Original Article https://doi.org/10.47748/tjvr.1069768

TJVR 2022; 6 (1): 15-18

Turkish Journal of Veterinary Research

https://dergipark.org.tr/tr/pub/tjvr

e-ISSN: 2602-3695

Monitoring of oxidative stress and TNF-α status during the healing process in hair goats with metritis

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Received: 08.02.2022 Accepted: 08.03.2022

ABSTRACT

Objective: This study aimed to monitor oxidative stress parameters including total antioxidant status (TAS) and total oxidant status (TOS), and tumor necrosis factor-alpha (TNF-α) level during the healing process in hair goats with metritis.

Materials and Methods: This study was carried out on a total of 25 hair goats with metritis (n=10; Group 1) and healthy (n=15; Group 2). The beginning of the study was accepted as day 0. In both Group 1 and Group 2, blood samples were collected on days 0 (first measurement day), 14 (second measurement day) and 28 (third measurement day) of the study for TAS, TOS and TNF-α analyzes. In addition, oxidative stress index (OSI) was calculated using TAS and TOS values.

Results: It was found that TAS level decreased in Group 1 compared to Group 2 at the first measurement day ($p<0.05$). However, TOS, OSI and TNF-α levels increased in Group 1 compared to Group 2 ($p<0.01$). At the second measurement day, TOS and OSI values were higher in Group 1 than Group 2 ($p<0.05$).

Conclusion: In conclusion, the antioxidant system weakened, oxidative stress and TNF-α levels increased in animals with metritis compared to healthy animals. However, at the third measurement time, all parameters became similar between groups.

Keywords: *Hair goat, Metritis, Oxidative stress, TNF-α*

INTRODUCTION

Metritis is defined as inflammation of the uterus (Kurt et al., 2019). It is known that the most important causes of metritis are uterine contamination resulting from dystocia or placental retention (Majeed, 1994). It is among the main reproductive disorders in goats (Kulsum et al., 2020) and is considered the major cause of infertility (Majeed, 1994). This reproductive disorder is responsible for decreased milk yield and increased culling rate and therefore reduced profitability in goat herds (Sayeed et al., 2020). It has an incidence of 23.4% (Kulsum et al., 2020) and a prevalence of 4.5% (Sayeed et al., 2020). The disease is well recognized with clinical findings such as fever and fetid watery red-brown uterine discharge. It is associated with immune dysfunction in the postpartum period (Kurt et al., 2019). On the other hand, there is a relationship between the inflammation and oxidative stress (Celi, 2010), and is closely linked to the occurrence of metritis (Mikulková et al., 2020), as well as many diseases (Lykkesfeldt and Svendsen, 2007). Oxidative stress occurs when the antioxidant defense capacity is exceeded by the production of ROS in the body (Lykkesfeldt and Svendsen, 2007; Sordillo and Aitken, 2009; Kurt et al., 2021).

Oxidative stress can cause oxidative damage to components of cells such as DNA, lipids and proteins (Lykkesfeldt and Svendsen, 2007; Cecchini et al., 2018), can result in cell death by apoptosis and necrosis or structural tissue damage. Therefore, it can increase susceptibility to diseases (Lykkesfeldt and Svendsen, 2007). Oxidative stress may be associated with uterine infections in the periparturient period (Yazlık et al., 2019; Mikulková et al., 2020). On the other hand, it is known that cytokines such as TNF-α are closely linked to the inflammation caused by the infection. Moreover, TNF has several roles in reproductive function, and it is involved in many biological events such as stimulation of cell proliferation and differentiation, induction of cell apoptosis (Skarzynski et al., 2009). It is also stated that the level of TNF-α increases in animals that experience uterine infection (Williams et al., 2008). For the reasons mentioned above, it is thought that both oxidative stress and TNF levels may differ in animals with uterine infection. It is also estimated that there may be differences in the level of those parameters during the healing process. Therefore, the present study aimed to monitor oxidative stress parameters including total antioxidant status (TAS) and total oxidant status (TOS), and tumor necrosis factor-alpha (TNF-α) level during the healing process in hair goats with metritis.

