

Determination of thiol/disulphide homeostasis as a new indicator of oxidative stress in dairy cows with subclinical endometritis

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ABSTRACT

The objective of this study was to determine thiol/disulphide homeostasis (TDH) in infertile cows with subclinical endometritis (SCE). Endometrial cytological samples were collected using a cytobrush to diagnose SCE in 36 infertile cows. According to the results of the cytology examination, those with acute endometritis were classified as Group I (n = 20) and those with chronic endometritis were classified as Group II (n = 16). A control group was formed of heifers as Group III (n = 20). Blood samples were taken from each group on the day of diagnosis (day 0) to analyse TDH. In the cytology examination, both the Giemsa method and immunocytochemical staining were applied to determine chronic inflammation and activity status. In 55.55% (20/36) of the infertile cows with cytological endometritis, the inflammation was determined to be active, and in 44.44% (16/36) it had become chronic. The native thiol and total thiol levels were found to be statistically significantly lower in the acute ($206.54 \pm 8.30 \mu\text{mol/L}$; $227.11 \pm 9.30 \mu\text{mol/L}$) and chronic SCE cases ($225.15 \pm 11.89 \mu\text{mol/L}$; $247.96 \pm 10.80 \mu\text{mol/L}$) compared to the healthy control group ($308.47 \pm 13.59 \mu\text{mol/L}$; $336.83 \pm 15.5 \mu\text{mol/L}$ respectively) ($P < 0.001$). Disulphide levels, disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios were similar in all the groups ($P > 0.05$). The diagnostic accuracy of native thiol, which can be used in the diagnosis of SCE, was 92.8%, that of total thiol was 89.3% and that of disulphide was 64.3% according to the ROC curve analysis. These results demonstrate that TDH is a reliable and sensitive indicator of oxidative stress in cow SCE, and that abnormal TDH might play a role in SCE pathogenic mechanisms. This is the first study to evaluate thiol/disulphide homeostasis in dairy cows with SCE as a new indicator of oxidative stress.

Key words: cow; oxidative stress; ROC; subclinical endometritis; thiol/disulphide

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Introduction

Infertility is one of the major problems affecting reproductive performance in dairy farms. By extending the period between calving and re-conception, failure in reproduction, the feeding of infertile animals in vain, and incurring extra labour, infertility in cows leads to economic losses (SENÜVER and NAK, 2015). The presence of subclinical endometritis (SCE) is one of the etiological factors of infertility (KASIMANICKAM et al., 2004; GILBERT et al., 2005). SCE is an inflammation of the endometrium without the clinical signs of endometritis (SHELDON et al., 2006; ORUC et al., 2015). Studies in literature have demonstrated that SCE in dairy cows has a negative impact on subsequent reproductive performance (GILBERT et al., 2005; KASIMANICKAM et al., 2004).

Thiols, known as functional sulfhydryl (-SH) groups, are of vital importance in preventing oxidative stress (OS) forming in cells (KEMP et al., 2008). While thiol components are mainly formed of albumin and other proteins, a small proportion consist of low molecular weight thiols such as: cysteine, glutathione, homocysteine and γ - glutamyl cysteine (TURELL et al., 2013). In proteins, thiol groups of amino acids (methionine, cysteine) which include sulphur are the primary targets of reactive oxygen species (ROS). Thiol groups presenting with ROS in the setting are oxidised and converted into disulphide bonds, also known as sulphur bridges. This transformation is an indicator of protein oxidation and, under this OS, thiol / disulphide homeostasis (TDH) is disrupted (JONES and LIANG, 2009). Just as this disruption may directly lead to certain diseases, some diseases may also result in such a disruption. Thiols and disulphides have significant roles in detoxification, apoptosis, antioxidant defence, enzyme activity regulation, receptors, transporters, Na-K channel and transcription (BISWAS et al., 2006; CIRCU and AW, 2010). In humans, abnormal TDH, which is a part of the antioxidant defence, has been examined in the pathogenesis of some diseases, such as pre-eclampsia (ÖZLER et al., 2015), diabetes mellitus (ATEŞ et al., 2016), some anomalies that occur during pregnancy such as recurrent miscarriage

