Original Article

Use of Certain Cardiac Biomarkers in Neonatal Calves With Acute Respiratory Distress Syndrome

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ABSTRACT

Background: Acute respiratory distress syndrome (ARDS), characterized by heightened respiratory effort, impaires oxygen exchange, and consequent hypoxia, is a leading cause of mortality in calves. Hypoxia associated with respiratory distress compromises cellular oxygen delivery and reduces tissue perfusion, thereby adversely affecting cardiac function.

Objectives: This study aimed to evaluate cardiac injury and the compressive stress load induced by hypoxia in neonatal calves with ARDS, thereby offering insights into potential management strategies.

Methods: Out of 68 neonatal calves presented to the animal hospital for diagnosis, treatment, or routine examination, 25 were included in this study (15 in the ARDS group, 10 in the healthy group). After clinical examinations, arterial blood samples were collected from all animals for ARDS diagnosis. In contrast, venous blood samples were obtained for cardiac troponin I (cTnI) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) measurements.

Results: Results indicated that arterial pH, partial pressure of oxygen, and oxygen saturation levels in calves with ARDS were significantly lower compared to healthy ones, whereas partial pressure of carbon dioxide and lactate levels were significantly elevated. Serum cTnI levels were significantly higher in calves with ARDS compared to healthy calves, while NT-proBNP levels were elevated, though not statistically significant. Although NT-proBNP did not demonstrate statistical significance in diagnosing ARDS, cTnI showed significant diagnostic value for ARDS, with an optimal cut-off value of 86.61 pg/mL (sensitivity: 72.7%, specificity: 100%).

Conclusion: Cardiac damage, as indicated by elevated cTnI levels, occurs in neonatal calves with ARDS. Although NT-proBNP levels are not significantly elevated, suggesting a lack of severe ventricular stress, cTnI levels show strong diagnostic relevance in identifying cardiac impairment.

Keywords: Calf, Cardiac troponin I (cTnI), Respiratory distress, Neonatal, NT-proBNP

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Introduction

Respiratory diseases, which contribute to treatment costs, impaired growth, and increased mortality (Beheshtipour & Raeeszadeh, 2020), are among the leading causes of calf deaths and significant economic losses (Sasaki et al., 2022). In newborn calves, the most prevalent respiratory conditions include postnatal hypoxia-hypercapnia, pulmonary hypertension, aspiration pneumonia, as well as bacterial and viral pneumonias (Valles, 2010).

Acute respiratory distress syndrome (ARDS) is a clinical syndrome of diffuse lung inflammation and oedema that commonly causes acute respiratory failure. ARDS can be precipitated by several causes, including both infectious and non-infectious triggers; these triggers can injure the lung directly through local inflammation or indirectly through systemic mediators of inflammation and injury (Bos & Ware, 2022). Respiratory distress syndrome (RDS) and its associated complications are the primary causes of calf mortality. In calves, RDS is characterized by increased respiratory effort, insufficient oxygen exchange, and resulting hypoxia (Ider et al., 2023; Bleul, 2009). Hypoxia resulting from respiratory distress impairs oxygen delivery to cells and reduces tissue perfusion, thereby significantly impacting cardiac function (Hanedan et al., 2015; Aydogdu et al., 2016). Therefore, cardiac biomarkers can be widely used to monitor and diagnose cardiac disorders in both humans and animals. Of these, cardiac troponin I (cTnI) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) are critical biomarkers for the early detection of cardiac disorders in calves (Hanedan et al., 2015; Aydogdu et al., 2016; Ayvazoğlu et al., 2024; Değirmençay, 2023).

The interaction between the heart and lungs within the thoracic cavity is well recognized during both spontaneous and mechanical ventilation. Dysfunction in one of these organs can significantly impact the function of the other. It is well known that ARDS is associated with cardiac subphenotypes that may influence mortality risk. Additionally, hypoxia linked to ARDS can result in pulmonary hypertension and right ventricular dysfunction. Furthermore, inflammatory biomarkers and infection can directly injure the myocardium in cases of ARDS secondary to infectious causes. Understanding the cause-and-effect relationship between ARDS and cardiac dysfunction is essential for effective management (Zainab et al., 2023). While previous studies have examined cardiac injury in premature and weaned calves with respiratory distress, the extent of cardiac injury and or compressive stress due to hypoxia in neonatal calves with ARDS remains unknown. This study aimed to assess cardiac injury and compressive stress load potentially induced by hypoxia in neonatal calves with ARDS and to provide insights into effective management strategies.

