase ($p<0.05$) in the levels of cortisol and estradiol pre and during lambing compared to after lambing, with a significant decrease ($p<0.05$) in the levels of progesterone after lambing compared to before lambing. This study concluded that during lambing, ewes were under stress factors, and an internal mechanism represented by enzymatic antioxidants like SOD, CAT, and GSH-Px acted actively during this period to neutralize the side effects of stress.

Keywords: Antioxidant, Ewes, Lambing, Pregnancy

INTRODUCTION

The enzymatic antioxidants are a fundamental constituent of the intra-cellular redox balance being able to render harmless dangerous reactive oxygen species (ROS) and hence protecting the cells against oxidative stress (Fridovich, 1999).

The reproductive organs consisting of ovaries and uterus are very active metabolically and are exposed to environmental agents such as oxidants (free radicals) likely to damage the DNA and proteins of the affected tissues (Fridovich, 1999; Rios *et al*., 2017). It has been shown that ewes enzymatic antioxidants play an important role in the female reproductive processes, and they affect reproductive performance in many ways (Rios *et al*., 2017).

Blood supplies oxygen to the tissues and at the same time removes all the toxic substances produced by the metabolism. SOD is one enzymatic antioxidant that is responsible for catalyzing the conversion of superoxide radicals into hydrogen peroxide (Bayr, 2005). Several isoforms for SOD like CuZn-SOD and Mn-SOD

and of them have been detected in many tissues and organs (Bayr, 2005). The SOD activity was crucial in the fulfillment of the normal gestation stage and lactating period in ewes (Al-Hassan *et al*., 2016; Nawito *et al*., 2016).

The oxidative state in the body of animals during pregnancy, parturition, and lactation and each showed several changes (Al-Hassan *et al*., 2016; Nawito *et al*., 2016); they revealed many changes during these physiological states. On the other hand, enzymatic antioxidant like GSH-px plays an important role as a scavenger for lipid hydro-peroxides and hydrogen peroxide (Ursini and Bindoli, 1987). GSH-px has been detected in the tissues of the reproductive system in ewes, especially in placentomes which act to maintain their redox balance. Garrel *et al*. (2010) reported a positive correlation between the GSH-px activity with normal pregnancy. A high positive correlation was discovered between normal postpartum and lactation period in animals with the activity of enzymatic antioxidants, especially GSH-px, which improves the performance of the reproductive system during this stage (Rios *et al*., 2017; Al-Hassan *et al*., 2016).

Furthermore, Wang *et al*. (2017) report revealed a clear reduction in catalase activity in anestrus ewes' ovaries which suggests a certain association between catalase and reproductive success. Ewes' lambing is initiated by a sharp dropping in the progesterone release. Before lambing a significant increase in the secretion of cortisol by the fetal adrenal gland (Miura *et al*., 2019). Cortisol stimulate the placental enzymes to form estrogen from progesterone which is followed by stimulating the release of prostaglandin F2alpha (PGF2α) from the uterus to increase myometrial response to oxytocin to induce contractions.

On the other hand, estrogen levels gradually increase during late pregnancy and peak just before lambing. This rise in estrogen prepares the reproductive tract for parturition and initiates maternal behaviors. It plays a role in uterine involution and the resumption of estrus cycling (Neal Schrick *et al*., 1993). The main aim of this study is to evaluate the enzymatic antioxidant activity (SOD, CAT, GSH-Px, GSH, and MDA) and reproductive hormones (progesterone, estrogen, and cortisol) in pregnant local breed ewes before and after parturition.

MATERIALS AND METHODS

Experimental Animals

Twenty pregnant ewes (aged from 2-4 years) were used for the current study. The animals were maintained and housed under similar conditions of feeding and management and the health condition was controlled by veterinarians. The study was conducted from November 2022 to May 2023 in Basrah, Iraq. This study received an approval from Ethical Committee of University of Basrah, College of Veterinary Medicine under the license 9/37 under the license dated 28-04-2022.