(ERKENEKLI et al., 2016; KARAMAN et al., 2016; KORKMAZ et al., 2016), cardiovascular diseases (ALTIPARMAK et al., 2016) and cancer (PRABHU et al., 2014). The parameters of TDH include native thiol and total thiol, disulphide and disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios. Although it has been possible to measure one side (reductive thiol) of these bilaterally balanced variables since 1979 (ELLMAN and LYSKO, 1979), with the recent development of a new method (EREL and NESELIOGLU, 2014), the level of both these two variables can be calculated separately and in total, and the ratios between both sides of the system can also be analysed.

To the best of our knowledge, there have been no studies in the literature to date concerning TDH in infertile cows. Therefore, this study provides the first report on this subject. The aim of this study was to determine thiol/disulphide homeostasis in infertile dairy cows with SCE, and to compare these with healthy heifers. The hypothesis of the study was that this homeostasis might have a role in the etiopathogenesis mechanisms of infertility problems in cows with SCE.

Materials and methods

Animals, housing and feeding. This study included 40 Holstein-Friesian dairy cows, aged 3-8 years, that had not become pregnant despite 3 inseminations and had no anomalies (abnormal uterine discharge, pyometra, urovagina, pneumovagina, perineal defects) upon the gynaecological examination. The study also included a control group of 20 healthy heifers aged 11-16 months. The present study was conducted on a private dairy farm where the cows were housed in semi-open free-range barns throughout the whole year, and were fed twice daily with a feed mixture which included corn and grass silage, hay, triticale, canola and a balanced grain ration, and water intake was ad libitum. The herd consisted of 350 milking cows. Primiparous and multiparous cows were housed together and milked twice daily.

Dairy records and anamnesis. Data related to the animals' age, calving date and count, birth-first

insemination time, insemination date and count, and incidence of puerperal period problems were collected from the dairy records and the anamnesis.

Study design and grouping. For determination of the cows without clinical endometritis and ovarian problems, a gynaecological examination was conducted of the vulva, tail and perineum inspection by transrectal palpation, vaginoscopy and ultrasonography. Endometrial smear samples were taken from the cows that were not found to have any clinical or anatomic anomalies in the examination (n = 40).

The infertile cows with no pathological problems in the clinical examinations were evaluated according to the cytology examination results (n = 40). Those with acute endometritis were included in Group I (n = 20) and those with chronic endometritis were included in Group II (n = 16). A total of 4 samples were not readable. Group III was the control group, consisting of healthy heifers with no previous gynaecological anomalies (n = 20). These heifers were used as the control group because it is difficult to select cows that are not bred at approximately 200 days postpartum as SCE.

Cytological sampling and evaluation. The cytology samples of cows with infertility problems were taken using an endocervical brush using the method suggested by KASIMANICKAM et al., (2004). The collected samples were placed on slides and transferred to the laboratory after fixation. The smear samples were stained using the Giemsa method, and a proportion of 5% neutrophils or greater was defined as the threshold for SCE (MELCHER et al., 2014). Inflammatory cell presence and inflammation characteristics were evaluated according to the design by POLAT et al., (2015). To determine the inflammatory status according to this method, the percentage of polymorphonuclear (PMN) cells and lymphocytes (LYM) were calculated. Using these criteria, cytopathological classifications were determined (PMN+LYM \geq 5%: acute SCE; LYM \geq 5%, chronic SCE). Within the cytology examination, immunocytochemical staining was also applied to determine chronic inflammation and activity status. The collected samples were incubated for 20 min

with Bovine Serum Albumin (BSA) solution (1 g BSA in 100 ml PBS). To determine T lymphocytes and active inflammation, the smear samples were incubated for 20 min with CD3 (Sigma, Catalog no: C7930, Dilution rate: 1/200) and IFN γ (AbdSerotec, Catalog no: MCA1964, Dilution rate: 1/300) primary antibodies, respectively. For dilution, antibody diluent solution (Catalog no: 003118, Thermo Fischer Scientific) was used. Secondly, a Mouse and Rabbit Specific HRP/DAB Detection IHC Kit (Abcam, Catalog no: Ab80436) was used. The preparates were then applied with a reverse staining process using Mayer's Haematoxylin, and they were exposed to a graded alcohol-xylol series. Finally, these sections were covered with mounting medium (Entellan[®], Merck, 107960) and analysed under a light microscope. The ratio of immunopositive lymphocytes to all lymphocytes was evaluated.