Materials and Methods

Study animals

Between February and October 2024, 25 out of 68 neonatal calves presented to the Animal Hospital at Harran University, Faculty of Veterinary Medicine, Eyyubiye Campus, Şanlıurfa, Türkiye, for diagnosis, treatment, and or routine examination were included in this study based on predefined inclusion criteria. Upon admission, a detailed history was obtained from the farmer or breeder, covering the type and duration of symptoms. Clinical examinations were subsequently conducted, and the necessary biological samples were collected.

Clinical examinations and blood sampling

The clinical examination included auscultation of the lungs and heart, palpation of lymph nodes, evaluation of mucosal membranes, and measurement of pulse, respiratory rate, and rectal body temperature. Venous blood samples (5 mL) were collected from all calves via jugular venipuncture following standard aseptic techniques. The samples were transferred into serum separation tubes for measurements of cardiac injury and compressive stress load biomarker concentrations. Additionally, arterial blood samples (1 mL) were obtained by puncturing the caudal auricular artery, as described by Nagy et al. (2001). After sample collection, it was ensured that no air bubbles were present and that the anticoagulant was neither insufficient nor excessive. The specimens were then analyzed immediately.

Arterial blood gas analyses

Arterial blood pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), and oxygen saturation (SaO_2) were measured in the central laboratory of the animal hospital using an automatic blood gas analyzer (epoc® Blood Analysis System, Siemens, Germany).

Diagnostic criteria for ARDS

The criteria for the presence of ARDS included hypoxia (PaO_2 <60 mm Hg), respiratory acidosis, hypercapnia ($PaCO_2$ >45 mm Hg), tachypnea (respiratory rate >45 breaths/min), and wheezing with abdominal respiration (Bleul, 2009). Among these parameters, the presence of at least two criteria, along with PaO_2 <60 mm Hg, was considered indicative of ARDS.

Formation of research groups

The ARDS group (n=15) consisted of calves diagnosed with ARDS based on clinical examinations and arterial blood gas analyses, with no other underlying diseases. The healthy group (n=10) included calves that did not have ARDS or any other disease and were brought in for routine health checks. Premature calves, identified through anamnesis and insemination date data, were excluded from the study. Calves with congenital anomalies, diarrhea, or those receiving any treatment were also excluded. Furthermore, calves diagnosed with gastrointestinal, decubitus, or umbilical infections during the clinical examination were excluded from the study. Thus, 25 of the 68 calves examined according to the study's inclusion and exclusion criteria were included, and research groups were formed.

Biomarker analyses

Venous blood samples were centrifuged at 2500× g for 10 min. The serum was then separated, transferred to Eppendorf tubes, and stored at -80 °C for approximately 5 months until biomarker analysis. The concentrations of cTnI and NT-proBNP were measured in the serum samples to assess cardiovascular involvement, following the manufacturer's instructions (Bovine cTn-I ELISA Kit, Sandwich ELISA, Double Antibody, Wuhan Fine Biotech®, China; NT-proBNP ELISA Kit, Sandwich ELISA, Double Antibody, Wuhan Fine Biotech®, China).

Statistical analysis

Statistical analysis was performed in SPSS software, version 25.00 (Windows®, USA). A sample Kolmogorov-Smirnov test was used to investigate the distribution of the data. Parametric data were presented as Mean±SEM, and nonparametric data as median and range (min-max). Data analysis for the two groups was performed using one-way ANOVA or the Kruskal-Wallis test, depending on data distribution. Receiver operator characteristic (ROC) analysis, with the area under the curve (AUC), was also performed to examine the diagnostic efficacies of serum cTnI and NT-proBNP concentrations. The optimal cut-off values were those with the fewest misclassifications, and for these, sensitivity and specificity were determined. Within the scope of ROC analysis, it was accepted that an AUC of 0.5 suggests no discrimination (i.e. the ability to diagnose calves with and without the disease or condition based on the test), 0.6–0.8 acceptable, 0.8–0.9 excellent, and >0.9 outstanding (Hosmer & Lemeshow, 2000). A $P<0.05$ was considered statistically significant in all analyses.

