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Research Article

OXIDANT/ANTIOXIDANT STATE IN REPEAT BREEDER COWS

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ABSTRACT. In this study, oxidant and antioxidant status in pregnant and repeat breeder (RB) cows were evaluated. For this purpose, approximately 10 mL of blood was taken into Li-Heparin tubes from the V. jugularis of a total of 68 Holstein breed cows, consisting of two groups: pregnant (n=34) and repeat breeder (n=34), less than 10 years old, inseminated 3 or more times. Malondialdehyde (MDA) levels, superoxide dismutase (SOD) activities, total oxidant capacity (TOC), total antioxidant capacity (TAC), total and native thiol levels (TT and NT) were determined in plasma obtained from blood. While no difference was determined between RB and pregnant cows in terms of plasma MDA levels and SOD activities, TOC, TAC, TT and NT levels were found to be higher in RB cows than in pregnant cows. In conclusion; determination of the status of oxidants and antioxidants along with the concentrations of physiological and pathological reactive oxygen species in order to reliably evaluate oxidative stress; since cows that do not fertilize are under oxidative stress, the factors that may cause this should be investigated, eliminated and it was also concluded that it would be beneficial to provide adequate amounts of antioxidants, minerals and vitamins in the rations of these animals.

Keywords: *Cow, oxidative stress, repeat breeder.*

INTRODUCTION

Repeat breeder (RB) is a very important infertility problem caused by various factors, causing low milk production and a decrease in the number of calves per animal, causing economic losses at the farmer and industry level. Causes of RB include factors such as oxidative stress (OS), estrus detection failures, endocrine dysfunction, ovulation defects, poor fertilization rates and/or early embryonic loss [1, 2]. It has been emphasized in various literatures [3, 4, 5, 6, 7] that OS may be related to hormonal dysfunctions, infections, immunological factors, decreased reproductive performance, and may affect effective insemination and healthy fetal development in dairy cows. Free radicals also play a role in many infertility-related diseases. Free radicals are defined as molecular species containing one or more unpaired electrons that can exist independently. They occur as natural byproducts of oxygen metabolism, and serve the purpose of killing bacteria and removing substances from the organism, but when out of control they become toxic and begin to damage body tissues through a process called OS. Substances that neutralize the potential harmful effects of free radicals are called antioxidants or free

radical scavengers. The imbalance between oxidants and antioxidants causes tissue damage [2].

One of the reasons for low productivity in dairy cattle is RB cows. Studies have shown that RB cows have high OS levels. Oxidative stress occurs when there is a significant imbalance between the production of free oxygen radicals and the antioxidant defense mechanism in cells [1, 2, 8, 9]. When free radicals are produced excessively, the normal functions and metabolic activities of cells are negatively affected. The highest concentration of thiol groups as antioxidants is found in plasma, and this is explained by the amino acids methionine and cysteine, which are the primary source of thiol groups in plasma [10]. As antioxidants, thiols participate in the elimination of reactive oxygen species and are used in oxidative reactions within oxidative molecules by forming disulfide bonds [11]. Total thiols (TT) include reduced and oxidized thiols, while native thiols (NT) include only reduced thiols (SH). Dynamic thiol/disulfide balance (DD) has an important effect on the arrangement of enzymatic activity, cell signaling, apoptosis, transcription and antioxidant defense [12]. Determining the thiol and disulfide ratio can provide information about exogenous and endogenous OS in biological systems [13]. So far, no study has been found that evaluates TT and NT status in RB cows.

In this study, oxidant and antioxidant status in RB cows were evaluated. For this purpose, a total of 68 Holstein breed cows, consisting of two groups: those that were less than 10 years old, those that were inseminated 3 or more times and could not become pregnant, and those that became pregnant, located on the farm belonging to the Kurmel Agriculture and Livestock Joint Stock Company in Develi District of Kayseri Province, were used. Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities, as well as possible changes in total oxidant capacity (TOC), total antioxidant capacity (TAC), TT and NT levels were determined in plasma obtained from blood. In this study, in addition to other OS parameters in RB cows, the status of TT and NTs, which can provide information about exogenous and endogenous oxidative stress status in biological systems, will be determined for the first time; the data obtained will be able to contribute to farm owners and relevant veterinarians, as well as researchers who will study this subject in the future.

MATERIALS AND METHODS

Animals

For the study, 68 Holstein breed cows, less than 10 years old and inseminated 3 or more times, located on the farm belonging to the Kurmel Agriculture and Livestock Joint Stock Company in Develi District of Kayseri Province, were used. These animals consisted of two groups: RB and pregnant.

Blood collection and biochemical analyzes

Approximately 10 ml of blood from *V. jugularis* of Holstein cows was taken into anticoagulant tubes and centrifuged at 3000 *rpm* for 10 minutes, plasma was separated and stored at -80 ºC until analysis.