Collection of blood samples. Blood samples were collected from the coccygeal vein for all groups on the day of examination (day 0). The samples were put into vacuumed glass tubes of 10 ml that did not include any anticoagulants. The collected samples were centrifuged for 15 mins at 3000 revolutions/min to extract the serum, which was then placed in Eppendorf tubes and stored at -20 °C until analysis.

Determining thiol/disulphide homeostasis. Thiol/disulphide parameters were determined in the collected blood samples according to EREL and NESELIOGLU, (2014). TDH was analysed by a fully automated method, which allows the evaluation of the two sides of thiol/disulphide homeostasis. This technique uses sodium borohydride (NaBH₄) for reduction of the dynamic disulphide bonds to functional thiol groups. Formaldehyde was used to eliminate all the unused NaBH₄, to prevent extra reduction of the 5,50-dithiobis-2-nitrobenzoic acid (DTNB) and extra reduction of the formed disulphide bond, produced after the DTNB reaction. After taking the measurements of the native thiol, total thiol and disulphide levels, the disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios were calculated (EREL and NESELIOGLU, 2014). The results were obtained as μ mol/L.

Animal rights statement. In this study, the Ethics Committee report was received in accordance with the directions of the Dollvet Inc. Experimental Animals Local Ethics Board (2018/01).

Statistical methods. Data obtained in the study were analysed statistically using NCSS 9.1 software. Conformity of the data to normal distribution was determined using the Shapiro-Wilk test. Evaluation of the significance of differences between the study groups related to the serum TDH profiles was conducted using One-way ANOVA, and post hoc Tukey tests.

Results

The study population consisted of one herd of 20 heifers, 11 primiparous (first lactation) and 29 multiparous (>second lactation) cows.

Cytology findings. During the study period, a total of 40 cows without vaginal discharge were included in the study. Cytological samples were obtained from 36 cows, and 4 samples were not readable. After the cytology examination, SCE was cytopathologically classified as acute or chronic according to evaluation of the cells in the collected samples. In cows determined to have acute endometritis, dense neutrophils were observed together with prismatic epithelium. In cows with chronic endometritis, the number of lymphocytes were higher (Fig. 1). In 55.55% (20/36) of the infertile cows with subclinical endometritis, the inflammation was determined to be active, and in 44.44% (16/36), it had become chronic.

Receiver-operating characteristic (ROC) analysis was applied to calculate the optimal positivity threshold for TDH parameters used in the diagnosis of SCE disease. Therefore, each value measured for antioxidants was considered as the cutoff point. Sensitivity, specificity and accuracy rates were calculated for each cut-off point and ROC curves were created. Summary statistics of variables were reported as mean ± standard deviation (SD) values. A value of P<0.05 was accepted as statistically significant.

Calculating thiol/disulphide homeostasis. A statistically significant difference was determined between the control, acute and chronic SCE groups in respect of native thiol and total thiol variables (P<0.001). The mean native thiol and total thiol values in the acute and chronic SCE groups were lower than those of the control group. The disulphide level, disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios were found to be similar in all the groups (P>0.05). When the acute and chronic SCE groups were compared, the levels were determined to be statistically similar (P>0.05). In Groups I and II, TDH was found to be reduced when compared with control group. The serum TDH profiles of the cows are summarized in Table 1.