Results

Clinical examination findings

Anamnestic data indicated that all calves in the ARDS group exhibited symptoms for 2 to 3 days (2.33±0.11). Furthermore, all calves included in this study, which were housed in individual pens, were naturally born on the farm (gestation >280 d). The body weights of the calves in both groups were similar ($P>0.05$). Clinical examinations revealed that heart rate, respiratory rate, and body temperature were significantly higher in calves with ARDS compared to healthy calves ($P<0.001$). Additionally, some calves with ARDS (7 out of 15) exhibited sinus arrhythmia on cardiac auscultation, along with abnormal lung findings such as crackles and wheezing (Khanamir et al., 2020). The clinical examination findings are presented in Table 1.

Arterial blood gas analysis findings

Statistically significant differences were observed in pH, $PaCO_2$, PaO_2, SaO_2, and lactate levels. Specifically, pH, PaO_2, and SaO_2 levels in calves with ARDS were significantly lower compared to the healthy calves ($P<0.05$, $P<0.001$, $P<0.001$, respectively), while $PaCO_2$ and lactate levels were significantly higher ($P<0.001$). The results of arterial blood gas analyses are presented in Table 2.

Biomarker analysis

Serum cTnI levels of calves with ARDS were statistically significantly higher than those of healthy calves ($P<0.005$). In comparison, NT-proBNP levels were numerically higher; no statistically significant difference was detected ($P=0.081$). The results of cardiac biomarker measurement are presented in Table 3.

The Spearman correlation analysis revealed a strong positive correlation between cTnI and NT-proBNP ($r=0.831$). No statistically significant correlations were found between cardiac biomarkers and arterial blood gas parameters. However, significant correlations were observed among arterial blood gas parameters (Table 4).

Table 1. Comparison of clinical findings between study groups

Parameter	Median (Min-Max)		P
	Healthy Group (n: 10)	ARDS Group (n: 15)	
Body weight (kg)	48 (42-55)	47 (34-54)	0.163
Heart rate (beats/min)	99 (86-120)	150 (78-178)	0.000
Body temperature (°C)	38.55 (37.6-39.4)	40 (38.8-41.2)	0.000
Respiratory rate (breaths/min)	42 (36-55)	70 (52-90)	0.000

ADRS: Acute respiratory distress syndrome.

ROC analysis revealed that NT-proBNP did not achieve statistical significance for diagnosing ARDS (P=0.121). In contrast, cTnI was statistically significant for diagnosing ARDS (P<0.05; AUC=0.818), with a cut-off value of 86.61 pg/mL (sensitivity: 72.7%; specificity: 100%). The ROC-based diagnostic efficacy analysis results are presented in Table 5 and Figure 1.

Discussion

This study assessed arterial blood pH, $PaCO_2$, PaO_2, SaO_2, lactate, and venous cTnI and NT-proBNP levels in neonatal calves with and without ARDS. The results indicated that pH, PaO_2, SaO_2, and cTnI levels were significantly decreased, whereas $PaCO_2$ and lactate levels were elevated in calves with ARDS compared with healthy calves. However, NT-proBNP levels were the same between the groups. These findings indicate that cTnI is a valuable biomarker for diagnosing cardiac involvement in neonatal calves with ARDS. It was concluded that severe ventricular stress was absent in calves with ARDS, as indicated by NT-proBNP levels, likely because of the higher prevalence of sinus arrhythmia than atrial fibrillation, which can enlarge the left atrium in these calves (Savarese et al., 2016). However, cardiac injury was evident, likely resulting from decreased tissue perfusion due to hypoxia, as reflected by the elevated cTnI levels. Therefore, understanding the role of cardiac abnormalities in ARDS may offer valuable insights into optimizing effective treatment strategies.

Evidently, 30% of livestock losses and economic damage are attributable to ruminant diseases, with respiratory diseases among the most significant contributors to economic losses worldwide. Additionally, approximately 90% of calf losses on farms are due to diarrhea and pneumonia, highlighting the need to prioritize these two conditions (Şentürk, 2015). Respiratory diseases are among the leading causes of calf mortality, with common respiratory conditions in newborn calves, including postnatal hypoxia-hypercapnia, pulmonary hypertension, aspiration pneumonia, as well as bacterial and viral pneumonias. Respiratory distress is recognized as a central factor underlying these conditions (Valles, 2010). Persistent hypoxia has been reported in calves suffering from respiratory diseases that cause respiratory distress,

