



RESEARCH ARTICLE

Comparison of blood gases, hematological and monitorization parameters and determine prognostic importance of selected variables in hypotensive and non-hypotensive calves with sepsis

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Hipotansif ve hipotansif olmayan sepsisli buzağlarda kan gazları, hemotolojik ve monitarizasyon parametrelerinin karşılaştırılması ve seçilmiş değişkenlerin prognostik öneminin belirlenmesi

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Öz

Amaç: Sunulan çalışmada hipotansif ve hipotansif olmayan sepsisli buzağlarda kan gazı, hemogram ve monitorizasyon parametrelerini karşılaştırmak ve mortalite tahmininde olası prognostik değişkenleri belirlemek amaçlanmıştır.

Gereç ve Yöntem: Araştırmaya sepsis kriterlerini taşıyan 22 adet buzağı dahil edildi. Hipotansiyon için sistolik kan basıncının (SBP) <90 mmHg ve / veya ortalama arteriyel kan basıncının (MAP) <65 mmHg kriterleri göz önünde bulunduruldu.

Bulgular: Çalışma sırasında 7 (%58) hipotansif ve 4 (%40) hipotansif olmayan buzağı öldü. Klinik olarak hipotansif buzağların vücut ısısı, SBP ve MAP düzeyleri hipotansif olmayanlara göre daha düşüktü. Hipotansif buzağların glikoz düzeyleri hipotansif olmayan buzağlara göre önemli oranda düşük, RBC ve HCT düzeyleri yüksekti. Ölen buzağların venöz pO₂ ve SO₂ düzeyleri hayatta kalan buzağlara göre daha düşük, laktat düzeyleri daha yüksekti. ROC analizi sonucunda; eğri altında kalan alan (AUC) 0,991 (% 95 güven aralığı (CI): 0.962-1.000; p=0,000), % 100 sensitivite ve % 99 spesifite ile laktat düzeyinin 6,7 mmol/L Cut-off değeri mortalite tahmini açısından en iyi prognostik gösterge olarak bulundu.

Öneri: Sonuç olarak, mortalite oranı hipotansif buzağlarda hipotansif olmayan buzağlara göre daha yüksektir. Bulgularımız, septik buzağlarda mortalitenin en güçlü belirleyicisinin doku hipoksisi olduğunu gösterdi. % 100 sensitivite ve % 99 spesifite ile 6.7 mmol/L Cut-off değerinde laktat düzeyleri sepsisli ölen ve hayatta kalan buzağları ayırt etmede en iyi prognostik göstergedir.

Anahtar kelimeler: Laktat, sepsis, buzağı, hipotansiyon, doku hipoksisi

Abstract

Aim: The aim of the present study was to comparison of blood gases, hematological and monitorization parameters in hypotensive and non-hypotensive calves with sepsis and also, determine the probably prognostic variables to prediction mortality.

Materials and Methods: Twenty-two calves were met the criteria of sepsis were enrolled in the study. Hypotension was defined as presence of systolic blood pressure (SBP) <90 mmHg and/or mean arterial pressure (MAP) <65mmHg.

Results: During study 7 (58%) hypotensive and 4 (40%) non-hypotensive calves died. Clinical findings showed that hypotensive calves had a lower body temperature, SBP and MAP than hypotensive calves. Glucose levels were significantly lower, and RBC and HCT levels were higher in hypotensive calves than non-hypotension calves. Venous pO₂ and SO₂ were lower and lactate was higher in non-survivor calves than survivor calves. The findings of receiver operating characteristic curve (ROC) showed the area under the curve (AUC) of 0.991 (p=0.000, 95% CI=0.962-1.000), sensitivity of 100% and a specificity of 99% for prediction of mortality at optimum cut off point of 6.7 mmol/L propound the lactate as best prognostic indicator.

