Slov Vet Res
DOI 10.26873/SVR-2325-2026

Early View

Original Research Article

Effects of Ischemia and Reperfusion Induced by Uterine Prolapse on Cardiac Biomarkers in Sheep

Key words

cardiac biomarker;
ischemia;
reperfusion;
sheep;
uterine prolapse

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Abstract: The aim of this study was to determine the cardiac damage caused by uterine prolapse in sheep, to reveal the cardiac changes that occur depending on the time elapsed since the event, and to evaluate the ischemia-reperfusion (I/R) injury induced by reperfusion following treatment using a prolapsed uterine model. The animal material of the study consisted of a total of 35 Awassi sheep that had given birth at least once and were within the first 24 hours postpartum. The sheep were divided into five groups according to the mode of delivery and the time of onset of uterine prolapse. The control group (Group 1, n=7) consisted of sheep that gave birth normally without any intervention. The groups that developed uterine prolapse were as follows: The study consisted of sheep that developed prolapse within 0-6 hours (Group 2, n=7), 6-12 hours (Group 3, n=7), 12-18 hours (Group 4, n=7), and 18-24 hours (Group 5, n=7) depending on the duration of prolapse. Cardiac troponin (cTnI), creatine kinase myocardial band (CK-MB), superoxide dismutase (SOD), catalase (CAT), malondealdehyde (MDA), interleukin 6 (IL-6), and tumor necrosis factor-alpha (TNF-α) levels were determined using a commercial ELISA kit from blood samples taken within one hour after birth from the sheep in the control group and before and 4 hours after treatment from the prolapsed uteri groups. Evaluations performed before and after prolapse treatment revealed significant differences both within and between groups in heart rate, respiratory rate, body temperature, WBC, cTnI, CK-MB, AST (lactate dehydrogenase), LDH, SOD, CAT, MDA, IL-6, and TNF-α ($P<0.001$). In conclusion, uterine prolapse causes serious cardiac and systemic effects in sheep, exacerbating ischemia-reperfusion injury. Therefore, early diagnosis and rapid intervention are critical for minimizing cardiac damage.

Received: 30 October 2025
Accepted: 4 January 2026

Introduction

Uterine prolapse is the protrusion of a retroverted uterus through the vagina and beyond the vulvar lips. It is most commonly seen in cows and sheep but can occur in all domestic animal species. This condition typically occurs immediately or within a few hours after birth, when the cervix is open and uterine contractions are inadequate (1). While its etiology is not fully understood, factors such as hormonal imbalances, hypocalcemia, mineral deficiencies, injury or overdistension of the birth canal, excessive traction during difficult labor, and forced expulsion of the fetal membranes are reported to play a role in its development (1, 2). In cases of uterine prolapse, the uterus appears as a large mass protruding from the vulva, often extending to the animal's knee (2). This situation disrupts uterine circulation, making the caruncles susceptible to trauma and infection. Furthermore, the development of thrombosis, ulceration, and necrosis can lead to anorexia, toxemia, rapid deterioration in general condition, and even death (3). Therefore, urgent treatment is necessary in cases of uterine prolapse; otherwise, edema, ischemia, internal bleeding, prostration, and shock can develop, leading to a prognosis ranging from poor to hopeless (4).

Cardiac biomarkers such as cardiac troponin I (cTnI) and creatine kinase myocardial band (CK-MB) can be considered important indicators in the early diagnosis of cardiac damage caused by uterine prolapse. Cardiac troponin I is widely used as one of the most sensitive and specific markers of myocardial damage in humans (5). Troponin is a protein complex composed of three subunits (T, I, and C) that participate in the contractile process. While troponin C is present in both cardiac and skeletal muscle,

troponin T and I are considered the subunits with the highest cardiac specificity (5). Additionally, CK-MB and lactate dehydrogenase (LDH) are specific isoenzymes that increase in various cardiac disorders (6).

Ischemia is defined as the reduction or complete cessation of blood flow to organs or tissues for any reason. Restoration of blood flow after ischemia is called reperfusion; however, this process can exacerbate the damage caused by ischemia. Ischemia/reperfusion (I/R) injury contributes to high morbidity and mortality in many clinical situations, including organ transplantation, myocardial infarction, cerebrovascular disease, major surgery, thrombolytic therapy, hemorrhagic shock, and resuscitation (7). Ischemia/reperfusion injury is a complex process characterized by cytokines, adhesion molecules, neutrophil infiltration, and the release of proinflammatory mediators. During this process, excessive production of reactive oxygen species (ROS) causes cellular damage through lipid peroxidation, protein degradation, and DNA oxidation (8). Due to the high reactivity of ROS, they are difficult to measure directly; therefore, malondialdehyde (MDA), the end product of lipid peroxidation, is a widely used indirect marker (9). Increased blood flow with reperfusion leads to the formation of excess free radicals, which cannot be eliminated by antioxidant defense mechanisms such as superoxide dismutase (SOD) and catalase (CAT). This paradoxical situation causes reperfusion to further exacerbate damage in ischemic tissue and is defined as reperfusion injury (10).