Table 2. Comparison of arterial blood gas analyses between study groups

Parameter*	Mean±SEM		P
	Healthy Group (n: 10)	ARDS Group (n:15)	
pH	7.38±0.03	7.23±0.18	0.006
$PaCO_2$ (mm Hg)	27.04±5.4	56.22±11.95	0.000
PaO_2 (mm Hg)	86.12±4.42	24.46±7.87	0.000
SaO_2 (%)	94.03±4.54	34.77±15.1	0.000
Lactate (mmol/L)	0.59±0.28	8.15±3.5	0.000

Abbreviations: ADRS: Acute respiratory distress syndrome; $PaCO_2$: Partial pressure of carbon dioxide; PaO_2: Partial pressure of oxygen; $SatO_2$: Oxygen saturation.

*Measured from arterial blood samples.

Table 3. Comparison of cardiac biomarkers between study groups

Parameter*	Mean±SEM		P
	Healthy Group (n:10)	ARDS Group (n:15)	
cTnI (pg/mL)	54.66±21.45	174.56±131.94	0.013
NT-proBNP (pg/mL)	39.21±28.07	94.76±92.27	0.081

Abbreviations: ADRS: Acute respiratory distress syndrome; cTnI: Cardiac troponin I; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.

*Measured from venous blood samples.

Table 4. Correlations of arterial blood gas analyses with cardiac biomarkers in study groups

Parameters	pH	$PaCO_2$	PaO_2	SaO_2	Lac	cTnI	NT-proBNP
pH	1.000	-0.53**	0.389	0.615**	-0.723**	-0.044	0.051
$PaCO_2$		1	-0.76**	-0.806**	0.808**	0.225	0.04
PaO_2			1	0.886**	-0.713**	-0.368	-0.184
$SatO_2$				1	-0.754**	-0.331	-0.179
Lac					1	0.268	0.14
cTnI						1	0.831**
NT-proBNP							1

Abbreviations: ADRS: Acute respiratory distress syndrome; $PaCO_2$: Partial pressure of carbon dioxide; PaO_2: Partial pressure of oxygen; SaO_2: Oxygen saturation; Lac: Lactate; cTnI: Cardiac troponin I; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.

**Correlation is significant at the 0.01 level (2-tailed).

such as pneumonia and aspiration pneumonia (Poulsen & McGuirk, 2009). In a case report, a calf with bronchopneumonia was documented to develop hypoxia (PO_2=21.8 mm Hg) and associated respiratory acidosis (Souza et al., 2018). In another study on 30 calves with aspiration pneumonia, hypoxia due to respiratory distress was observed (Akyüz et al., 2022). A study of 128 calves with respiratory disease categorized them by symptom severity (mild, moderate, severe) and compared their oxygen levels with those of healthy calves, revealing significantly lower mean oxygen levels than those of the healthy group (Soltesova et al., 2015). A recent study found that arterial blood gas analysis of 24 calves with aspiration pneumonia revealed reduced oxygen levels compared to healthy calves (Gülersoy et al., 2023). Reduced oxygen levels in calves with ARDS have been associated with clinical signs such as tachycardia, tachypnea, weakness, lethargy, mild to moderate

Table 5. ROC-based diagnostic efficiency of cardiac biomarkers in detecting the presence of ARDS

Parameters	AUC	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval		Cut-off	Sensitivity	Specificity
				Lower Bound	Upper Bound			
cTnI (pg/mL)	0.818	0.103	0.014	0.617	1	86.61	72.7%	100%
NTproBNP (pg/mL)	0.7	0.125	0.121	0.455	0.945	49.55	72.7%	80%

Abbreviations: cTnI: Cardiac troponin I; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; Std. Error: Standard error; Asymptotic Sig.: Asymptotic significance.

Note: Under the nonparametric assumption, bNull hypothesis: True area=0.5.