Table 1. The serum thiol/disulphide profiles of the groups

Parameters	Groups			P
	Acute (n = 20)	Chronic (n = 16)	Control (n = 20)	
Native thiol (µmol/L)	206.54 ± 8.30 ^b	225.15 ± 11.89 ^b	308.47 ± 13.59 ^a	<0.001
Total thiol (µmol/L)	227.11 ± 9.30 ^b	247.96 ± 10.80 ^b	336.83 ± 15.50 ^a	<0.001
Disulphide (µmol/L)	9.44 ± 1.16	11.40 ± 1.22	14.20 ± 1.48	0.244
Disulphide/native thiol (%)	4.66 ± 0.60	5.55 ± 0.78	4.53 ± 0.39	0.470
Disulphide/total thiol (%)	4.14 ± 0.50	4.87 ± 0.62	4.10 ± 0.32	0.503
Native thiol/total thiol (%)	91.16 ± 1.12	90.26 ± 1.23	91.80 ± 0.64	0.619

^{a,b} - Letters in the same row indicate differences between groups

A significant connection was determined between native thiol and total thiol variables and birth count ($P < 0.001$). In this correlation, the mean native thiol and total thiol values of the heifers were higher than in the primiparous and multiparous cows. No significant difference was observed in respect of the averages of disulphide level, disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios ($P > 0.05$) (Table 2).

As a result of the ROC curve analysis, each point on the curve represents the sensitivity and specificity pair of the test according to a cutoff point. Any increase in sensitivity is achieved by a reduction in specificity. As the curve in the ROC plane approaches the upper left corner, the area under the curve (AUC), and therefore the accuracy of the test, increases. The significance of the difference of the areas under the curve from 0.5 was assessed using Z statistical analysis. The areas under the ROC curve for the native thiol, total thiol and disulphide antioxidants in the animals

with SCE were significantly different (0.97, 0.98 and 0.65 respectively) ($P < 0.05$). No statistically significant difference was determined between the AUCs for disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios (0.54, 0.55 and 0.54 respectively) ($P > 0.05$). (Table 3; Fig. 2).

Sensitivity, specificity and diagnostic accuracy ratios were determined for native, total thiol and disulphide values, and were found to be usable in the diagnosis of SCE according to the AUC results and showed a statistically significant increase related to the disease. The highest sensitivity and specificity values were 280.7 $\mu\text{mol/L}$ for native thiol, 288.3 $\mu\text{mol/L}$ for total thiol and 8.7 $\mu\text{mol/L}$ for disulphide, which were determined as the optimal positivity thresholds. According to these threshold values, the diagnostic accuracy of native thiol, which can be used in the diagnosis of SCE, was calculated as 92.8%, that of total thiol was 89.3% and that of disulphide was 64.3% (Tables 4 to 6).

Table 2. The serum thiol/disulphide profiles according to cows parity

Parameters	Groups			P
	Heifer (n = 20)	Primipar (n = 11)	Multipar (n = 29)	
Native thiol ($\mu\text{mol/L}$)	308.47 \pm 13.59 ^a	186.56 \pm 12.90 ^b	225.24 \pm 6.59 ^b	<0.001
Total thiol ($\mu\text{mol/L}$)	336.83 \pm 15.50 ^a	209.00 \pm 13.09 ^b	246.11 \pm 6.95 ^b	<0.001
Disulphide ($\mu\text{mol/L}$)	14.20 \pm 1.48	11.22 \pm 1.55	9.85 \pm 0.94	0.149
Disulphide/native thiol (%)	4.53 \pm 0.39	6.30 \pm 1.02	4.48 \pm 0.47	0.289
Disulphide/total thiol (%)	4.10 \pm 0.32	5.45 \pm 0.80	4.00 \pm 0.39	0.125
Native thiol/total thiol (%)	91.80 \pm 0.64	89.09 \pm 1.60	91.61 \pm 0.85	0.209

^{a,b}: Letters in the same row indicate differences between groups

Table 3. The size of area under the ROC curve and importance controls of thiol/disulphide homeostasis parameters

