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Research Article

Determination of Dynamic Thiol Disulfide Homeostasis in Cows with Dystocia

Oğuz MERHAN[1], Lale BAŞER[1*], Kadir BOZUKLUHAN[2], Abdulsamed KÜKÜRT[1], Necdet Cankat LEHİMCİOĞLU[3], Emine ATAKİŞİ[1], Ömer ÇELİK[4]

[1] Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye
[2] Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye
[3] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye
[4] Institute of Health Sciences, Kafkas University, Kars, Türkiye

ABSTRACT

Pregnancy is a complex physiological process in animals that affects multiple mechanisms and leads to oxidative stress due to the increased production of free radicals. Thiols, which contain sulfhydryl (-SH) groups, play a critical role in sulfur-based biochemical processes and act as potent antioxidants that protect cells from oxidative damage. The aim of this study was to investigate thiol/disulfide balance in cows experiencing dystocia. A total of 30 Simmental cows were included, comprising 15 cows with normal calving and 15 cows with dystocia. Blood samples were collected in non-anticoagulant tubes immediately after parturition. Biochemical analyses revealed that cows with dystocia had significantly lower levels of total thiol ($P<0.001$), native thiol ($P<0.001$), and the native thiol/total thiol ratio ($P<0.001$) compared to cows with normal calving, whereas disulfide levels were markedly elevated ($P<0.01$). Furthermore, the disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the dystocia group ($P<0.001$). These findings indicate that thiol/disulfide homeostasis is disrupted in cows experiencing dystocia, reflecting severe oxidative stress and cellular redox imbalance. The results suggest that thiol/disulfide homeostasis could serve as a biomarker for oxidative disturbances associated with dystocia. Furthermore, antioxidant strategies aimed at reducing oxidative stress may complement existing clinical interventions and improve recovery in affected animals. In conclusion, this study highlights the critical importance of thiol/disulfide balance during the parturition process and emphasizes its potential both as a diagnostic marker and as a target for therapeutic strategies aimed at mitigating oxidative stress. It is concluded that further research is warranted to elucidate the mechanistic links between redox imbalance and dystocia and to evaluate the effectiveness of antioxidant support in clinical applications.

Keywords: *Antioxidants, cows, dystocia, oxidative stress, thiol/disulfide homeostasis.*

Güç Doğum Yapan İneklerde Dinamik Tiyol-Disülfid Homeostazının Belirlenmesi

ÖZET

Gebelik, hayvanlarda birçok mekanizmayı etkileyen karmaşık bir fizyolojik süreçtir ve serbest radikallerin artmış üretimine bağlı olarak oksidatif strese yol açmaktadır. Sülfhidril (-SH) grubu içeren tiyoller, kükürt temelli biyokimyasal süreçlerde kritik görevler üstlenir ve hücreleri oksidatif hasara karşı koruyan güçlü antioksidanlardır. Bu çalışmanın amacı, güç doğum (distosia) yapan ineklerde tiyol/disülfid dengesini incelemektir. Çalışmaya 15 normal doğum yapan ve 15 güç doğum yapan olmak üzere toplam 30 Simmental ırkı inek dahil edilmiştir. Doğumdan hemen sonra antikoagülansız tüplere kan örnekleri alınmıştır. Biyokimyasal analizler sonucunda, güç doğum yapan ineklerde toplam tiyol ($P<0.001$), doğal (native) tiyol ($P<0.001$) ve doğal tiyol/toplam tiyol ($P<0.001$) düzeylerinin normal doğum yapanlara göre anlamlı derecede azaldığı; buna karşılık disülfid seviyelerinin belirgin şekilde yükseldiği saptanmıştır ($P<0.001$). Ayrıca disülfid/doğal tiyol ve disülfid/toplam tiyol ($P<0.001$) oranlarının da güç doğum yapan grupta belirgin derecede arttığı görülmüştür ($P<0.001$). Bu bulgular, güç doğum yapan ineklerde tiyol/disülfid dengesinin bozulduğunu ve bunun şiddetli oksidatif stres ile hücresel redoks dengesizliğini yansıttığını göstermektedir. Çalışma sonuçları, tiyol/disülfid homeostazının güç doğuma bağlı oksidatif bozuklukların belirlenmesinde biyobelirteç olarak kullanılabileceğini düşündürmektedir. Ayrıca oksidatif stresin azaltılmasına yönelik antioksidan yaklaşımlar, mevcut klinik uygulamalara destek sağlayarak etkilenen hayvanlarda iyileşmeyi artırabilir. Sonuç olarak, bu araştırma tiyol/disülfid dengesinin doğum sürecindeki kritik önemini ortaya koymakta ve hem tanısal bir belirteç hem de oksidatif stresin azaltılmasına yönelik tedavi stratejileri için bir hedef olabileceğini vurgulamaktadır. Redoks dengesizliği ile güç doğum arasındaki mekanizmal bağlantıların daha ayrıntılı araştırılması ve antioksidan desteğinin klinik uygulamalardaki etkinliğinin değerlendirilmesi gerektiği sonucuna varılmıştır.