To our knowledge, there are no studies investigating the presence of myocardial damage in sheep with prolapsed uterus. The aims of this study were i) to demonstrate the diagnostic significance of cardiac biomarkers such as cTnI, CK-MB, LDH, and AST in determining cardiac damage caused by prolapsed uterus; ii) to evaluate the relationship between the time elapsed since the prolapsed uterine episodes and cardiac damage; iii) to reveal the relationship between cardiac damage markers that change due to prolapsed uterus and proinflammatory cytokines and oxidative stress markers; and iv) to investigate I/R injury caused by reperfusion following treatment in the prolapsed uterine model. The hypothesis of our study was that circulatory impairment developing in the prolapsed uterus and increased oxidative stress markers and cytokines due to the organ being left in the external environment would lead to changes in cardiac markers; in addition, cardiac damage will be further exacerbated by the sudden entry into systemic circulation of cytokines and reactive oxygen species accumulated in the tissue due to circulatory impairment following the placement of the uterus.

Materials and methods

Selection of animal material

The animal material for this randomized controlled clinical study consisted of 35 Awassi sheep brought to the Animal Hospital of the Faculty of Veterinary Medicine, Harran University, that had given birth at least once and were within the first 24 hours postpartum. The animals included in the study were selected from sheep that had given birth to a single lamb in their previous pregnancies, had no postpartum complications, had a body condition score between 3 and 4 (1 = very thin, 5 = obese) (11), and were between 3 and 6 years old. Adequacy of sample size was determined using the G*Power method. Animals were divided into five groups based on mode of delivery and developmental time for uterine prolapse. The control group (Group 1, n=7) consisted of sheep that gave birth normally without any intervention. Sheep with uterine prolapse were divided into four groups according to the duration of uterine extrusion: (Group 2, n=7) 0-6 hours, (Group 3, n=7) 6-12 hours, (Group 4, n=7) 12-18 hours, and (Group 5, n=7) 18-24 hours. The control group consisted of sheep from the same flock who gave birth normally and underwent health checks and showed no pathological findings. Since changes in edema and necrosis levels in the caruncles were observed depending on the duration of uterine extrusion in cases of uterine prolapse, the grouping was made based on the history taken from the patient owners (12). In the prolapsed uterine group, after the first blood sample was taken, the prolapsed uterus was placed in its anatomic position, and only 2 mL of intravenous oxytocin (Vetaş Oxytocin/Vetaş®, containing 10 IU of oxytocin per mL) was administered until the second blood sample was taken to prevent recurrence. Those who had received any treatment within the last month, those who presented to the clinic with a recurrence following owner or veterinary intervention, and those who had been admitted more than 24 hours or who were brought in comatose were excluded from the study.

Hematological and biochemical analyses

Blood samples were taken from all sheep before any treatment. In the prolapsed uterine groups, a second blood sample was collected at 4 hours following treatment to determine ischemia/reperfusion injury (13). Before each blood collection, body temperature, heart rate, and respiratory rate were recorded. In the control group, blood samples were taken within the first hour after birth. Blood samples were collected in tubes containing coagulant activator (BD Vacutainer, Plymouth, UK) and without it (BD Vacutainer) for hematological and biochemical analyses. Hematological analyses were performed within one hour of blood collection. Serum tubes were kept at room temperature for approximately 30 minutes and then centrifuged at 3000×g for 10 minutes. The resulting serum samples were divided into three separate 0.5 mL Eppendorf tubes and stored at -20°C for up to two months.

Serum parameters were analyzed using commercial ELISA kits, cTnI (Sheep Cardiac Troponin 1 ELISA Kit, MBS745008, MyBioSource, USA), CK-MB (Sheep Creatine Kinase MB isoenzyme ELISA Kit, MBS736314, MyBioSource, USA), SOD (Super Oxidase Dismutase ELISA Kit, MBS1602165, MyBioSource, USA), CAT (Sheep Catalase ELISA Kit, MBS017839, MyBioSource, USA), MDA (Sheep Malondialdehyde ELISA Kit, MBS741484, MyBioSource, USA), IL-6 (Sheep Interleukin 6 ELISA Kit, MBS020400, MyBioSource, USA) and TNF-α (Sheep TNF-alpha ELISA Kit, LDH and AST levels were determined using a biochemistry analyzer (Seamaty SMT-120V, Notavet, İzmir, Turkey), and WBC values

were determined using an automatic blood counting device (Sysmex Europe, pocH-100iV Diff, Germany).

Statistical analysis

Statistical analyses were performed using SPSS version 26 software. The Shapiro-Wilk test was used to assess the normal distribution of variables, and the Levene test was used to assess homogeneity of variances. Differences between pre- and post-treatment measurements in the prolapsed uterine groups were examined using a paired t-test. One-way ANOVA was used for intergroup comparisons. Two-Factor Repeated Measures ANOVA was applied to evaluate time (pre-post) and group (control and prolapse groups) factors together. If significant differences were detected, Tukey's multiple comparison test was used as a post-hoc test. A p-value of <0.05 was considered statistically significant in all analyses.

Results

Mean heart rate, respiratory rate, body temperature, and WBC values of the study groups are shown in Table 1 and Figure 1. In the evaluations made before and after prolapse treatment, significant differences were detected in these parameters both within and between groups (P<0.001). While pre-treatment heart rate was higher in G2 and G3 compared to G1, the lowest value was recorded in G1 and the highest value was recorded in G4 (P<0.001). After treatment, heart rate was determined to be higher in G3 compared to G2; the lowest value was recorded in G2, and the highest value was recorded in G4 (P<0.001). While pre-treatment respiratory rate was higher in G2, G3, and G4 compared to G1, the lowest value was recorded in G1, and the highest value was recorded in G5 (P<0.001). After treatment, respiratory rate was determined to be higher in G3 and G4 compared to G2; the lowest value was detected in G2 and the highest value was detected in G5 (P<0.001). Before treatment, body temperature was higher in G2 compared to G1 and in G4 compared to G3. The lowest value was recorded in G1, and the highest value was recorded in G5 (P<0.001). After treatment, body temperature was higher in G3 and G4 compared to G2; the lowest value was found in G2 and the highest value was found in G5 (P<0.001). Pre-treatment WBC values were higher in G3, G4, and G5 compared to G2; the lowest value was recorded in G1, and the highest value was recorded in G5 (P<0.001). Post-treatment WBC values differed significantly among all groups; the lowest value was determined in G2, and the highest value was determined in G5 (P<0.001).