Conclusion: Mortality rate in hypotensive calves is higher than non-hypotensive calves. Our findings indicated that tissue hypoxia is the strongest determinant of mortality in septic calves. Blood lactate levels with sensitivity of 100% and specificity of %99 at cut-off point of 6.7 mmol/L is the best prognostic indicator to differentiate between survivor and non-survivor calves with sepsis.

Keywords: Lactate, sepsis, calf, hypotension, tissue hypoxia





Introduction

Sepsis is the systemic inflammatory response (SIRS) due to the any microbial infection (viral, bacteria, fungal, etc.), and can lead to extensive clinical problems with significant mortality rate (Martin et al 2003). The exact pathophysiology of sepsis is multifactorial and has not been elucidate until now. Infection triggers both pro- and anti-inflammatory cascades that contribute to the clearance of infection and also, tissue damage and organ dysfunction (Russel 2006, Angus and van der Poll 2013). When infection invade the body, causes a number of profound physiologic responses including abnormal body temperature (fever/hypothermia), anorexia, leukocytosis followed by leukopenia, altered heart rate (tachycardia/bradycardia), reduce cardiac output (also reduction of stroke volume) and blood pressure (systolic and/or mean arterial pressure), changes in hematologic variables, alterations in blood coagulation (hypo- or hyper-coagulation), hyperglycemia followed by profound hypoglycemia, comatose state and death (Constable et al 2016). It is known that the hemodynamic alterations in heart, macrovascular and microvascular levels can be occurred during episodes of sepsis. Previous studies in septic animals revealed cardiovascular changes, including systolic and diastolic alterations of left ventricular (Ince et al 2019) and right ventricular (Akar 2017) function and marked peripheral vasodilation (Naseri et al 2019). Sepsis-related cardiovascular changes can lead to the maldistribution of blood flow and contribute to mortality in septic humans, calves, and puppies (Groeneveld et al 1988, Naseri et al 2019, Ince et al 2019).

In our acknowledge, there is no literature information about comparison of these variables in hypotensive and non-hypotensive calves with sepsis. However, there are some studies about hematological and electrolyte and acid-base distribution in calves with diarrhea (Sen et al 2013, Brar et al 2015), sepsis and septic shock (Naseri et al 2019) and evaluation of prognostic indication of related parameters in calves with various pathological conditions (Yildiz et al 2017). For this reason, the purposes of the present study were to comparison of blood gases, hematological and monitorization parameters in hypotensive and non-hypotensive calves with sepsis and also, determine the probably prognostic variables in the sample population.

Material and Methods

Selection of calves with sepsis

Twenty-two Holstein calves (12 hypotensive; 10 non-hypotensive) admitted to the Large Animal Hospital of the Faculty of Veterinary Medicine, Selcuk University with a history of diarrhea, anorexia and lethargy were included in the study. The all-enrolled calves were met the criteria for sepsis. Sepsis was defined as SIRS plus suspicion of infection that

described in adult humans (Levy et al 2003) and pediatrics (Goldstein et al 2005) and calves (Constable et al 2016, Trefz et al 2017, Naseri et al 2019). For definition of SIRS all calves had two or more of the following abnormalities: abnormal leukocyte count (leukocytosis or leukopenia, or band neutrophils >10%), abnormal body temperature (<38.5 or >39.5 °C), abnormal heart rate (HR) (<100 or >120 beats per minute), and abnormal respiratory rate (RR) (>36 breaths per minute). Hypotension was defined as presence of systolic blood pressure (SBP) <90 mmHg and/or mean arterial pressure (MAP) <65mmHg (Naseri et al 2019). The treatment and monitorization of septic calves were performed in neonatal calves intensive care unit (Naseri et al 2019).

Sample collection

Two mL of blood was collected by jugular venipuncture at the time of admission. For blood gas 1 mL of collected sample was anaerobically transferred into sodium heparin containing plastic syringes and analysis performed immediately. One more mL of the blood was put into the tubes containing K₃EDTA and complete blood count (CBC) analysis was performed immediately.