Anahtar kelimeler: *Antioksidanlar, distosia (güç doğum), inekler, oksidatif stres, tiyol/disülfid dengesi.*

***Corresponding author:** *Lale BAŞER, Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye. lalebesli10@gmail.com*

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Introduction

Fertility is one of the most important factors for a successful farm operation and inefficiency in fertility is one of the most costly problems. Environmental factors, management, diseases and genetic factors are very important for reproductive success (Abdela and Ahmed, 2016). The main objective of cattle management is to achieve high levels of productivity without compromising the health of the calf and its mother. Therefore, it is extremely important to plan births throughout the year, conceptually define, track and record normal and difficult birth cases (Mekonnen and Moges, 2016).

Dystocia can be defined as a health problem that is frequently seen in dairy cows and causes significant problems for national economies (Abdela and Ahmed, 2016). Calving difficulty, called dystocia, is generally defined as difficult calving and assistance is needed at this stage (Wondie, 2017). Dystocia (difficult birth) can result from both indirect factors related to the dam or fetus and induced factors such as assisted or medically initiated labor (Hossein-Zadeh, 2016). Indirect factors include maternal age, fetal weight and position, pelvic structure, and hormonal changes, all of which may contribute to complications during parturition (Hossein-Zadeh, 2016). Reactive oxygen species (ROS), primarily originating from mitochondria and phagocytes, act as key regulators in cellular signaling and immune responses under physiological conditions, whereas their imbalance, particularly through the formation of •OH radicals, leads to severe oxidative damage (Kükürt et al., 2021). In oxygen-dependent respiration, incomplete reduction of oxygen may occur, resulting in the generation of reactive oxygen species (ROS), primarily superoxide anions and hydrogen peroxide (Ulrich and Jakob, 2019). Direct causes include maternal factors such as improper feto-pelvic proportion, lack of cervical width, uterine torsion, inertia; offspring factors include oversized fetus, breech position, incorrect posture, multiple pregnancy. Indirect factors include calf weight at birth, sex, gestation period, body composition of the cow, weight and age of the cattle, climate, nutrition, diseases, mobility status, genetic effects (Mekonnen and Moges, 2016; Zaborski et al., 2019). Dystocia is more common in cows giving birth for the first time than in cows that have given birth many times. This is because their body composition is smaller and their pelvic development is slower. However, the risk factors for dystocia differ in these two different cow types (Abdela and Ahmed, 2016).The incidence is higher in dairy cattle than beef cattle. Maternal dystocia occurs less frequently than dystocia caused by fetal causes (Wondie, 2017).

Reactive oxygen species (ROS), primarily originating from mitochondria and phagocytes, act as key regulators in cellular signaling and immune responses under physiological conditions, whereas their imbalance, particularly through the formation of •OH radicals, leads to severe oxidative damage (Kükürt et al., 2021). In oxygen-dependent respiration, incomplete reduction of oxygen may occur, resulting in the generation of reactive oxygen species (ROS), primarily superoxide anions and hydrogen peroxide (Ulrich and Jakob, 2019). Antioxidant systems are essential to eliminate ROS that damage the building blocks of the cell (Ray et al., 2012; Forman et al., 2014; Bolduc et al., 2019).

Thiols, represented as R-SH, are biological mercaptans, and biothiols are further categorized into small free thiols and large protein thiols. Cellular and plasma thiols have a pivotal function in modulating antioxidant defense. Plasma contains protein sulfhydryl groups, cysteine, glutathione (GSH), homocysteine, and their disulfide forms. The GSH/GSSG system constitutes the low-molecular-weight sulfhydryl/disulfide pool within cells, whereas in plasma, the disulfide form (cysteine/cystine) predominates. The dynamic thiol–disulfide balance is essential for antioxidant system function. Thiols are highly reactive and act as potent antioxidants by scavenging free radicals as electron donors. Their antioxidant potential is influenced by environmental, structural, and catalytic factors. Cysteine can be endogenously synthesized from methionine and serves as a precursor for bioactive thiols such as GSH and taurine. Structurally, it can bind to metals and is critical for enzymatic functions (Kükürt et al., 2021). While moderate levels of cysteine support cellular signaling pathways, elevated plasma or intracellular concentrations are associated with cardiovascular and neurological diseases as well as oxidative DNA damage (Erel and Neselioglu, 2014; Kükürt et al., 2021). Given that dystocia induces oxidative stress, evaluating thiol/disulfide homeostasis can provide insights into the oxidative status and antioxidant defense in affected cows.