Table 2 and Figure 2 present the cTnI, CK-MB, AST, and LDH values of the study. Significant differences were detected in this effect before and after prolapse treatment, both within the groups and between the groups (P<0.001). Pre-treatment cTnI levels were higher in G2, G3, G4, and G5 compared to G1, with the lowest value recorded in G1 and the highest value in G5 (P<0.001). Post-treatment cTnI levels were higher in G3, G4, and G5 compared to G2, with the lowest value recorded in G2 and the highest value again in G5 (P<0.001). Pre-treatment CK-MB levels were higher in group G4 compared to group G2; the highest value was recorded in group G5, and the lowest value was recorded in group G1 (P<0.001). After treatment, CK-MB levels were higher in groups G3 and G4 compared to group G2; the highest value was found in group G5, and the lowest value was found in group G2 (P<0.001). Before treatment, AST and LDH levels were determined to be higher in G3, G4, and G5 compared to G1, with the lowest value being recorded in G1 and the highest value being recorded in G5 (P<0.001). After treatment, AST and LDH levels were determined to be higher in G3 and G5 compared to G2, with the lowest value being detected in G2 and the highest value being detected in G5 (P<0.001).

SOD, CAT, and MDA values of the study groups are given in Table 3 and Figure 3. Before and after prolapse treatment, significant differences were detected in these parameters both within and between groups (P<0.001). While pre-treatment SOD level was lower in G2, G3, and G5 compared to G1, the lowest value was recorded in G5 and the highest value was recorded in G1 (P<0.001). Post-treatment SOD level was lower in G4 and G5 compared to G2; the lowest value was again detected in G5, and the highest value was detected in G1 (P<0.001). Pre-treatment CAT level was lower in G2, G3, G4, and G5 compared to G1; the lowest value was recorded in G5, and the highest value was recorded in G1 (P<0.001). Post-treatment CAT level was lower in G2, G3, G4, and G5 compared to G1; the lowest value was recorded in G5, and the highest value was recorded in G1 (P<0.001). Post-treatment CAT level was lower in G3, G4, and G5 compared to G2; the lowest value was detected in G5, and the highest value was detected in G1 (P<0.001). Pre-treatment MDA levels were higher in G2, G3, G4, and G5 compared to G1; the lowest value was recorded in G1, and the highest value was recorded in G5 (P<0.001). Post-treatment MDA levels were higher in G3, G4, and G5 compared to G2; the lowest value was detected in G2, and the highest value was detected in G5 (P<0.001).

The IL-6 and TNF-α values for the study groups are presented in Table 4 and Figure 4. Significant differences were detected in these parameters before and after prolapse treatment, both within and between groups (P<0.001). Pre-treatment IL-6 levels were higher in G2, G3, G4, and G5 compared to G1, with the lowest value recorded in G1 and the highest value recorded in G5 (P<0.001). Post-treatment IL-6 levels were higher in G4 and G5 compared to G2, with the lowest value recorded in G2 and the highest value recorded in G5 (P<0.001). Pre-treatment TNF-α levels were higher in G2, G3, G4, and G5 compared to G1, with the lowest value recorded in G1 and the highest value recorded in G5 (P<0.001). Post-treatment TNF-α levels were higher in G3, G4, and G5 compared to G2; the lowest value was found in G2, and the highest value was found in G5 (P<0.001).

Table 1. Heart rate, respiratory rate, body temperature, and WBC values in sheep with prolapsed uterus and after treatment (Mean ± Std. Deviation)

Groups		HR (min)		RR (min)		Temperature (◦C)		WBC (x103 /µL)		P Paired Samples Test
		Pre-T	Pos-T	Pre-T	Pos-T	Pre-T	Pos-T	Pre-T	Pos-T	
G1	7	108.7±3.1[a]		45.4±2.6[a]		38.5±0.1[a]		7.3±0.07[a]		
G2	7	118±4.1[A,b]	120.4±4.1[B,x]	58.7±2.6[A,b]	63.4±2.5[B,x]	38.8±0.3[A,b]	39.2±0.1[B,x]	7.6±0.1[A,ab]	9.3±0.3[B,x]	
G3	7	129±2.7[A,c]	132.5±2.6[B,y]	65.1±2.2[A,c]	69.8±1.6[B,y]	39.1±0.2[A,bc]	39.5±0.2[B,y]	9.5±0.4[A,c]	11.4±0.4[B,y]	<0.001
G4	7	130±1.8[A,cd]	133.1±2.6[B,yz]	71.1±4.3[A,d]	73.5±2.9[B,z]	39.9±0.1[A,d]	40.2±0.2[B,z]	11.04±0.5[A,d]	15.1±0.9[B,z]	
G5	7	128±3.6[A,cde]	132.2±3.3[B,yzt]	72.7±3.7[A,de]	74.8±2.3[B,zt]	40.1±0.2[A,de]	40.4±0.1[B,zt]	16.31±1.20[A,e]	20.03±1.5[B,t]	
P value						<0.001				