Parameters	The size of area under curve of AUC	Standart deviation of AUC	Z Test value AUC>0.5	95% confidence interval of AUC		P
				Min	Max	
Native thiol	0.9694	0.0197	23.814	0.8939	0.9914	<0.001
Total thiol	0.9750	0.0169	28.172	0.9079	0.9934	<0.001
Disulphide	0.6528	0.0760	2.011	0.4782	0.7777	0.022
Disulphide/native thiol	0.5438	0.0783	0.559	0.3728	0.679	0.288
Disulphide/total thiol	0.5417	0.0782	0.533	0.3709	0.677	0.297
Native thiol/total thiol	0.5542	0.0779	0.695	0.3833	0.6884	0.2435

Table 4. Specific proportions of the optimal positive values for native thiol level

Native thiol	A	B	C	D	Sensitivity A/(A+C)	Specificity D/(B+D)	Accuracy (A+D)/(A+B+C+D)
≤ 266.50	32	3	4	17	0.8889	0.8500	0.8750
≤ 275.50	33	3	3	17	0.9167	0.8500	0.8929
≤ 278.60	34	3	2	17	0.9444	0.8500	0.9107
≤ 280.70	35	3	1	17	0.9722	0.8500	0.9286
≤ 281.70	35	4	1	16	0.9722	0.8000	0.9107
≤ 297.70	36	4	0	16	1.0000	0.8000	0.9286
≤ 304.00	36	5	0	15	1.0000	0.7500	0.9107

Table 5. Specific proportions of the optimal positive values for total thiol level

Total thiol	A	B	C	D	Sensitivity A/(A+C)	Specificity D/(B+D)	Accuracy (A+D)/(A+B+C+D)
≤ 271.40	29	1	7	19	0.8056	0.9500	0.8571
≤ 272.30	30	1	6	19	0.8333	0.9500	0.8750
≤ 280.20	31	1	5	19	0.8611	0.9500	0.8929
≤ 281.10	31	2	5	18	0.8611	0.9000	0.8750
≤ 288.30	32	2	4	18	0.8889	0.9000	0.8929
≤ 289.70	32	3	4	17	0.8889	0.8500	0.8750
≤ 292.80	33	3	3	17	0.9167	0.8500	0.8929

Table 6. Specific proportions of the optimal positive values for disulphide level

Disulphide	A	B	C	D	Sensitivity A/(A+C)	Specificity D/(B+D)	Accuracy (A+D)/(A+B+C+D)
≤ 8.50	14	0	22	20	0.3889	1.0000	0.6071
≤ 8.65	15	0	21	20	0.4167	1.0000	0.6250
≤ 8.70	16	0	20	20	0.4444	1.0000	0.6429
≤ 9.00	16	1	20	19	0.4444	0.9500	0.6250
≤ 9.80	16	2	20	18	0.4444	0.9000	0.6071
≤ 9.90	16	3	20	17	0.4444	0.8500	0.5893
≤ 10.10	16	4	20	16	0.4444	0.8000	0.5714