Blood gases and complete blood count

Venous blood gas analysis which included pH, the partial pressure of carbon dioxide (pCO₂), partial pressure of oxygen (pO₂), oxygen saturation (SO₂), potassium (K), sodium (Na), calcium (Ca), glucose, lactate, base excess (BE), bicarbonate (HCO₃) was performed using an automatic blood gas analyzer (ABL 90 Flex, Radiometer, USA). CBCs including total leukocytes (WBC), lymphocytes (Lym), granulocytes (Gran), monocyte (Mon), erythrocytes (RBC), mean corpuscular volume (MCV), hematocrit (HCT), hemoglobin (Hgb) and platelets (THR) were performed using an automatic cell counter (MS4e, Melet Schlosing Laboratories, France).

Blood pressure measurement

Systolic blood pressure (SBP) and mean arterial pressure (MAP) were measured indirectly using an oscillometric technique (Compact 7, Medical Econet, Germany) using by a cuff on the coccygeal artery and restraining in lateral recumbency (Naseri et al 2019).

Statistical analyses

Because of small sample population nonparametric test (Mann-Whitney U) was used to compare hypotensive and non-hypotensive calves and being stated as median and range. To seek correlations between pO₂, SO₂, lactate, SBP, MAP and body temperature on linear regression analysis and Spearman rank test was used. The prognostic values of pO₂, SO₂ and lactate were evaluated using receiver operating cha-



racteristic (ROC) curve analysis to determine the prognostic cut-off values for the best differentiation between survivor and non-survivor septic calves. Statistical significance was considered as $p < 0.05$ and $p < 0.01$.

Results

The hypotensive and non-hypotensive calves had various ages (median 5, (2-20 days); median 7, (1-15 days), respectively). There were no statistically significant differences in

age between the groups ($p > 0.05$). Five hypotensive and 6 non-hypotensive calves survived and were discharged from the hospital 48 hours after the admission and were followed for the next 7 days by calling the owners. During hospitalization period 7 (58%) hypotensive and 4 (40%) non-hypotensive calves died.

Clinical findings showed that hypotensive calves had a lower body temperature, SBP and MAP than hypotensive calves ($p < 0.05$). The HR and RR did not differ between hypotensive

Table 1. Comparison of selected physiologic, acid-base, and hematologic variables in the hypotensive (n:12) and non-hypotensive calves (n: 10) at admission. Data are presented as median and range in parentheses. Please see text for explanation of abbreviations

Parameters	Hypotension	Non-hypotension	p value
Age (day)	5 (2-20)	7 (1-15)	0.917
HR (bpm)	100 (50-180)	132 (60-190)	0.148
RR (breath/min)	36 (16-70)	28 (12-120)	0.219
Temp (°C)	35.10 (33-37.20)	38.40 (35.60-41)	0.001
SBP (mm Hg)	82.50 (73-97)	124 (96-141)	0.000
MAP (mm Hg)	59.50 (46-59)	77 (65-104)	0.000
pH	7.01 (6.80-7.33)	7.12(6.93-7.70)	0.095
pCO ₂ (mm Hg)	46.90 (25-73.10)	44.55 (16-51.10)	0.29
pO ₂ (mm Hg)	23.55 (8-43)	22 (18-45)	0.808
SO ₂ (%)	23.40 (3-84)	33 (14-58)	0.345
K (mmol/L)	7.60 (3.60-9.90)	5.80 (3.40-8.0)	0.058
Na (mmol/L)	138.50 (126-167)	140 (124-151)	1.00
Ca (mmol/L)	0.97 (0.74-1.34)	0.95 (0.38-1.19)	0.754
Glucose (mg/dL)	31 (5-105)	82 (1-156)	0.034
Lactate (mmol/L)	6.95 (0.40-24)	5.90 (0.30-14.70)	0.554
Base excess (mmol/L)	-18.0 (-26.20-8.70)	-12.90 (-28.40-2.40)	0.422
HCO ₃ (mmol/L)	11.40 (5.0-29.80)	13.50 (4.0-27.70)	0.554
WBC (cells/mL)	22.33 (4.83-73.54)	18.83 (9.93-26.99)	0.422
Lym (cells/mL)	9.72 (1.17-81.30)	3.63 (3.30-10.71)	0.069
Mon (cells/mL)	0.62 (0.11-2.28)	0.88 (0.41-1.62)	0.169
Gran (cells/mL)	11.23 (3.55-35.20)	13.12 (5.97-21.19)	0.651
RBC ($\times 10^3$ cells/mL)	10.90 (8.44-15.24)	9.10 (7.32-11.79)	0.041
MCV (fl)	39.10 (31.40-48.0)	37.70 (33.0-43.70)	0.917
HCT (%)	45.50 (31.50-66.20)	29.40 (18.80-49.60)	0.023
Hgb (g/dL)	13.35 (10.40-20.60)	12.60 (7.40-14.80)	0.193
THR (cells/mL)	436 (202-897)	291 (138-455)	0.051