Malondialdehyde (BTLab, catalog no: E1371Hu, China) levels, SOD (BTLab, catalog no: E4502Hu, China) activities, TOC (Rel Assay, catalog no: RL0024, Turkey) [14], TAC (Rel Assay, catalog no: RL0017, Turkey) [15], TT (Rel Assay, catalog no: RL0192, Turkey) and NT (Rel Assay, catalog no: RL0185, Turkey) levels in plasma were

determined with ELISA (Bio-Tek, ELx50, USA) by using commercial kits. In addition, oxidative stress index (OSI), expressed as a percentage of the ratio of TOC levels to TAC levels, was also calculated [16]. The dynamic disulfide level was determined by subtracting the NT level from the TT level and taking half of the value obtained [17].

Statistical analysis

The analysis data of the samples were given as arithmetic mean±standard error. Student Independent T Test (SPSS 20 program for Windows) was applied to determine the statistical difference in the data of pregnant and RB animals.

RESULTS AND DISCUSSION

In the study, it was determined that MDA levels and SOD activities in plasma of RB cows were not statistically different from the MDA levels and SOD activities of pregnant cows (P>0.05). On the other hand, plasma TOC, TAC, TT and NT levels of RB cows were found to be statistically higher than those of pregnant cows (P<0.001). However, no statistically significant difference was determined between the groups in terms of oxidative stress index (OSI) and dynamic thiol/disulfide balance (DD) values (P>0.05; Table 1).

Table 1. *Oxidative stress parameters in pregnant and RB cows (Mean ± Standart error)*

Parameters	Pregnant N=34	RB N=34	P
MDA (nmol/ml)	9.13±0.64	8.99±0.91	-
SOD (ng/ml)	20.14±0.95	20.18±1.17	-
TOC (µmol/L)	0.43±0.05^{a}	0.71±0.04^{b}	***
TAC (mmol/L)	1.15±0.07^{a}	1.82±0.06^{b}	***
OSI (arbitrary unit)	0.46±0.10	0.40±0.03	-
TT (µmol/L)	889.93±85.88^{a}	1436.61±111.14^{b}	***
NT (µmol/L)	481.56±32.83^{a}	879.46±99.88^{b}	***
DD (µmol/L)	204.19±44.52	278.57±46.37	-

*$^{a-b}$: Values within each row with different superscripts differ significantly. -: not significant; ***: P<0.001*

Repeat breeder is a multifactorial situation; therefore, a multifaceted role of OS can be predicted. Oocyte metabolism and steroid production cause an increase in the production of reactive oxygen species (ROS) [18]. The resulting oxygen deprivation is necessary for stimulation of angiogenesis and follicular growth and development. Balance is maintained by the antioxidant system, but uncontrolled ROS release causes stress. The resulting OS damages nucleic acids, proteins and lipids and therefore impairs oocyte quality [19]. Oxidative damage to the oocyte is thought to be a cause of persistently poor oocyte quality. Stress can cause excessive production of free radicals that attack polyunsaturated fatty acids, thereby causing changes in lipoproteins, resulting in changes in progesterone production [20, 21].

Oxidative stress results from the overproduction of ROS depending on the level of antioxidant defenses and indicates an imbalance between the body's pro-oxidants and antioxidant mechanisms. Increased stress levels affect the physiological functions of

female reproduction. Studies on OS have now become an active field of research in animal species, particularly in veterinary medicine in general and reproduction in particular. This is because the incidence of infertility problems in livestock is increasing and will perhaps continue as an emerging area of research in the coming years. Oxidative stress is thought to play a role in the pathophysiology of female infertility, and from a clinical perspective, it is of great importance to have knowledge about the effect of OS on bovine infertility. Reactive oxygen species target cellular macromolecules such as lipids, proteins, and nucleic acids, ultimately resulting in cellular death [21].

Malondialdehyde, an index of lipid peroxidation, is used as an indicator of OS in cells and tissues. It is well known that the healthy animal has well-balanced ROS and antioxidants, and when the balance is disrupted, leading to overproduction of their ROS leads to the occurrence of OS. Reactive oxygen species have the ability to trigger pathological processes in the female gonads, accessory glands and reproductive system, affecting many physiological processes from oocyte maturation to fertilization, embryo development and pregnancy, leading to longer gestation interval from calving, poor pregnancy rate and fertility failure [22]. It has been emphasized that since the imbalance observed between oxidant and antioxidant negatively affects the physiological events responsible for the control of the estrous cycle, it may disrupt the physiological events that enable ovulation and lead to cystic ovarian disease [23]. In the presented study, oxidative stress status in pregnant and RB cows were evaluated.