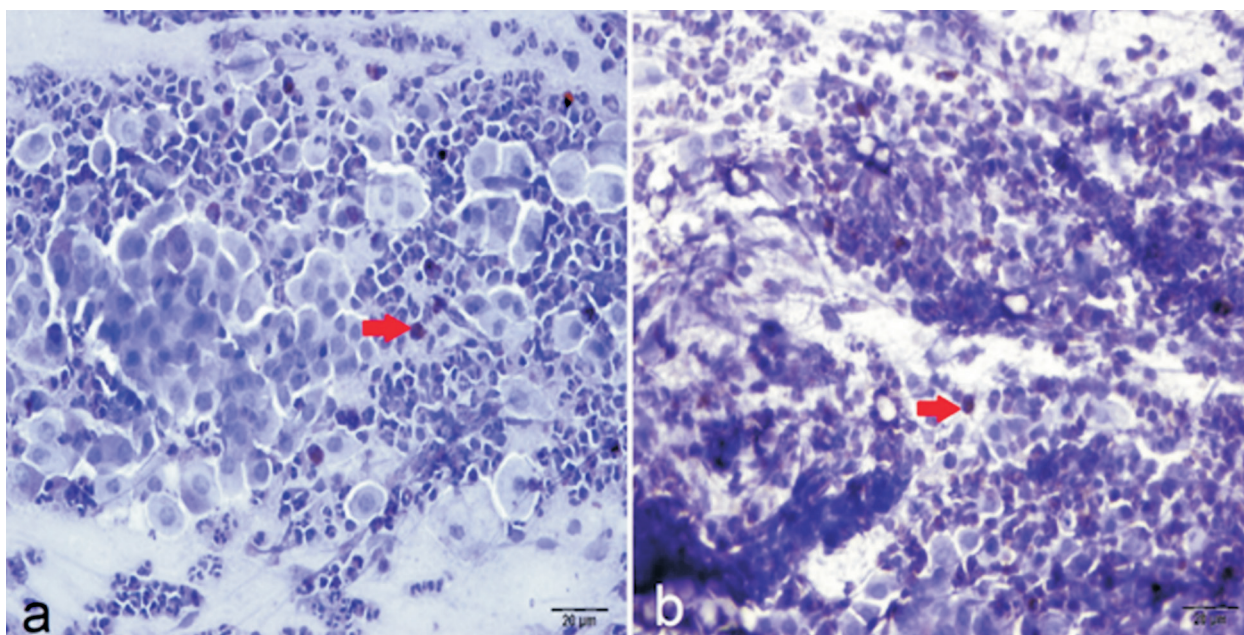


Fig 1. The image of epithelial cells recovered from the endometrium via cytobrush. a) CD3 immunopositive cells, b) IFN γ immunopositive cells. ICC; $\times 20$

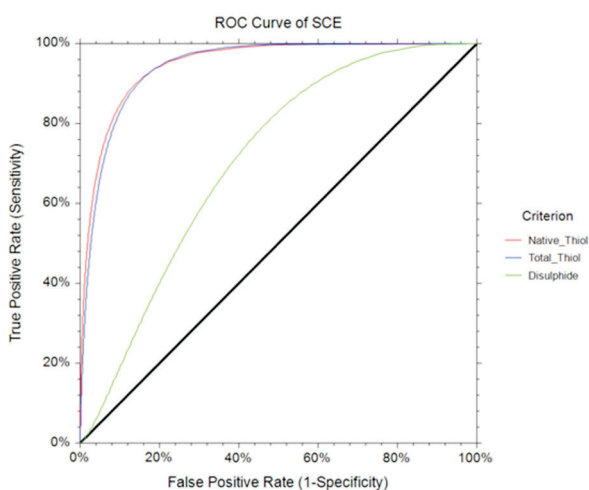


Fig. 2. ROC curves comparing native thiol, total thiol ve disulphide variables for prediction of SCE

Discussion

Subclinical endometritis is an inflammatory disease and is one of the major problems affecting reproductive performance (KASIMANICKAM et al., 2004; GILBERT et al., 2005; SHELDON et al., 2006). In many inflammatory diseases, an increase in the production of pro-inflammatory cytokines has been associated with an increase in

oxidative stress (OS) mediators. OS is known to play an important role in the pathogenesis of many reproductive events such as: embryonic losses, endometritis, follicular cysts and repeat breeder syndrome in cows (ANNE and JACQUEZ, 2002; CELI et al., 2011; EMRE et al., 2017; RIZZO et al., 2007; TALUKDER et al., 2014). In many studies, it has been suggested that in cases of uterus infections, Hp, SAA and ceruloplasmin levels were significantly higher compared to those of healthy animals (BISWAL et al., 2014; CHAN et al., 2004; KAYA et al., 2016). HEIDARPOUR et al., (2012) reported that serum MDA levels were higher in cows with endometritis. In another study, NO plasma concentrations were found to be higher in cows with clinical and subclinical endometritis than in healthy control cows (LI et al., 2010). Similarly, KRISHNAN et al., (2014) identified a relatively higher plasma concentration of NO and LPO as well as hydrogen peroxide production in SCE. MUSAL et al., (2004) found that albumin, Hp and SAA levels were higher in cows with endometritis than those of healthy animals. BRODZKI et al., (2015) showed that the serum levels of cytokines and acute phase proteins were higher in cows with subclinical endometritis compared to healthy cows.