[a, b, c, d, e and x, y, z, t:] Different letters within the same column represent time-dependent statistical differences within the group. [A, B:] Different letters within the same row represent time-dependent statistical differences between groups.; HR; heart rate, RR; respiratory rate, WBC; white blood cells

Table 2. CTnI, CKMB, AST and LDH values before and after treatment of uterine prolapse in sheep (Mean ± Std. Deviation)

Groups		CTnI (ng/mL)		CKMB (U/L)		AST (U/L)		LDH (U/L)		P Paired Samples Test
		Pre-T	Pos-T	Pre-T	Pos-T	Pre-T	Pos-T	Pre-T	Pos-T	
G1	7	0.39±0.007[a]		114.2±8.6[a]		110.1±7.5[a]		451.4±18.6[a]		
G2	7	0.185±0.02[A,b]	0.294±0.02[B,x]	134.7±8.32[A,ab]	146.7±5.4[B,x]	121.5±7.9[A,ab]	150.1±9.8[B,x]	471.4±11.3[A,ab]	605.7±66.03[B,x]	
G3	7	0.291±0.01[A,c]	0.440±0.03[B,y]	150.7±9.01[A,bc]	160.0±7.0[B,y]	157.2±8.03[A,c]	184.8±12.6[B,y]	577.1±57.3[A,c]	730.0±52.3[B,y]	<0.001
G4	7	0.463±0.04[A,d]	0.676±0.06[B,z]	191.5±12.5[A,d]	192.8±7.5[AB,z]	181.5±11.4[A,d]	201.2±11.4[B,yz]	671.4±55.7[A,d]	807.1±77.7[B,yz]	
G5	7	0.662±0.07[A,e]	0.869±0.07[B,t]	200.0±15.0[A,de]	205.2±14.1[AB,zt]	209.1±10.7[A,e]	243.5±19.3[B,t]	771.4±80.3[A,e]	987.1±50.7[B,t]	
P value						<0.001				

[a, b, c, d, e and x, y, z, t:] Different letters within the same column represent time-dependent statistical differences within the group. [A, B:] Different letters within the same row represent time-dependent statistical differences between groups. CTnI; Cardiac troponin I, CKMB; Creatinine kinase myocardial band, AST; Aspartate amino-transferase, LDH; Lactate dehydrogenase

Table 3. SOD, CAT, and MDA values before and after treatment of uterine prolapse in sheep (Mean ± Std. Deviation)

Groups		SOD (ng/ml)		CAT (ng/ml)		MDA (ng/ml)		P Paired Samples Test
		Pre-T	Pos-T	Pre-T	Pos-T	Pre-T	Pos-T	
G1	7	70.4±3.07[a]		10.82±0.2[a]		0.51±0.03[a]		
G2	7	59.03±7.27[A,b]	46.45±4.15[B,x]	9.25±0.8[A,b]	6.85±0.7[B,x]	0.63±0.04[A,b]	0.77±0.04[B,x]	
G3	7	48.03±5.2[A,c]	40.7±6.4[B,xy]	7.11±0.6[A,c]	4.72±0.5[B,y]	0.75±0.05[A,c]	1.05±0.09[B,y]	<0.001
G4	7	47.17±5.07[A,cd]	31.6±1.81[B,z]	4.53±0.5[A,d]	2.98±0.7[B,z]	0.92±0.10[A,d]	1.15±0.08[B,z]	
G5	7	35.31±3.91[A,e]	24.6±4.23[B,t]	2.82±0.7[A,e]	1.86±0.1[B,t]	1.04±0.05[A,e]	1.30±0.06[B,t]	
P value				<0.001				

[a, b, c, d, e and x, y, z, t:] Different letters within the same column represent time-dependent statistical differences within the group. [A, B:] Different letters within the same row represent time-dependent statistical differences between groups, SOD; Superoxide dismutase, CAT; Catalase, MDA; Malondialdehyde

Table 4. IL-6 and TNF-α values before and after treatment of uterine prolapse in sheep (Mean ± Std. Deviation)

Groups		IL-6 (pg/ml)		TNF-α (pg/ml)		P Paired Samples Test
		Pre-T	Pos-T	Pre-T	Pos-T	
G1	7	16.87±1.3^{a}		8.0±0.7^{a}		
G2	7	25.87±2.3^{A,b}	32.30±1.8^{B,x}	12.42±1.41^{A,b}	16.14±1.17^{B,x}	
G3	7	35.73±3.9^{A,c}	46.44±4.6^{B,xy}	18.28±1.55^{A,c}	23.00±2.03^{B,y}	<0.001
G4	7	50.59±3.4^{A,d}	54.44±19.7^{B,yz}	24.42±1.3^{A,d}	29.71±1.38^{B,z}	
G5	7	58.73±5.6^{A,e}	74.87±5.4^{B,t}	31.42±2.9^{A,e}	38.28±1.69^{B,t}	
P value				<0.001		

a, b, c, d, e:and x, y, z, t: Different letters within the same column represent time-dependent statistical differences within the group. $^{A, B}$: Different letters within the same row represent time-dependent statistical differences between groups. IL-6; Interleukin 6, TNF-α; Tumor necrosis factor-alpha