In light of these findings, it is expected that the determination of thiol/disulfide levels will provide valuable insights into various physiological and pathological conditions. This study aimed to investigate thiol/disulfide homeostasis in healthy and dystocia-affected cows, and to explore its potential association with stress and metabolic disturbances.

Materials and Methods

Ethical Approval

This study was approved by the Local Ethics Committee for Animal Experiments of Kafkas University (KAU-HADYEK, Protocol No: KAU-HADYEK/2025-140).

Animals

The study was conducted on a total of 30 Simmental cows (*Bos taurus*), including 15 cows with dystocia and 15 cows with normal parturition. All cows were female, aged between 3–5 years, and had at least one previous calving. All animals included in the study were clinically healthy at the time of sampling, with no signs of disease or subclinical infection. The animals were housed and managed in a private enterprise in Kars city center, Turkiye. Dystocia was defined as cases requiring assisted delivery, while normal parturition referred to spontaneous delivery without any assistance. Blood samples were collected from the jugular vein of the cows between 3 and 48 hours after parturition, using anticoagulant-free tubes.

Sample Preparation

Blood samples taken from the vena jugularis were collected in anticoagulant-free tubes and brought to the Department of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, and centrifuged at 3000 rpm for 10 min in the Biochemistry Application Laboratory. The separated serum samples were transferred into labeled, sterile eppendorf tubes and stored at -20 °C until the analyses were performed.

Biochemical Analyses

Total thiol and native thiol in the obtained serum samples were measured colorimetrically (Epoch, Biotek, USA) using a commercial test kit (Rel Assay Diagnostics, Turkiye). Disulfide = (Total thiol (Rel Assay Diagnostics, RL0192, Turkiye) – Native thiol (Rel Assay Diagnostics, RL0185, Turkiye))/2, Disulfide/Native Thiol (%) = (Disulfide × 100)/Native thiol, Disulfide/Total Thiol (%) = (Disulfide × 100)/Total thiol, and Native Thiol/Total Thiol (%) = (Native thiol × 100)/Total thiol were calculated using these formulas (Erel and Neselioglu, 2014).

Statistical Analyses

GraphPad Prism 8.0.2 program was used for statistical analysis of the data obtained from the studies. Shapiro-Wilk Test were performed to determine whether the data were normally distributed. Total thiol, native thiol, native thiol/total thiol, disulfide, disulfide/native thiol and disulfide/total thiol levels data were analyzed by Independent samples t test $P<0.05$ level was considered statistically significant.

Results

Among the biochemical parameters, total thiol ($P<0.001$), native thiol ($P<0.001$), and native thiol/total thiol ($P<0.001$) levels were significantly decreased in cows with dystocia compared to the normal calving group. Disulfide ($P<0.01$) level was significantly increased compared to the normal calving group. In addition, disulfide/native thiol and disulfide/total thiol ($P<0.001$) levels were significantly increased compared to the normal calving group, as shown in Table 1 and Figure 1.

Discussion

Dystocia, a common problem in dairy farms, is reported to occur in 3% to 25% of cattle, with incidence being higher in first-time calving cows (approximately 30%) than in cows with multiple calvings (approximately 10%) (Tenhagen et al., 2007). Many factors contribute to difficult labor, including parity, calf size and sex, calf weight, maternal body composition, metabolism, and environmental conditions. Farm management practices also play a crucial role in the development of dystocia. Difficult parturition in cattle is one of the most important complications of pregnancy, leading to reduced overall performance and substantial economic losses (Yokus et al., 2007).

ROS are physiologically produced continuously as a result of metabolic processes and the amount of this production may increase due to various reasons (Yokus et al., 2007). Pregnancy, which is a physiological process involving many different mechanisms, causes oxidative stress due to the increase of free radicals in the organism because it affects many mechanisms in animals (Petersen et al., 2004; Merhan and Bozukluhan, 2022). Increased metabolic activity during pregnancy increases oxidative stress and oxidative stress also contributes to pregnancy pathology (Yokus et al., 2007).