However, in another study conducted on pasture-based cows, there was no association of peripartum Hp with endometritis (BURKE et al., 2010).

TDH is one of the important markers for oxidative stress (SEN, 1998; BISWAS et al., 2006). According to our review of the literature, no previous study has investigated TDH as a marker for OS in animals, except for rats. However, these parameters have been analysed in human medicine as the pathogenesis of many problems. For example, in studies on the relationships between facial paresis and TDH, native thiol and total thiol levels were found to be significantly lower, while the disulphide level was calculated to be higher compared to the control group (BABADEMEZ et al., 2017; DEMIR et al., 2018). When compared with control groups, serum thiol levels have also been determined to be significantly lower in obstetric diseases, such as pre-eclampsia, gestational diabetes mellitus, pregnancies complicated by idiopathic recurrent pregnancy loss and idiopathic intrauterine growth restriction (ÖZLER et al., 2015; ERKENEKLI et al., 2016; KORKMAZ et al., 2016; CETIN et al., 2018a). ERGIN et al., (2015) found that native and total thiol levels decreased and disulphide levels increased in pregnancies with hyperemesis gravidarum. In another study, there was an increase in the disulphide/thiol ratio in patients with idiopathic recurrent pregnancy loss, although there was no difference in disulphide levels (ERKENEKLI et al., 2016). However, in another study, no difference was found between patients with pregnancy complicated by preterm prelabor membrane rupture and a control group, in respect of maternal thiol/disulphide profiles (CETIN et al., 2018b).

In the current study, native thiol and total thiol levels were determined to be lower in the acute and chronic SCE groups of cows than in the healthy control group of heifers. However, it should also be taken into account that the control group of heifers may have higher thiol levels and better antioxidant status than healthy multiparous cows. In addition, in both the acute and chronic SCE cases, native thiol and total thiol levels were lower than those of the healthy control group ($P < 0.001$), whereas

the disulphide levels, disulphide/total thiol, native thiol/total thiol and disulphide/native thiol ratios were similar in all the groups ($P > 0.05$). In the comparison between the acute and chronic SCE groups, thiol levels were also similar ($P > 0.05$). It was observed that OS may decrease natural thiol and total thiol levels due to the increase in OS in SCE groups. This indicates a higher OS level in SCE groups than in the healthy control group. However, unlike some previous studies (ERGIN et al., 2015; GÜMÜŞYAYLA et al., 2016; AKTAŞ et al., 2017; BABADEMEZ et al., 2017), when the disulphide level was analysed in the current study, it was found to be at a similar level to that of the control group. BERNABUCCI et al., (2005) reported that plasma and erythrocyte SH levels were a better measure of oxidative status in transition dairy cows. They examined only the level of total thiol in their study, while in our study, besides the total thiol, native thiol and 4 different parameters were also examined. This is the first study which has measured the parameters of thiol/disulphide homeostasis in dairy cows with SCE. In addition, the results of our study suggest that the OS in SCE might be a mechanism independent of disulphide levels. In the light of these findings, it can be said that the results of the test might reveal a role in the etiopathogenesis mechanism of infertility problems. These findings are compatible with previously published data in clinical studies. Therefore, it may be possible to decrease or prevent the impact of oxidative stress on SCE through treatment.

ROC curve analysis is a commonly used method for capacity evaluation and comparison of diagnostic tests. Using specificity and sensitivity values, this method identifies the best cut-off points for the categorization of experimental groups. The accuracy of the categorization depends on the size of the area under the ROC curve. This is a commonly used criterion for selection of the correct diagnostic test (GARDNER and GREINER, 2006; GÜRCAN and BABAK, 2013). ROC curve analysis has been shown to be a complementary calculation method to identify the degree of chronic endometritis (GÜRCAN and BABAK, 2013). The results of the current study showed the diagnostic accuracy of native thiol, which can be used in the diagnosis

of SCE, to be 92.8%, while that of total thiol was 89.3% and that of disulphide was 64.3%. UELAND et al. (1996) suggest that altered redox thiol status in vascular patients should be considered in the light of antioxidant cardiovascular disease. ELMAS et al. (2017) also showed that TDH can identify obese children with cardiovascular inflammation with adequate sensitivity and specificity. From the results obtained in the current study, it was concluded that thiol parameters could be used as an auxiliary diagnostic method in the diagnosis of SCE.