Blood Samples

In three periods, blood samples were collected from the jugular vein using a 16 G needle in a sterile centrifuge tube from the pregnant experimental animals. The first period (prelambing), the second period (immediately after lambing), and the third period (after two weeks from lambing). Serum was obtained by centrifuging blood samples at 3000 rpm for 20 min, then it was labeled and stored at −20 ^{o}C before being analyzed.

Antioxidants Analysis

Based on Flohé and Günzler (1984), the activity of SOD in serum was determined by using a special kit (Sza kits, Germany) at 480 nm immediately and in the temperature of 37^{o} C. The activity of CAT enzyme in serum (units/ml) was measured depending on Beers and Sizer (1952), which was modified by Aebi (1984) through (Sza kits, Germany) at 240 nm after 30 seconds. GSH-px activity was measured depending on Flohé and Günzler (1984) on a particular kit (Sza kits, Germany) at 412 nm and 37° C. The level of GSH was evaluated (Mmole/L) by the procedure of Tietz *et al*. (1994) by special kit (Sza kits, Germany) at 37^{o} C, and the results were recorded at 412 nm. MDA level (Mmole/L) was determined depending on Beuge and Aust (1978) by using Sza Kits (Germany), and the results were measured at 532 nm at 25^{o} C. All analysis was done by spectrophotometer, apple, Japan.

Hormone Analysis

Estradiol E2, Progesterone P4, and cortisol concentration were estimated using the enzyme-

linked immunosorbent assay (ELISA) technique and used a specific ovine kit (Yingxin Laboratory, China) according to the manufacturer's instructions.

Statistical Analysis

The results were analyzed using the analysis of variance (ANOVA) test by SPSS computer package (Version 21, IBM, USA). Differences were compared by Tukey's multiple-comparison post hoc test. All data were presented as means ± SEM, and the differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results showed a significant increase in the antioxidant enzymes (catalase and SOD) occurred two weeks after lambing compared to pre and during lambing (Figure 1). Also, both enzymes revealed a non-significant decrease during lambing compared to before lambing in experimental animals. Parturition is regarded as an essential stage in mammals after pregnancy which is accompanied by many events that tightly connect to complete this stage (Liggins *et al*., 1973). Enzymatic and non-enzymatic antioxidant levels are considered important for parturition in mammals (Erişir *et al*., 2009). The current study observed an apparent decline in the activity of antioxidant enzymes, especially catalase, and SOD, which agrees with many researchers that involved animal parturition (Khudhair *et al*., 2021). Catalase and SOD are regarded as essential antioxidant enzymes that neutralize the oxidation processes that occur during stress factors (Rios *et al*., 2017; Al-Hassan *et al*., 2016; Nawito *et al*., 2016). A stress factor occurred during parturition, which started with the fetus completing the delivery process (Erişir *et al*., 2009). During this stage, a high withdrawal of enzymatic and non-enzymatic antioxidants challenges the formation of hydroxyl radicals or superoxide anions radicals and then lipid peroxidation (Kankofer, 2001). In the current study, increasing catalase and SOD may be attributed to the inflammatory process following parturition and the lactation period (Bradford *et al*., 2105), which requires high levels of antioxidants to enhance protection from stress and return the animal to normal condition (Madhi *et al.,* 2022).

Furthermore, our results showed a significant increase in the activity of GSH-px in two weeks after lambing compared to before and during lambing. Also, they revealed a significant increase during lambing compared to before (Figure 2). Furthermore, the current study confirmed a significant increase in GSH before and