Table 2. Comparison of pO₂, SO₂ and lactate survivor and non-survivor septic calves. Data are presented as median and range in parentheses

Parameters	Survivor	Non-survivor	p value
pO ₂ (mm Hg)	27 (21-45)	18.40 (8-26.60)	0.000
Lactate (mmol/L)	2.20 (0.30-5.90)	11 (5.50-24.0)	0.000
SO ₂ (%)	47.30 (10-84.60)	19.40 (3-58)	0.043

Table 3. The area under the curve (AUC), standard error, confidence interval (95%), optimum cut-off values of pO₂, SO₂, lactate, and respective sensitivity and specificity of mortality prediction in septic calves

Variable	AUC	Standard error	p value	Asymptotic 95% confidence interval		Sensitivity	Specificity	Cut-off value
				Lower band	Upper bound			
pO ₂	0.932	0.055	0.001	0.824	1.000	90	90	22.90
Lactate	0.991	0.015	0.000	0.962	1.000	100	99	6.70
SO ₂	0.764	0.112	0.41	0.546	0.982	81	80	32.85

Table 4. Spearman correlation coefficients of selected variables in 22 septic calves

	pO ₂	Lactate	SO ₂	SBP	MAP	Temp
pO ₂	1	-0.83**	0.718**	-0.12	0.05	-0.08
Lactate		1	-0.67**	0.04	-0.02	-0.01
SO ₂			1	-0.10	0.05	0.15
SBP				1	0.77**	0.62**
MAP					1	0.81**
Temp						1

**p<0.01

and non-hypotensive calves with sepsis (p>0.05) (Table 1). The results also revealed that at the time of admission hypotensive calves were severely ill than the non-hypotensive calves. Higher respiration rate, lower heart rate, severe dehydration, prolonged capillary refill time (CRT), stuporous state were the more characteristic features between the hypoten-

sive and non-hypotensive calves with sepsis. The blood gases analysis showed that the only glucose levels in hypotensive calves were significantly lower than non-hypotension calves (31 (5-105) and 82 (1-156), respectively) (p<0.05). CBC results demonstrated higher levels of RBC and HCT compare to the non-hypotensive calves (p<0.05) (Table 1).