When the age distribution of TDH was evaluated in the study, naturally, the levels were found to be higher in heifers (healthy animals). However, this study demonstrated that parity had no significant influence on TDH. Since parity did not change TDH, it can be said that SCE resulted in similar OS conditions in both primiparous and multiparous cows. However, native and total thiol concentrations were found to be numerically lower in primiparous cows when compared to multiparous cows ($P>0.05$). This could stem from the adaptation processes of heifers after their first birth to metabolic changes or the formation of a defence mechanism against free oxygen radicals, which are produced in a larger amount in this period.

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Conclusions

This is the first research to show thiol/disulphide homeostasis in Holstein dairy cows with SCE. The findings show that infertility in cows can be associated with their thiol balance. TDH was defined as useful indicator of oxidant/antioxidant imbalance and may be used as a practical marker in the diagnosis of SCE. However, the reason for the higher thiol level in the control group may have been due to the better antioxidant status of the selected heifers than the healthy multiparous cows. Nevertheless, to be able to clarify potential correlations and examine the cause and effect relationships that have been proposed in previous studies, and confirmed in the current study, there is a need for further studies, with a greater number of cases, which can present more comprehensive data and more detailed analyses.

Conflict of Interests Statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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SAŽETAK

Cilj istraživanja bio je odrediti tiol-disulfidnu (TDH) homeostazu u neplodnih krava sa supkliničkim endometritisom (SCE). Endometralni citološki uzorci prikupljeni su u 36 neplodnih krava. Prema rezultatima citološke analize, krave s akutnim endometritisom svrstane su u skupinu I (n = 20), dok su one s kroničnim endometritisom svrstane u skupinu II (n = 16). Kontrolnu skupinu činile su junice u skupini III (n = 20). Na dan dijagnoze (dan 0) u sve tri skupine uzeti su uzorci krvi za analizu TDH-a. U citološkoj analizi provedeno je i Giemso i imunocitokemijsko bojenje, za određivanje kronične upale i njezine aktivnosti. U 55,55% (20/36) neplodnih krava s citološkim endometritisom upala je bila aktivna, a u 44,44% krava (16/36) ona je postala kronična. Nativni tiol i njegova ukupna razina bili su statistički znakovito niži u krava s akutnim endometritisom ($206,54 \pm 8,30 \mu\text{mol/L}$; $227,11 \pm 9,30 \mu\text{mol/L}$) i u slučajevima kroničnog SCE-a ($225,15 \pm 11,89 \mu\text{mol/L}$; $247,96 \pm 10,80 \mu\text{mol/L}$) u usporedbi s kontrolnom skupinom ($308,47 \pm 13,59 \mu\text{mol/L}$; $336,83 \pm 15,5 \mu\text{mol/L}$ zasebno) ($P < 0,001$). Omjer razina disulfida, disulfida i ukupnog tiola, nativnog i ukupnog tiola te disulfida i nativnog tiola bili su slični u svim skupinama ($P > 0,05$). Prema ROC krivulji, dijagnostička točnost nativnog tiola, koja se može primijeniti u dijagnostici SCE-a, bila je 92,8 %, ukupnog tiola 89,3%, odnosno 64,3 % u slučaju disulfida. Ovo je prvo istraživanje tiol-disulfidne homeostaze u mliječnih krava sa SCE-om kao novim pokazateljem oksidacijskog stresa, a rezultati pokazuju da je TDH pouzdan i osjetljiv pokazatelj oksidacijskog stresa u krava sa SCE-om i da abnormalni TDH može utjecati na patogenezu SCE-a.

Ključne riječi: krava; oksidacijski stress; ROC; supklinički endometritis; tiol-disulfid