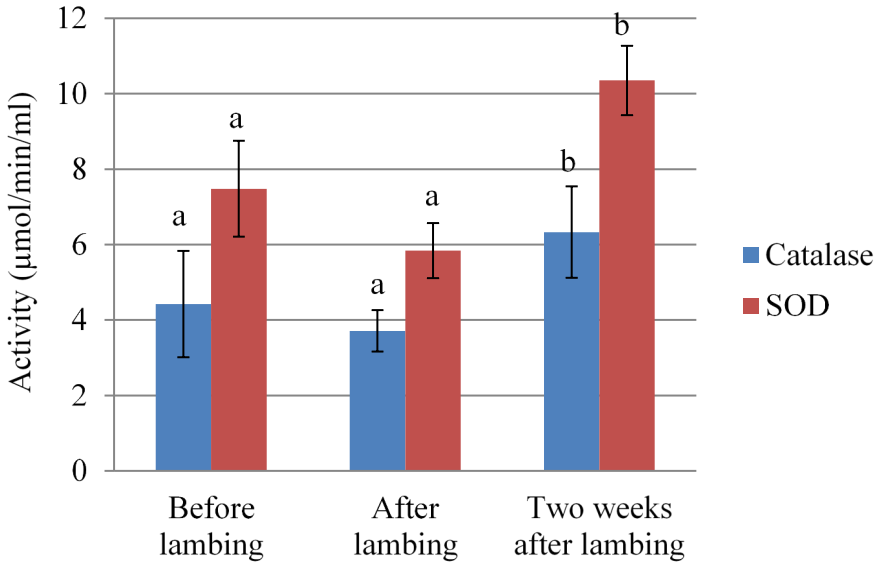

Figure 1. Catalase and SOD activity in ewes before, after, and two weeks after lambing. Different litters indicate significant differences at $P<0.05$.

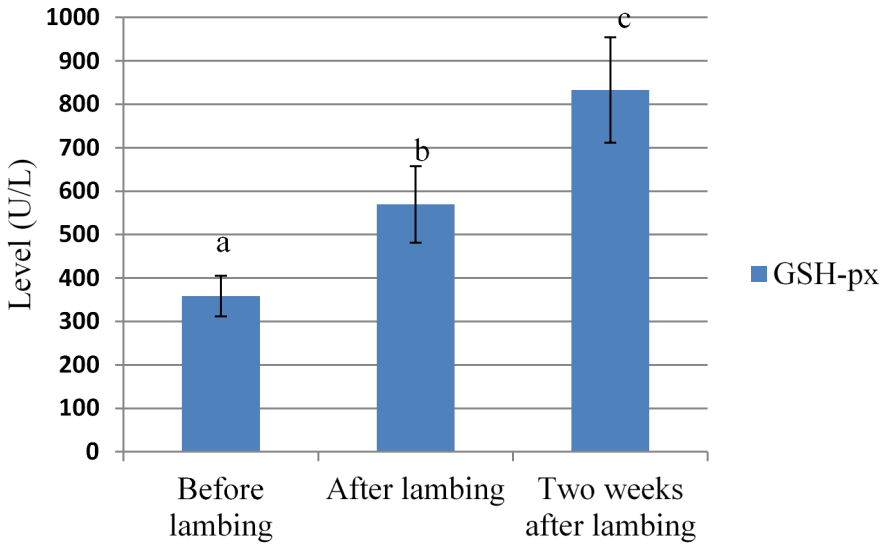

Figure 2. GSH-px activity in ewes before, after, and two weeks after lambing. Different litters indicate significant difference at P<0.05.