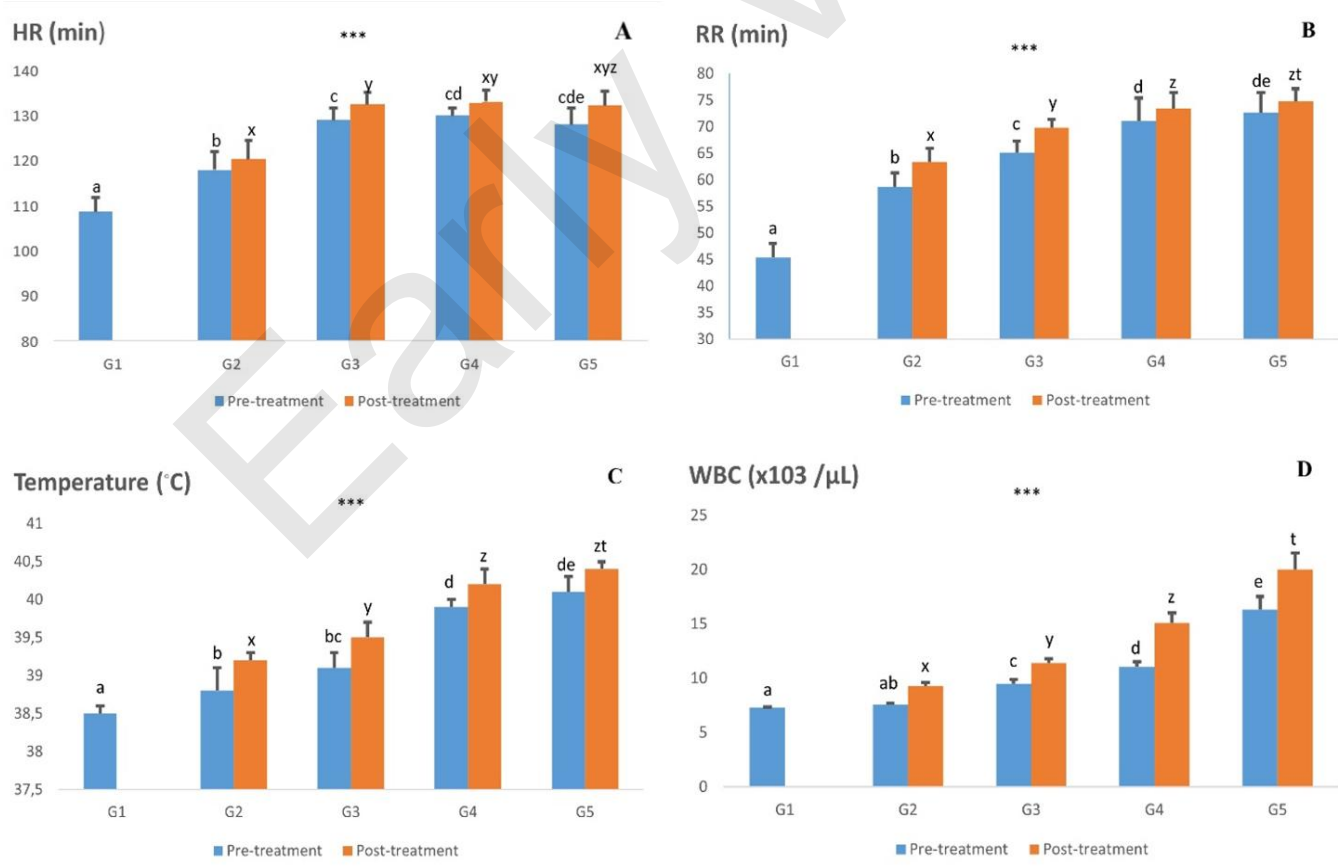

Figure 1. The study focuses on the changes in heart rate (A), respiratory rate (B), body temperature (C), and WBC (D) values during and after treatment for prolapsed uterus in sheep, both within and between groups. P = between groups < 0.001, *** = within group P < 0.001