MATERIALS and METHODS

Ethical approval

This study was approved by the Ethics Committee of Ceyhan Veterinary Faculty, Cukurova University, Adana, Turkey (approval number: 01/01 and 24.01.2022).

Animals and study design

This study was carried out on a total of 25 hair goats with healthy (n=15) and metritis (n=10). All goats had similar age, body condition score and parity, and they were within the first two weeks postpartum at the start of the study. The goats were subjected to clinical examination including vaginal examination before the study. Metritis was characterized by fetid watery red brown uterine discharge, high fever and systemic signs of diseases (Sheldon et al., 2006). Animals with high fever, odorous watery red-brown vaginal discharge were defined as metritis. Animals that did not show any signs of disease as a result of clinical examination were considered healthy. The goats with metritis and healthy were recorded in Group 1 and Group 2, respectively. Goats with metritis were used systemic broad-spectrum antibiotics and fluid therapy according to farm's routine treatment protocol, and corticosteroids were also given when necessary. Animals that did not recover from the disease during the study were not included in the study. The beginning of the study was accepted as day 0. In both Group 1 and Group 2, blood samples were collected from the jugular vein into sterile serum tubes with clot activator on days 0 (first measurement day), 14 (second measurement day) and 28 (third measurement day) of the study. All blood samples were centrifuged at 1500 x g for 10 min, then serum samples were collected and stored at -20°C until analysis.

Laboratory analysis

Determination of TAS, TOS and TNF-α levels

Serum TAS and TOS levels were measured with the Enzyme Linked Immunosorbent Assay (ELISA) devive (Stat Fax-2100, Awareness®, USA) using commercial kits (Rel Assay Diagnostics®, Turkey). Serum TNF-α level was measured using an ELISA device (Stat Fax-2100, Awareness®, USA) and commercial kit (TNF-α ELISA kits, Bt Lab®, China). Oxidative stress index (OSI) calculated as previously defined (Kurt et al., 2021).

Statistical analysis

Statistical analyzes were performed using the SPSS package program. The obtained data were analyzed statistically according to the non-parametric Mann-Whitney U test. All results were presented as mean ± standard deviation (mean ± SD) and $p<0.05$ was considered significant.

RESULTS

It was determined that the TAS level decreased in Group 1 compared to Group 2 at the first measurement day ($p<0.05$). However, other parameters within TOS, OSI and TNF-α significantly increased in Group 1 compared to Group 2 ($p<0.01$). On the second measurement day, TOS and OSI values were higher in Group 1 than Group 2 ($p<0.05$), but there was no difference between groups in terms of TAS and TNF-α values ($p>0.05$). At the time of the third measurement, it was found that TAS, TOS, OSI, and TNF-α levels were similar between Group 1 and Group 2 ($p>0.05$). TAS, TOS, OSI, and TNF-α results obtained in the two groups are presented in detail in Table 1.

Table 1. TAS, TOS, OSI and TNF-α results measured in Group 1 and Group 2.

Parameters	Groups	First measurement (Mean ± SD)	Second measurement (Mean ± SD)	Third measurement (Mean ± SD)
TAS (mmol/L)	Group 1	1.29±0.64^{a}	1.42±0.44	1.38±0.23
	Group 2	1.80±0.42^{b}	1.44±0.26	1.36±0.26
TOS (umol/L)	Group 1	10.08±4.20^{a*}	6.33±2.07^{a}	5.66±0.78
	Group 2	5.26±1.09^{b*}	5.05±0.58^{b}	5.03±1.28
OSI	Group 1	0.97±0.53^{a*}	0.49±0.21^{a}	0.43±0.12
	Group 2	0.31±0.10^{b*}	0.36±0.07^{b}	0.39±0.14
TNF-α (ng/L)	Group 1	1.63±0.24^{a*}	0.93±0.29	0.89±0.31
	Group 2	1.01±0.17^{b*}	0.96±0.29	0.91±0.29

Different letters in the same column represent statistical difference for similar parameters ($p<0.05$). *$p<0.01$ TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, TNF-α: tumor necrosis factor-alpha.