Oxidative stress causes depletion of the body's antioxidant resources, and physiological adaptation, negative balance in energy, increased metabolic reactions and the presence of ketone bodies contribute to the reduction of antioxidant reserves during pregnancy (Sahoo et al., 2009). Even physiologically, parturition causes stress and the occurrence of dystocia adds to the normal stress of the body (Bansal et al., 2011).

Studies in cows with dystocia have reported an increase in MDA level, an indicator of lipid peroxidation, and a decrease in antioxidant enzyme levels (Thangamani et al., 2019; Merhan et al., 2020). It has been reported that an imbalance between ROS production and removal from the body in dairy cows may cause problems with parturition (Büyükoğlu and Aslan, 2018).

As a mercaptan, thiol consists of a sulfur atom and a hydrogen atom bound to a carbon atom, and it contains a sulfhydryl group (-SH). By participating in an oxidation reaction with oxidants, it can form disulfide (RSSR) bonds. This bond is known as a covalent bond and is also referred to as a disulfide bond. When oxidative stress

Table 1. Thiol/disulfide homeostasis in normal calving and dystocia cows (mean and standard error)

Parameters	Normal Calving	Dystocia	P
Total thiol (μmol/L)	472.67±9.17	438.25±13.26	P<0.001
Native thiol (μmol/L)	396.08±6.38	318.18±7.71	P<0.001
Disulfide (μmol/L)	38.30±4.47	60.04±7.59	P<0.01
Disulfide/Native thiol (%)	9.79±1.19	19.35±2.56	P<0.001
Disulfide/Total thiol (%)	7.97±0.78	13.35±1.19	P<0.001
Native thiol/Total thiol (%)	84.06±1.55	73.29±2.38	P<0.001