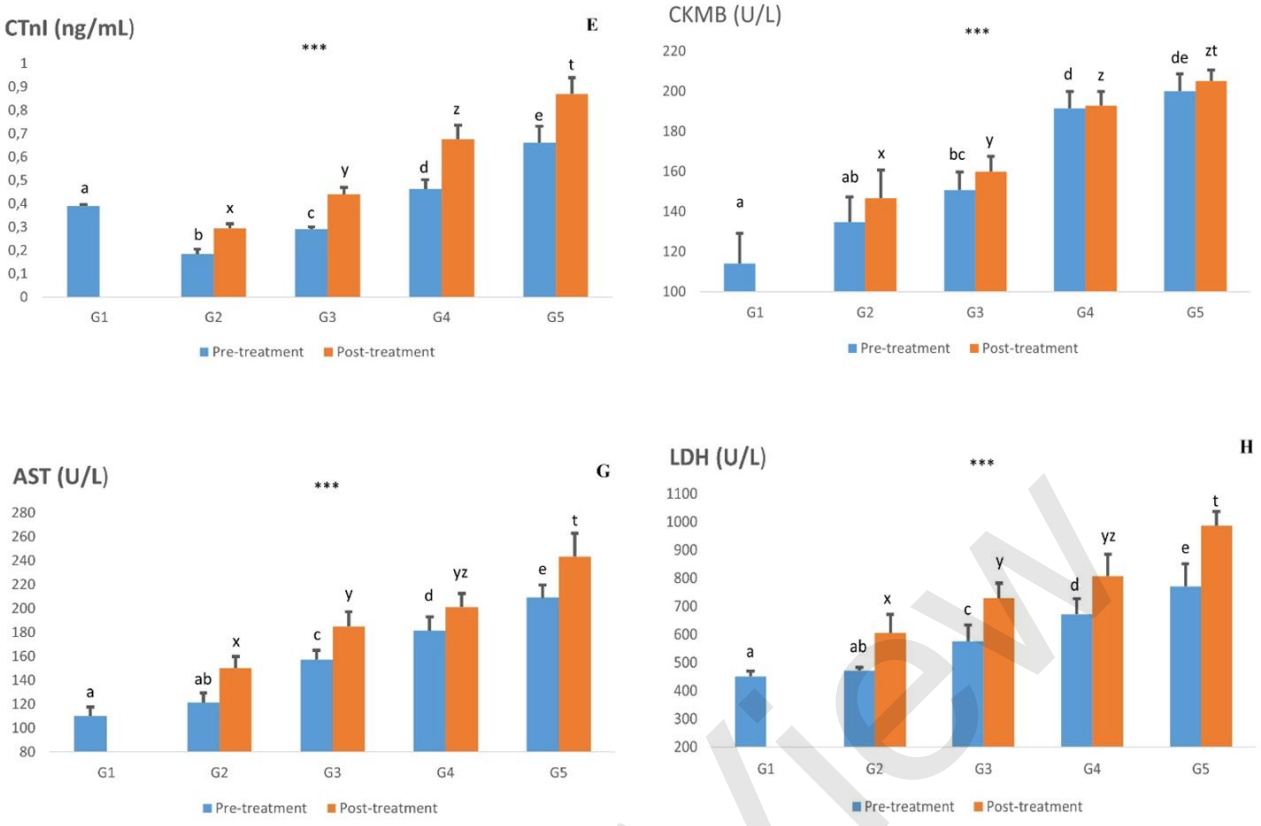

Figure 2. Intra-group and inter-group changes in CTnI (E), CKMB (F), AST (G), and LDH (H) values before and after treatment for prolapsed uterus in sheep. P = inter-group < 0.001, *** = intra-group P < 0.001

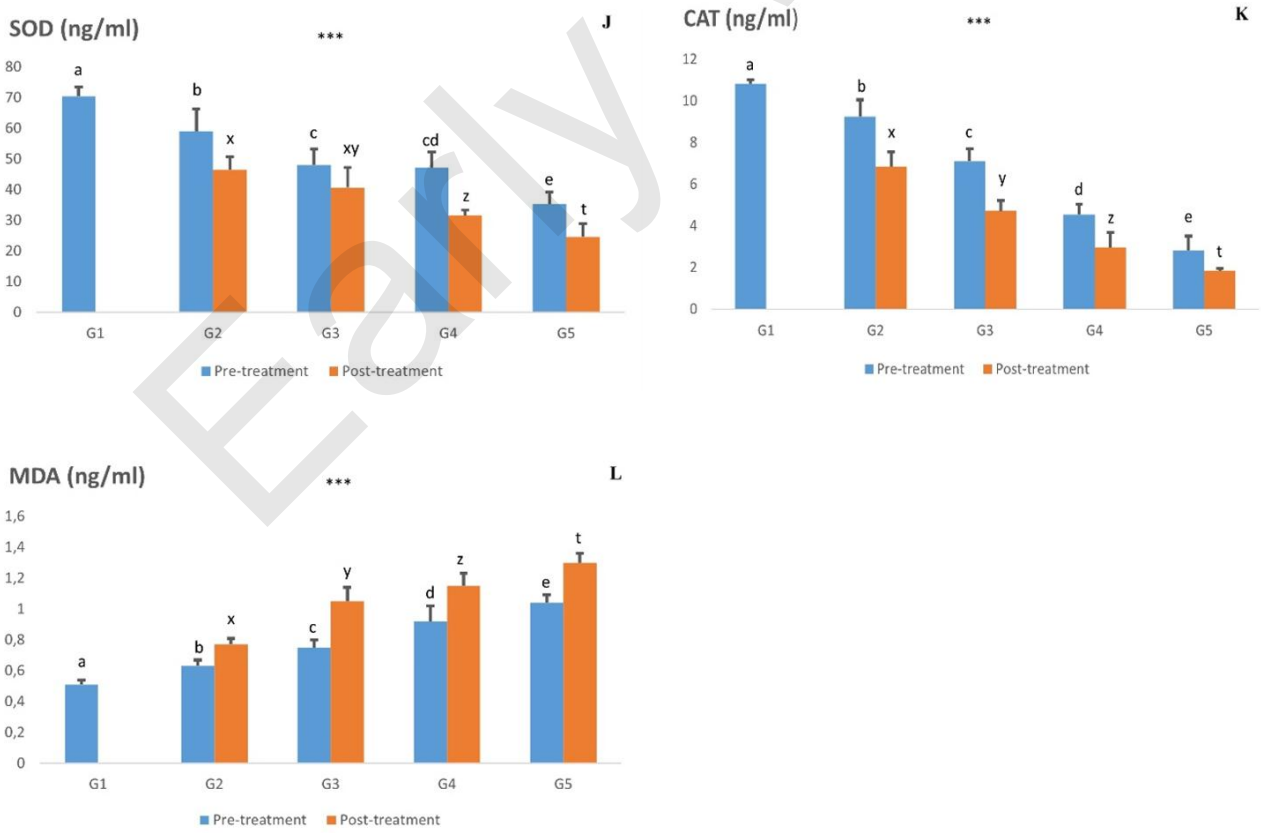

Figure 3. Intra-group and inter-group changes in SOD (J), CAT (K), and MDA (L) values before and after treatment for prolapsed uterus in sheep. P = inter-group < 0.001, *** = intra-group P < 0.001