DISCUSSION

The presented study investigated oxidative stress parameters including TAS, TOS and OSI, and TNF-α level during the healing process in Hair goats with metritis. In addition, a healthy group was formed to compare these results with healthy animals.

Metritis is known as an important cause of infertility (Majeed, 1994). Furthermore, various gynecological disorders, including metritis, are vital factors that cause economic losses in small ruminant breeding (Kulsum et al., 2020). Metritis can occur under the influence of many bacterial contaminations (Mikulková et al., 2020; Doumtsop et al., 2021), and can cause inflammation, leukocyte infiltration, edema and myometric degenerations in all layers of the uterine wall (Mikulková et al., 2020), and thus leads to fertility problems in many ways. Sheldon et al. (2006) stated that the occurrence of metritis is usually associated with deficiencies in the immune system in dairy cows. On the other hand, it has been informed that oxidative stress may predispose factor to metritis (Mikulková et al., 2020). Huang et al. (2021) reported that ROS production increased in goats during periparturient period due to metabolic adaptation process and catabolic energy metabolism due to energy deficit in this period. Therefore, it is known that this period is quite critical in terms of diseases and oxidative stress. Similarly, Mikulková et al. (2020) reported that oxidative stress was higher in cows with metritis during the early postpartum period. The oxidative stress findings of the presented study support this information. It was observed that oxidative stress increased on the day the disease was diagnosed (first measurement day) in goats with metritis. The reason for this situation was thought to be the weak antioxidant defense system, because TAS level was significantly lower in animals with metritis compared to healthy animals. In addition, in this study, it was revealed that the antioxidant level of the animals increased in parallel with the recovery from the disease. On the second measurement day, the animals recovered in general, but the oxidative stress level was still higher in Group 1 than in Group 2, although the antioxidant level returned to normal. No signs of diseases were observed in any animal on the third measurement day. In addition, there was no difference between Group 1 and Group 2 in terms of oxidative stress parameters in this period. This situation reveals the relationship between metritis and oxidative stress in goats. However, we think that oxidative stress level should be determined before metritis occurs. Thus, it can be revealed whether it can be used in the early diagnosis of the disease. However, in the presented study, differences in oxidative stress and TNF-α levels were observed only during the healing process. TNF-α is known as a cytokine associated with the inflammatory syste (Skarzynski et al., 2009; Kushibiki, 2011). Moreover, TNF-α has various immune system functions, including antimicrobial activity and mediation of inflammation (Kushibiki, 2011). Williams et al. (2008) stated that the level of TNF-α increased in animals with uterine infection. So, considering role of TNF-α in the inflammatory system, its level was investigated in goats with metritis. In the presented study, TNF-α level increased significantly on the first measurement

day in Group 1 compared to Group 2. We think that this is due to the activation of the inflammatory system due to metritis. This assumption is confirmed by the TNF-α level on the second and third measurement days. So, TNF-α level of Group 1 became similar to Group 2 in parallel with the recover of the disease. However, we think that TNF level should be investigated more comprehensively in animals with uterine infection and it should be determined whether it can be used in the early diagnosis of the metritis in goats.

CONCLUSION

This study investigated the changes in oxidative stress parameters and TNF-α levels during the healing process in hair goats with metritis. On the first day of measurement, the antioxidant system weakened, oxidative stress and TNF-α levels increased in animals with metritis compared to healthy animals. Similarly, oxidative stress was still high in animals with metritis at the second measurement time. On the other hand, at the third measurement time, all parameters became similar between groups. However, we think that this situation should be investigated comprehensively by including treatment protocols and pathogen species that cause metritis in future studies.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

Author's Contributions: SK and FE contributed to the study conception and design. LM contribution to laboratory work. Supervision/Consultancy (SK and FE), Data collecting (FE and SK), Literature research (SK and FE), Writing the article (SK and FE), Critical review (FE and SK). All authors read and approved the final manuscript. SK: Serdal KURT, FE: Funda EŞKİ, LM: Leyla MİS

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