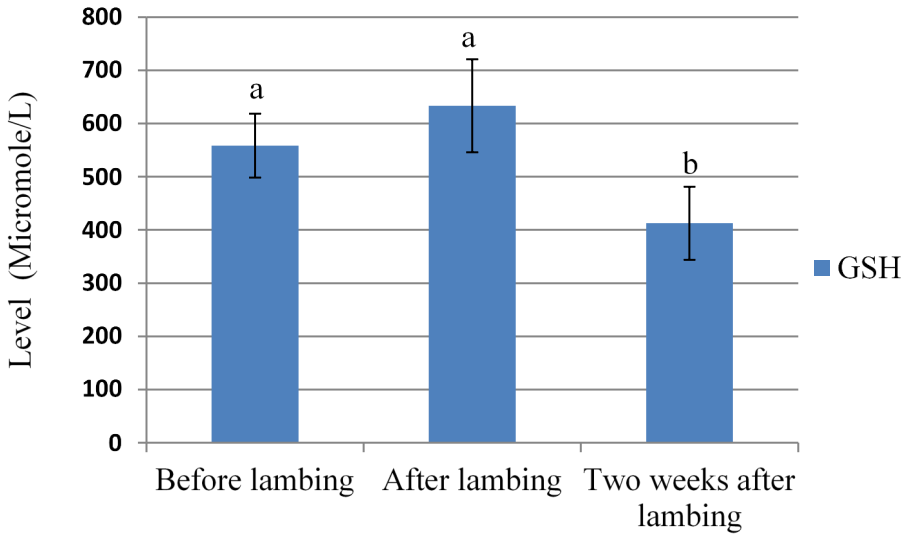

Figure 3. GSH level in ewes before, after, and two weeks after lambing. Different litters indicate significant difference at P<0.05.

after postpartum compared to two weeks after lambing (Figure 3). Moreover, the oxidation index (MDA) in the present study indicated a significantly high level occurred before and during lambing compared to two weeks from birth (Figure 4).

The study recorded an increase in the levels of GSH-px starting from lambing until two weeks after birth. GSH-px is one of the enzymatic antioxidants that reduce lipid hydroperoxides into their corresponding alcohols (Ursini and Bindoli, 1987). It also reduces free hydrogen peroxide in the water to protect the cells from oxidative breakdown (Ursini and Bindoli, 1987). Increasing GSH-px during and after lambing confirms the presence of stress factors, represented by the stress of lambing and the changes associated with postpartum, which require a continuous

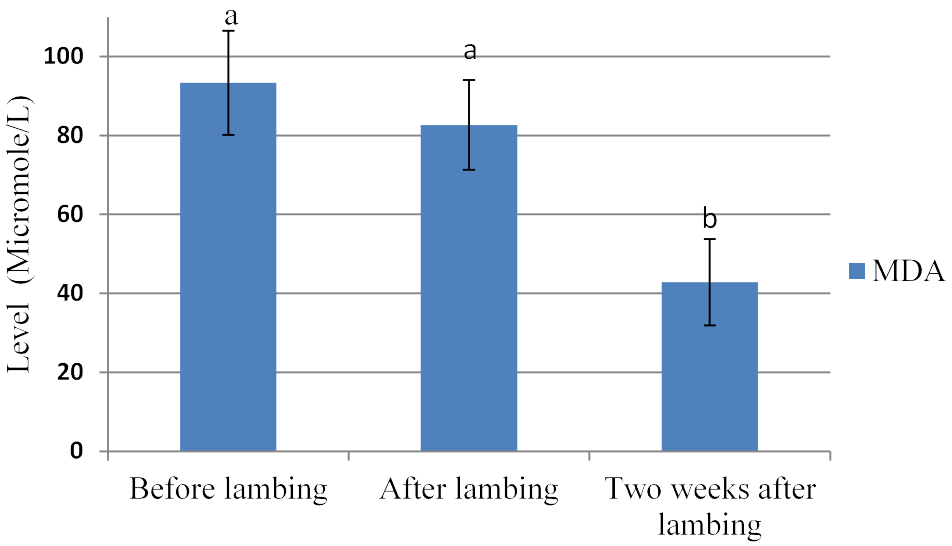

Figure 4. MDA level in ewes before, after, and two weeks after lambing. Different litters indicate significant difference at P<0.05.

supply of antioxidants like GSH-px to resist and protect the body from the harmful effect of oxidative stress.

In addition to enzymatic antioxidants, the current results showed a significant decrease in the level of GSH in ewes after two weeks from lambing, which came in agreement with another study conducted on ewes (Rios *et al*., 2017) and cows (Khudhair *et al*., 2021). Many cells produce GSH as a primary antioxidant for neutralizing ROS through its associated enzymes, like peroxidase, reductase, and transferase (Ghosh *et al*., 2015; Cruz *et al*., 2014). After lambing, the reduction in the GSH level confirms that the ewes were under oxidative stress, and there are continuous requirements for GSH and its associated enzymes to neutralize free radicals and resist oxidative stress during this stage (Ghosh *et al*., 2015; Cruz *et al*., 2014).