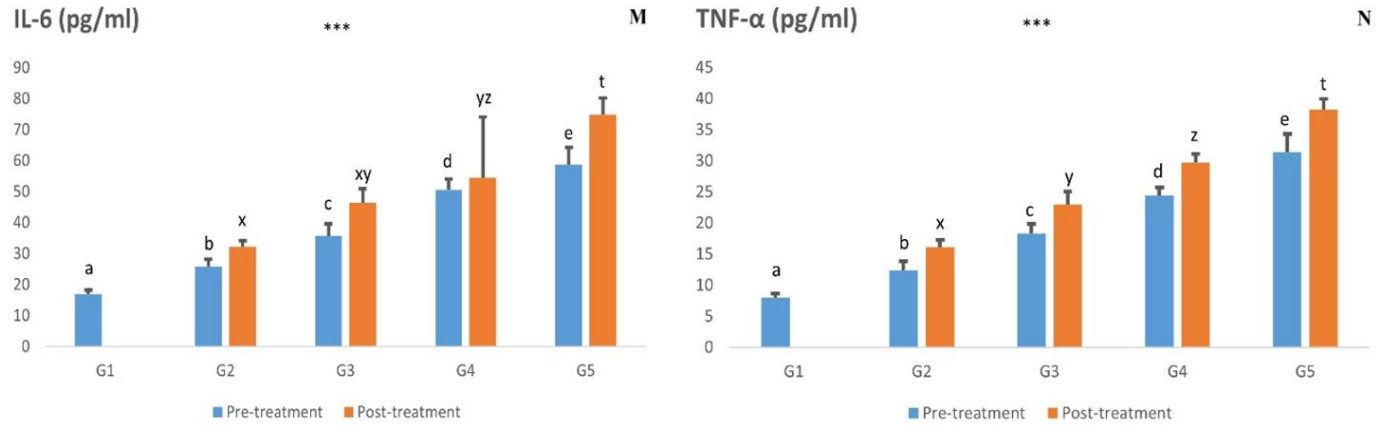

Figure 4. Intra-group and inter-group changes in IL-6 (M) and TNF-α (N) values before and after treatment for prolapsed uterus in sheep. P = inter-group <0.001, *** = intra-group P < 0.001

Discussion

Uterine prolapse usually occurs during the third stage of labor, when the fetus is expelled and the fetal membranes separate from the maternal caruncles. Edema, ischemia, lacerations, and internal hemorrhage in the prolapsed uterus can damage multiple organs and tissues, leading to exhaustion and shock; in some cases, death can occur (4). The present study demonstrates that cardiac damage increases with time since uterine prolapse, and after treatment, significant changes occur in clinical parameters, cardiac biomarkers, proinflammatory cytokines, and oxidative stress parameters, exacerbating cardiac damage.

In cases of uterine prolapse, particularly the severity of the prolapse, the duration of the prolapse, and complications with infection or trauma generally lead to increased respiratory rate (tachypnea), increased heart rate (tachycardia), and increased body temperature. The increases in respiratory rate, heart rate, and body temperature observed in the prolapsed uteri group are consistent with findings previously reported in cows with prolapsed uteri (14). The increase in these parameters observed with the time elapsed since the prolapsed uteri in this study is thought to be related to pain and secondary infections resulting from trauma to the external organ. An acute inflammatory response characterized by elevated WBC values frequently occurs in cases of uterine prolapse. This spike may be related to the stress caused by the prolapsed uteri or increased cortisol levels (15), or it may be explained by the ease of access of infectious agents to the uterus exposed to the external environment (16).

Cardiac troponins are considered reliable biomarkers for the detection of myocardial damage, particularly diseases that cause cardiac damage, in both humans (17) and animals (18). Cardiac troponin I (cTnI) is a structural and regulatory protein found in cardiomyocytes, and its increase in circulation is a highly sensitive indicator of myocardial damage in both humans and animals (19). Elevated values of specific (cTnI) and nonspecific (AST, CK-MB, and LDH) cardiac biomarkers in sheep affected by uterine prolapse indicate possible myocardial damage. In the present study, it was determined that cTnI, CK-MB, AST, and LDH concentrations were higher in sheep with prolapsed uterus compared to sheep that gave birth vaginally, and these values increased with the time elapsed since prolapse. These findings suggest cardiac damage due to circulatory compromise resulting from ventral torsion of the prolapsed uterus. Furthermore, trauma and secondary infections due to externalization of the uterus are thought to exacerbate the increase in cardiac parameters over time. Similarly, changes in cTnI, CK-MB, and LDH levels have been reported in sheep with torsion uteri (20). Increased serum cTnI concentrations were also found to be higher in sheep with experimental acute myocardial ischemia (21) and in lambs with myocarditis compared to healthy lambs (22). The higher AST and LDH levels in the prolapsed uterine groups in this study may be due to the sustained muscle activity of the uterus and skeletal muscles resulting from the prolonged prolapse of the uterus from the vulva (23, 24). Furthermore, the increase in these enzymes may be explained by leakage resulting from cellular damage following maximum muscle exertion during uterine torsion (25).