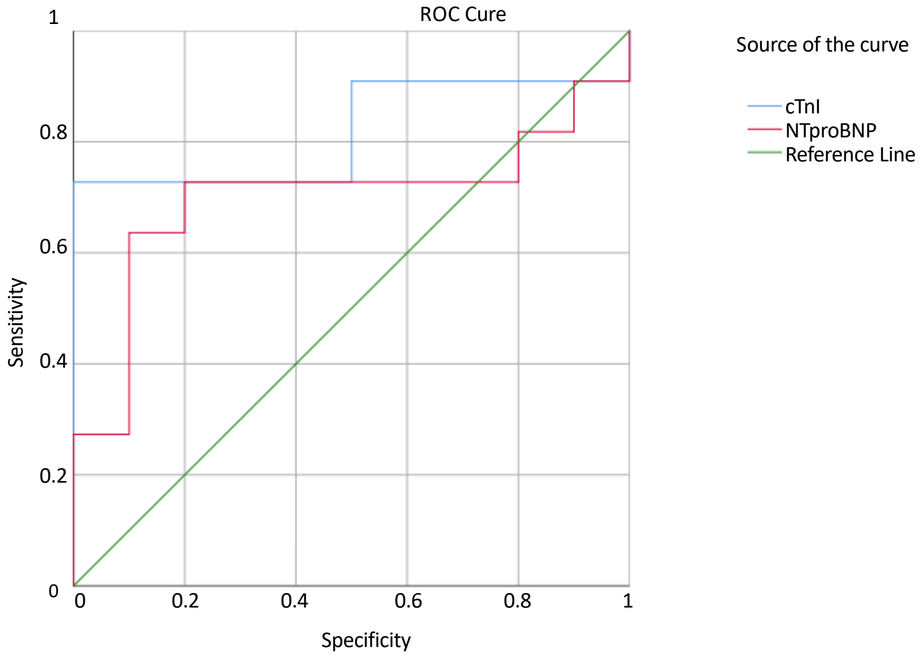

Figure 1. ROC curve showing the diagnostic efficacy of cardiac biomarkers for detecting ARDS

cyanosis, and hyperthermia (Ider et al., 2021; Gülersoy et al., 2023; Bleul, 2009). In this study, the clinical findings in calves with ARDS—specifically tachycardia, tachypnea, and hyperthermia—were consistent with and supported by similar findings in previous studies (Hanedan et al., 2015; Gülersoy et al., 2023) which are due to alveolar–capillary barrier injury with interstitial and alveolar oedema formation as a result of reduced oxygen levels (Bos & Ware, 2022).

Neonatal hypoxic ischemia is commonly associated with cardiovascular disorders (Aydogdu et al., 2016) and is believed to cause myocardial damage secondary to hypoxic ischemia (Sweetman et al., 2012). Numerous studies have demonstrated that myocardial damage develops in infants with perinatal asphyxia and RDS (Tapia-Rombo et al., 2000; Trevisanuto et al., 2000a, Trevisanuto et al., 2000b; Szymankiewicz et al., 2006). In a study of premature calves, all those with RDS were hypoxic, as evidenced by low PO_2 and SaO_2, suggesting that hypoxia led to myocardial damage (Aydogdu et al., 2016). Similarly, research on weaned calves with bovine respiratory disease (BRD) reported elevated cTnI levels, indicating that hypoxia-induced myocarditis may occur in calves with BRD (Hanedan et al., 2015). No specific biomarkers exist for diagnosing myocardial injuries in bovine medicine. While creatine kinase, lactate dehydrogenase, and their isozymes can detect myocardial damage in humans and animals, their usefulness is limited by low tissue specificity and sensitivity. Cardiac troponins (I or T) are the most reliable biomarkers, offering nearly absolute myocardial specificity and greater sensitivity than creatine kinase, lactate dehydrogenase, isozymes, and myoglobin (O'Brien, 2008). In the present study, cTnI levels were assessed in non-premature, unweaned neonatal calves with ARDS, and a significant increase in cTnI levels was observed ($P<0.05$), in line with previous findings. These results indicate that myocarditis may occur in calves of different ages and due to various causes of hypoxia (Aydogdu et al., 2016; Hanedan et al., 2015), suggesting that neonatal calves with ARDS are also at risk of developing myocarditis. In addition, since serum cTnI levels increase early in myocardial tissue damage due to both ischemic and non-ischemic injury, resulting from the degradation of contractile proteins, the increase in calves with ARDS in this study may therefore be attributed to non-ischemic damage (Solís et al., 2018).