The occurrence of oxidative stress in ewes pre, during, and after lambing was documented through the MDA level. MDA is a marker for lipid peroxidation and oxidative stress in tissue systems (Grotto *et al*., 2009). Our results revealed a significant increase in MDA pre and during lambing, which confirms the ewes were under oxidative stress that accompanied lambing, followed by a significant decrease after lambing. These results confirm our finding for the enzymatic antioxidants (SOD, CAT, GSH-px), which decreased before and during lambing due to the continuous withdrawal of antioxidants to neutralize the massive stress during this stage, which came in agreement with Ghosh *et al*. (2015) and Cruz *et al*. (2014). While after lambing, the MDA index significantly decreased and was accompanied by high levels of enzymatic antioxidants, which attributed to the puerperium inflammation and the lactation period (Rios *et al*., 2017), which requires high levels of antioxidants to enhance protection against stress and return the animal to normal condition (Rios *et al*., 2017; Al-Hassan *et al*., 2016; Nawito *et al*., 2016).

On the other hand, estradiol, progesterone, and cortisol among the three different periods in experimental animals, revealed a significant increase ($p< 0.05$) in the level of estradiol before and during lambing, compared its level after two weeks from birth (Table 1). Furthermore, progesterone levels in experimental animals indicated a significant decrease ($p< 0.05$) occurred after birth compared to the other periods (before and during lambing), as mentioned in Table 1. Moreover, it was also observed that there was an increase in cortisol levels before and immediately after lambing compared to its level two weeks from lambing (Table 1).

In the present study, the hormonal analysis revealed a significant increase in the concentration of cortisol pre and during lambing, which

Table 1. Concentration of Hormones (Estradiol, Progesterone, and Cortisol) in Ewes Before, After, and Two Weeks After Lambing.

Groups/ewes	Estradiol (pg/ml)	Progesterone (ng/ml)	Cortisol (pg/ml)
Before lambing	73.76±7.24 [a]	2.87±0.31 [a]	83.05±17.55 [a]
Immediately after lambing	67.53±9.70 [a]	1.97±0.28 [a]	79.33±10.62 [a]
Two weeks after lambing	44.53±5.28 [b]	0.89±0.23 [b]	43.33±6.48 [b]

Data are presented as number (mean ± SEM). [ab] different letters within each column indicate significant difference (P<0.05).

confirms our results about the animals' study were under stress factors, especially before and during lambing, which was in agreement with Brunet and Sebastian (1991). Cortisol has a role during stress factors as a response to protect the body from the side effect of stress by providing an energy substrate (Brunet and Sebastian, 1991; Magyar *et al*., 1980). This elevation was followed by a significant decrease after two weeks from lambing, which confirmed the disappearance of stress factors despite puerperium inflammation and lactation period.

On the other hand, the current study recorded a significant increase in estradiol before and during lambing, compared to its level after two weeks from birth. In addition, there was a significant decrease in progesterone occurred after birth compared to the other periods (before and during lambing), which came in agreement with many studies in ewes (Liggins *et al*., 1973) and cows (Khudhair *et al*., 2021). Many studies documented the role of cortisol before parturition for shifting the progesterone to estradiol which is responsible for uterine contraction and completion of the birthing process (Liggins *et al*., 1973). These hormonal have a tight relationship with enzymatic antioxidant, which controls the process of providing the antioxidants to minimize the adverse effect of stress factor during lambing in ewes.

CONCLUSION

The current study concluded the occurrence of stress factors before and during lambing in ewes, and an internal mechanism was represented by enzymatic antioxidants like SOD, CAT, and GSH-Px, which act actively during this period to neutralize the side effect of stress. Moreover, there is a tight relationship between hormonal before and after parturition with enzymatic antioxidants to control the side effect of stress during lambing in ewes.

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CONFLICT OF INTEREST

There are no stated conflicts of interest by the authors**.**

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