Multivariate regression analysis for mortality showed that venous pO_2 and SO_2 were significantly higher in survivor calves than non-survivor calves ($p < 0.05$). Also, levels of lactate were significantly high in non-survivor calves than survivors ($p < 0.05$) (Table 2). The findings of the ROC analysis for the utility of pO_2 , SO_2 and lactate in differentiating between the survivor and non-survivor calves shown in Table 3. The area under the curve (AUC) of 0.991 ($p = 0.000$, 95% CI = 0.962-1.000), sensitivity of 100% and a specificity of 99% for prediction of mortality at optimum cut off point of 6.7 mmol/L propound the lactate as best prognostic indicator (Table 3)

(Fig 1). Venous pO_2 with area under the curve (AUC) of 0.932 ($p = 0.001$, 95% CI = 0.824-1.000) had sensitivity of 90% and a specificity of 90% for prediction of mortality at optimum cut off point of 22.90 mmHg (Table 3) (Fig 2). And finally, venous SO_2 with area under the curve (AUC) of 0.764 ($p = 0.041$, 95% CI = 0.546-0.982) had sensitivity of 81% and a specificity of 80% for prediction of mortality at optimum cut off point of 32.85 percent (Table 3) (Fig 1). There was negative association between lactate and venous pO_2 and SO_2 . Body temperature had positive association with SBP and MAP. There was no significant correlation between lactate and MAP (Table 4) (Fig 2).

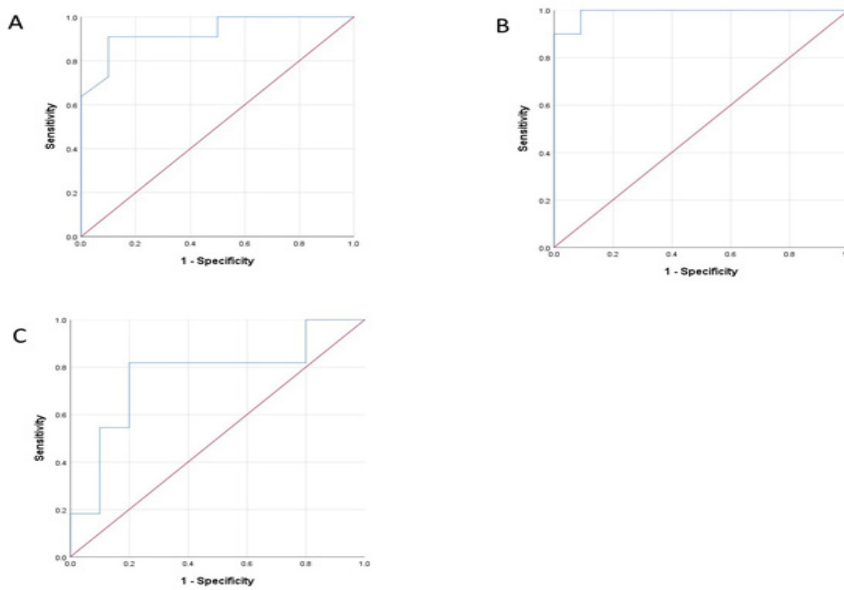


Figure 1. The receiver operating characteristic curve (ROC) for A) partial oxygen pressure (pO_2), B) lactate, and C) oxygen saturation (SO_2). Data shown that lactate are the best predictor of mortality in septic calves.