Natriuretic peptides play a crucial role in regulating fluid balance and blood pressure by inhibiting the renin-angiotensin-aldosterone system. Released in response to increased blood volume or vasoconstriction, they promote cardiac relaxation and dilation, as well as vasodilation, diuresis, and natriuresis (Rao et al., 2021). B-type natriuretic peptides, primarily produced by ventricular myocytes in response to ventricular dysfunction, hold diagnostic, therapeutic, and prognostic significance in critically ill patients. Brain natriuretic peptide (BNP) and

NT-proBNP, specifically, have been proposed as key biomarkers for diagnosing and predicting the progression of heart failure (McMurray et al., 2012). In summary, while troponins are highly sensitive and specific markers for detecting myocardial damage, natriuretic peptides provide valuable prognostic and diagnostic insights into cardiac dysfunction (Aygun & Yildiz, 2018). Currently, NT-proBNP is the most commonly used natriuretic peptide in veterinary medicine (Değirmençay, 2023). Although NT-proBNP levels have been extensively studied in neonatal calves with sepsis (Ayvazoğlu et al., 2024; Beydilli & Gökçe, 2020; Phipps, 2024), research concerning their levels in respiratory diseases remains limited. In a study on cattle with BRD, NT-proBNP levels were significantly elevated compared to healthy controls, suggesting it may be an important biomarker for diagnosing this disease (Değirmençay, 2023). The study concluded that pulmonary hypertension due to hypoxia likely contributed to these increased NT-proBNP levels. However, arterial blood samples were not analyzed to confirm hypoxia, and arterial oxygen saturation was not evaluated, limiting the scientific validity of associating increased NT-proBNP with hypoxia. This study marks the first evaluation of NT-proBNP in neonatal cases of ARDS. Although cTnI levels were significantly elevated, NT-proBNP levels did not show a statistically significant increase (P=0.081). This suggests that while secondary myocarditis may occur in neonatal calves with ARDS due to hypoxia (Sweetman et al., 2012), the level of pulmonary hypertension, including pulmonary artery hypertension (Naseri et al., 2020), and ventricular compressive stress from hypoxia may not reach a threshold that would impact NT-proBNP levels. Despite the lack of a statistically significant rise in NT-proBNP among ARDS-affected calves compared to the healthy ones, a strong positive correlation was found between NT-proBNP and cTnI levels, with cTnI showing a statistically significant increase (R=0.831). This finding indicates that myocarditis secondary to hypoxia in calves with ARDS may potentially increase ventricular stress or could initiate myocarditis when ventricular stress reaches a sufficient level.

Additionally, the ROC analysis showed that the cTnI biomarker is a valuable diagnostic tool for distinguishing healthy calves from those with ARDS (P=0.014, AUC=0.818, sensitivity: 72.7%, specificity: 100%, cut-off: 86.61 pg/mL). Although NT-proBNP was identified as a significant diagnostic tool in cattle with BRD in a previous study (P<0.001, AUC=0.975), our findings showed it was not statistically significant for diagnosing ARDS in calves (P=0.121, AUC=0.700). This discrepancy may be attributed to differences in disease severity and exposure duration in the subjects of the two studies. In this study, calves with ARDS were in the acute phase of the disease, while the earlier study did not differentiate between acute and chronic cases. It is known that the severity of pulmonary hypertension can vary with the disease process (Naseri et al., 2020), potentially leading to divergent diagnostic outcomes between studies. Future studies could mitigate these differences by categorizing cases by acute or chronic disease stage, thereby enhancing the consistency and reliability of diagnostic assessments.

The limitations of this study include the small sample size and the lack of electrocardiography, echocardiography, and additional biomarker evaluations to assess cardiac damage. Thus, future studies should include a larger sample and incorporate these examinations to provide a more comprehensive assessment.

Conclusion

This study demonstrated that neonatal calves with ARDS experience cardiac muscle damage, as indicated by elevated cTnI levels. The absence of a statistically significant elevation in NT-proBNP levels suggests that severe ventricular stress was not present. Moreover, cTnI appears to be a highly specific marker for detecting cardiac injury in ARDS. Recognizing the role of cardiac dysfunction in ARDS is crucial, as it helps clinicians assess mortality risk in BRD cases and optimize treatment strategies. Future studies comparing calves with ARDS to those with chronic RDS could provide valuable insights into disease pathophysiology and the prognostic value of cardiac biomarkers.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Experiments Local Ethics Committee of Harran University, Şanlıurfa, Türkiye (Code: 15.01.2024, 2024/001/008 – 01-14).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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