Biochemical markers of oxidative stress are sensitive indicators of tissue damage that can be detected much earlier than histological changes (26). Direct measurement of reactive oxygen species (ROS), with the exception of hydrogen peroxide, is difficult due to their high reactivity and short half-life (27). MDA is the end product of ROS-induced lipid peroxidation in the cell membrane and is one of the most widely used biomarkers of oxidative stress (28). Erişir et al. (29) reported a significant increase in plasma MDA concentrations in cows with a prolapsed uterus in their study evaluating these animals. In the present study, consistent with the literature, MDA levels were found to be higher in sheep with a prolapsed uterus compared to healthy sheep, and this increase continued with the time elapsed since the prolapse. Birben et al. (30) suggest that this increase in MDA is a reflection of increased oxidative stress and deficient antioxidant defense mechanisms, which in turn indicate cell and tissue damage. Furthermore, MDA and troponin I levels have been reported to be strongly associated with acute myocardial infarction and unstable angina, reflecting both oxidative stress and cardiac cell death (31). This finding is

considered significant evidence confirming that uterine prolapse causes cardiac damage in sheep.

Superoxide dismutase and catalase are the primary antioxidant enzymes that protect cells from oxidative stress by neutralizing ROS. Their coordinated activity plays a critical role in preventing cellular damage, particularly in cases of oxidative damage. In the present study, it was determined that SOD and CAT concentrations were lower in sheep with a prolapsed uterus compared to the control group, and this decrease worsened with time since the prolapse. This reduction is thought to contribute to the increased cardiac damage in sheep with prolapsed uterus. SOD converts superoxide radicals to hydrogen peroxide, while CAT prevents the accumulation of harmful ROS by decomposing the resulting hydrogen peroxide into water and oxygen. However, if SOD activity is not supported by sufficient CAT, the accumulated hydrogen peroxide is converted to highly toxic hydroxyl radicals via the Fenton reaction, which can exacerbate cardiac damage (32).

Inflammatory mediators such as IL-6 and TNF-α are acute-phase proteins that participate in the inflammatory response. Although the liver primarily synthesises these factors, adipose tissue also serves as a significant source (33). In the present study, TNF-α and IL-6 levels were significantly increased in sheep affected by uterine prolapse, and it was evaluated that this increase may be related to a myocardial depressant effect. TNF-α release can increase endothelial permeability at the level of myocardial cells, leading to cytoplasmic cTnI leakage without cardiomyocyte necrosis (34). Similarly, increases in TNF-α and IL-6 levels have been reported in sepsis and trauma patients (35, 36). These findings confirm that the increase in cTnI observed in sheep with prolapsed uteruses in our study is associated with an increase in TNF-α and IL-6 levels.

When blood flow is interrupted, free radicals cause significant tissue damage (37). Following reperfusion, the formation of large amounts of oxygen-derived free radicals causes tissue damage, and systemic complications can occur along with inflammatory responses (10). In the presented study, a prolapsed uterus, twisted ventrally and disrupted by blood flow, was evaluated as an ischemia model. In the literature review, no similar studies were found in ruminants, except for the manual correction of uterine torsion in buffaloes by Mahmoud et al. (38). Mahmoud et al. (38) performed measurements on the fifth day after treatment and reported improvements in some clinical and biochemical parameters, but they did not evaluate any cardiac damage. In contrast, the current study, instead of long-term follow-up, revealed the effects of proinflammatory cytokines accumulated in the restored uterus and the passage of inflammatory cells into the systemic circulation on cardiac damage using measurements taken 4 hours after treatment. Studies in laboratory animals have also reported increased serum cardiac injury markers (LDH, CK-MB, cTnI), increased oxidative and proinflammatory parameters (MDA, IL-6), and decreased antioxidant enzymes (SOD, CAT) following testicular and ovarian torsion, leading to cardiac dysfunction (39, 40). Consistent with the literature, the observed deterioration in measured parameters after treatment for uterine prolapse in the present study confirms the development of reperfusion injury. Furthermore, tissue damage during reperfusion is known to be more severe than that occurring during ischemia (Liu et al., 2007). The severity of ischemia-reperfusion injury depends on the balance between oxygen-derived free radicals and antioxidant defense systems (41).

The current study has several limitations. First, a small number of sheep was included in each group. The time course of uterine torsion was based on the history obtained from the producers. This may not accurately reflect the actual time of labor or the time elapsed since uterine prolapse. Furthermore, after uterine replacement, the sheep were observed and evaluated only briefly (4 hours) in the hospital. The short duration prevented us from assessing the long-term effects of ischemia/reperfusion. Therefore, changes in serum cTnI and other parameters in the days following uterine correction and completion of labor could not be assessed. Furthermore, due to the limited number of comparable studies on I/R damage in sheep, the discussion section focuses primarily on studies conducted on humans and laboratory animals.

This study found increases in serum cTnI concentrations in sheep with uterine prolapse. Furthermore, it was demonstrated that myocardial damage increased with increasing time since uterine prolapse, and ischemia/reperfusion injury became more pronounced after treatment. These findings demonstrate the critical importance of rapid intervention to minimize cardiac damage in cases of uterine prolapse. Further studies are needed to elucidate the potential mechanisms contributing to cTnI release from the myocardium into the circulation and to determine whether the myocardial damage caused by uterine prolapse is reversible or permanent. It is believed that future studies will increase treatment success by increasing the number of animals per group, extending the follow-up period to at least 7–14 days, and developing additional treatment protocols by investigating the effects of antioxidant or anti-inflammatory agents aimed at preventing or reducing damage to the heart and other organs after various types of torsion and prolapse.

Acknowledgements

This study was carried out based on the permission of the Harran University Animal Experiments Local Ethics Committee (HRÜ-HADYEK) and numbered 2025/002.

Declaration of interest

The authors have no conflict of in-terest to disclose

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