Siddique et al. [2] found that blood MDA and nitric oxide (NO) levels were higher in RB buffaloes compared to normal buffaloes, while SOD, CAT activities, glutathione and TAC levels were significantly reduced, and as a result, RB buffaloes were under OS; and they stated that sufficient amounts of antioxidants and minerals should be provided in the rations of these animals. Likewise, Ahmed et al. [24] found an increase in MDA and NO levels and a decrease in CAT, SOD activities, ascorbic acid, glutathione and TAC levels as oxidant/antioxidant markers in RB buffalo cows. In another study [9], plasma MDA, GSH levels and CAT, GSH-Px activities were determined in a total of 40 Holstein cows aged between 3 and 8 years, consisting of 20 RB and 20 control groups without fertilization problems; It was determined that MDA levels were statistically higher and GSH levels, GSH-Px and CAT activities were lower in RB cows. As a result, it was concluded that the increases in plasma MDA levels and decreases in plasma GSH levels, GSH-Px and CAT activities detected in RB cows were a significant deterioration of OS in the picture of non-fertility. However, in the present study, contrary to the researchers above, no statistically significant difference was detected between RB and pregnant cows in terms of plasma MDA levels and SOD activities. Serum TAC level provides a cumulative view of the status of both enzymatic and non-enzymatic antioxidants in the body, which will inactivate ROS and prevent their harmful effects [25, 26]. Soni et al. [1] found that serum MDA and NO levels were higher, but TAC and progesterone levels were lower, in RB cows than in normal cows, and they emphasized that these data reveal the role of OS in luteal insufficiency, which leads to pregnancy failure in RB cows. Similarly, another study [27] revealed that lower antioxidant status and higher MDA levels were determined in RB cows compared to those with healthy cycles. El-Amrawi et al. [28] also emphasized that serum TAC levels of cows with inactive ovaries are lower than those of cows with regular estrous cycles, and that the excessive production of free radicals or ROS and antioxidant/oxidant imbalance in these animals may cause depletion of antioxidant defense systems.

Studies evaluating the thiol-disulfide balance, which is one of various enzymatic and non-enzymatic defense mechanisms in animals, especially against the harmful effects of ROS, are quite limited [29, 30, 31]. Çamkerten et al. [29] determined that NT, TT and disulfide levels in sheep with sarcoptic mange were lower than in healthy sheep and there was no difference between the groups in terms of disulfide/native thiol, disulfide/total thiol and native thiol/total thiol ratios. They stated that sarcoptic mange affects the disulfide balance in infected sheep and that antioxidant molecules that will ensure this balance should be added to therapeutic options. Deveci and Erdal [31] reported that TT, NT and disulphide levels in dairy cattle with foot disease were higher than in healthy ones, and the thiol-disulfide balance was disrupted. However, only one study [30] was found in which TT and NT levels were evaluated in RB cows. In this study, which was reported to evaluate thiol/disulfide homeostasis as a new indicator of OS in infertile dairy cows with subclinical endometritis, serum TT and NT levels of infertile cows with acute and chronic endometritis were lower than healthy cows, and disulfide levels, disulfide/TT, NT/ TT ratios were determined to be similar in all groups. With the results obtained in this study, it was concluded that thiol/disulfide homeostasis is a reliable and sensitive indicator of OS in cow subclinical endometritis and that abnormal thiol/disulfide homeostasis may play a role in the pathogenic mechanisms of subclinical endometritis.

In the presented study, it was found that plasma TOC levels increased in RB cows compared to pregnant cows due to increased OS, and TAC levels were also higher to compensate for this increase. In addition, plasma TT and NT levels, which can provide information about exogenous and endogenous OS in biological systems, were found to be statistically higher in RB animals, supporting the increased TOC and TAC levels.

CONCLUSION

As a result, in this study, while no difference was detected between repeat breeder and pregnant cows in terms of plasma malondialdehyde levels and superoxidase dismutase activities, it was determined that total oxidant and antioxidant capacity and total and native thiol levels were higher in repeat breeder cows than in pregnant cows.

In conclusion; determination of the status of oxidants and antioxidants along with the concentrations of reactive oxygen species in order to reliably evaluate oxidative stress; since cows that do not fertilize are under oxidative stress, the factors that may cause this should be investigated, eliminated and it was also concluded that it would be beneficial to provide adequate amounts of antioxidants, minerals and vitamins in the rations of these animals.

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Ethical Approval. Ethical approval for the study was obtained from the Erciyes University Animal Experiments Ethics Commitee in Turkey (decision dated 04.01.2023 and numbered 23/001).

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: S.U., M.E., Design: S.U., M.E., Data Collection or Processing: S.U., M.Ş., ÇK.B., Analysis or Interpretation: S.U., M.E., M.Ş., ÇK.B., Literature Search: S.U., M.E., Writing: S.U., M.E.

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