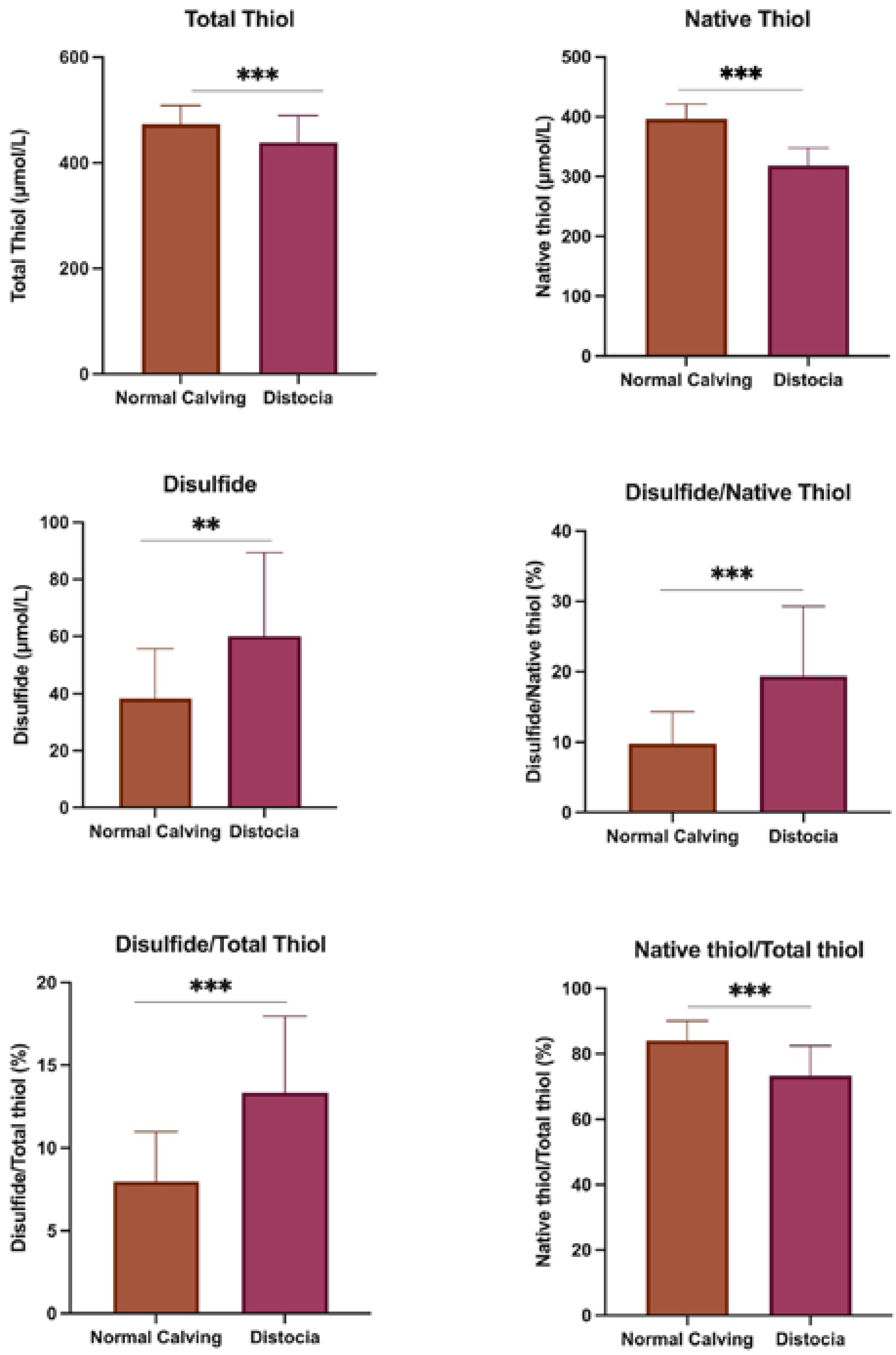

Figure 1. Thiol/disulfide homeostasis in normal parturient and dystocia cows.
The asterisks indicating statistical differences between groups represent the following: * = P<0.05, ** = P<0.01, *** = P<0.001. The study included the following sample sizes: Normal calving group (n = 15), distocia group (n = 15). Error bars represent the standard error of the mean (SEM).

occurs, protein thiols can undergo reversible bonding with low-molecular-weight thiols, producing disulfides that are later reduced, thus maintaining the dynamic thiol–disulfide system. This homeostasis plays a crucial role in many fundamental cellular processes such as antioxidant defense, detoxification, signal transduction, apoptosis, enzymatic activity, and regulation of transcription factors, as well as in various diseases, metabolic and pathological conditions. Therefore, the analysis of this homeostasis in blood may provide scientifically significant insights for numerous biochemical and pathological conditions (Erel and Neselioglu, 2014). Thiols, known as functional sulfhydryl (-SH) groups, are vital in preventing oxidative stress in cells (Büyükoğlu and Aslan, 2018). Free radicals in the environment are oxidized by thiol groups and converted into disulfide bonds, also known as sulfur bridges (Jones and Liang, 2009). Thiol/disulfide balance is used as a new and practical method that allows a complete assessment of the presence of oxidants and antioxidants. In human medicine, thiol/disulfide homeostasis is a novel biomarker used in diabetes, cardiovascular and many other conditions (Ates et al., 2016; Elmas et al., 2017; Aydın et al., 2021).

Although reported to play a role in the pathophysiology of many bacterial, viral and parasitic diseases in veterinary medicine, (Adıgüzel and Merhan, 2024; Aydın et al., 2023; Bölükbaşi and Merhan, 2024; Çamkerten et al., 2019.; Çenesiz et al., 2024; Değirmençay et al., 2021; Ertaş et al., 2023; Kaya and Merhan, 2024; Tarhan et al., 2023; Schmidt et al., 2021; Terzi et al., 2022) thiol/disulfide homeostasis, a biomarker used for oxidative stress, is a new parameter studied in veterinary medicine. It was reported that there was no statistical difference in disulfide, disulfide/native thiol, disulfide/total thiol and native thiol/total thiol levels, while native thiol and total thiol levels decreased statistically significantly in cattle with endometritis compared to the normal parturient group (Emre et al., 2021).

The findings of this study showed that total thiol, native thiol, and native thiol/total thiol levels were significantly decreased in dystocia cases compared to the normal calving group, whereas disulfide, disulfide/native thiol, and disulfide/total thiol ratios were significantly increased. These findings indicate that thiol/disulfide homeostasis is impaired in dystocia cows, which may reflect severe oxidative stress and disruption of the cellular redox balance. Consequently, thiol/disulfide homeostasis may serve as a potential biomarker in understanding the pathophysiology of dystocia.

Conclusion

In conclusion, the evaluation of thiol/disulfide homeostasis markers in dystocia cases may contribute to a better understanding of the disease pathogenesis and underlying mechanisms. These findings suggest that thiol/disulfide homeostasis could serve as a potential biomarker for dystocia. Furthermore, antioxidant therapies aimed at counteracting oxidative stress may be investigated in future studies as potential complementary approaches, and monitoring thiol/disulfide imbalance may have potential clinical relevance in dystocia management.

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Author contribution statement

OM, LB, and NCL designed the study. OM, KB, and NCL collected the samples. LB and ÖÇ performed the laboratory analyses. LB, EA, and AK evaluated the analysis results. OM and LB prepared the manuscript with contributions from all authors.

Conflict of interest

The authors declared that there is no conflict of interest.

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