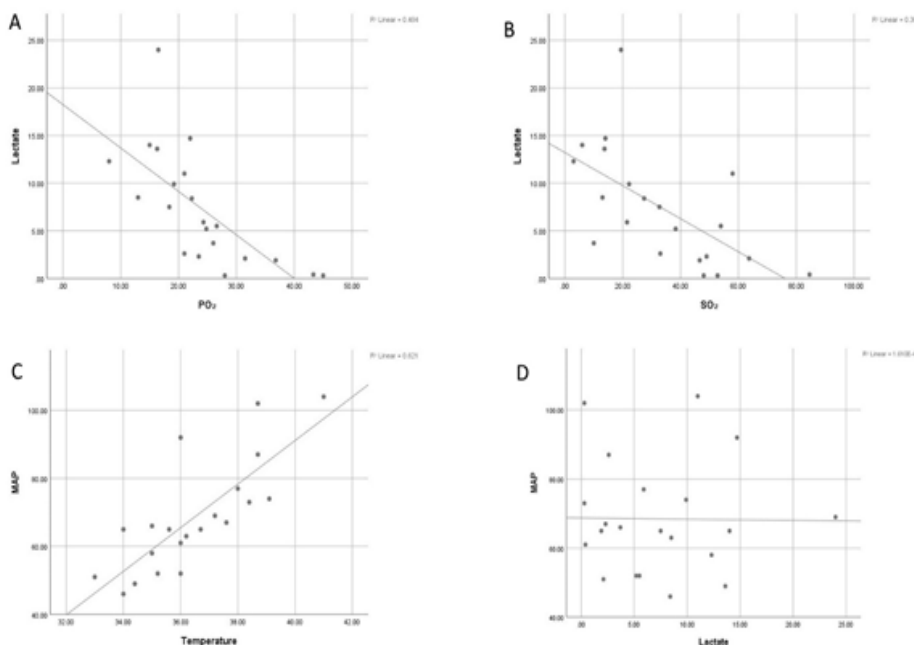


Figure 2. Grouped scatter graphs showed negative association between lactate, venous pO_2 (A) and SO_2 (B). Body temperature had positive correlation with MAP (C). There was no significant association between lactate and MAP (D).





Discussion

In neonatal calves, septicemia generally progress with high mortality and cause serious economic losses. Newborn calves are at great risk for the development of sepsis. Because the protection of calves from infections strongly depends on colostral antibodies. When calves are born in a contaminated environment, virulent pathogens multiply before normal intestinal flora occurs, causing the development of infection. The respiratory tract (inhalation), uterus, umbilical cord, and contaminated colostrum (digestion) are important entry routes for calves (Aldridge et al 1993, Constable 2016).

In newborn calves, sepsis and septic shock can affect the vital organs including central nervous system, hemostatic, metabolic, and cardiovascular systems functions (Irmak et al 2006, Fecteau et al 2009, Basoglu et al 2018, Naseri et al 2019). Decrease systemic vascular resistance is one of the most important cardiovascular system responses to sepsis and principally leads to hypotension. Previous study by Naseri et al (2017) showed that circulatory dysfunction, hypotension (reduce vascular tonicity) and volume depletion (hypovolemia) were the most important cardiovascular system abnormality in calves with sepsis and septic shock. Finding of the present study demonstrated that at time of admission hypotensive calves were severely ill than the non-hypotensive calves. Higher RR, lower HR and body temperature, severe dehydration, prolonged CRT, stuporous state were the more characteristic features between the hypotensive and non-hypotensive calves with sepsis. It is possible to achieve this result that hypotension is the one of most important clinical featured related to severity of illness in calves.

Similar study in septic hypotensive calves showed that body temperature is so lower in calves with sepsis and septic shock than healthy calves and there was no significant change in HR and RR between calves with sepsis and healthy calves (Naseri et al 2019). Parallel to this, our findings showed that body temperature was significantly lower in hypotensive calves than non-hypotensive calves and there was important association between variables of systemic arterial pressure (SBP and MAP) and body temperature. It seems that the over production and release of nitric oxide (NO) by inflammatory cells into the blood stream cause hypotension and can lead to the hypothermia in septic individuals (Pereira et al 2014). Any change in HR despite profound hypotension may be another reason of hypothermia in hypotensive calves (Naseri et al 2019).

Present study showed severe hypoglycemia in hypotensive calves. Trefz et al (2017) showed that severe hypoglycemia (<3.2 mmol/L) was present in 6.3% of calves but it was responded to the mortality rate of closely to the 80%. There were some mechanisms of hypoglycemia that described for cases of sepsis. Increase glucose utilization by liver (Lang and Dobrescu 1991, Maitra et al 2000) and decreased hepatic glucose production can lead to the hypoglycemia (Clemens et al 1983, Maitra et al 2000). The higher rate of hypoglycemia

in hypotensive calves may be explained by severity of illness, metabolic disorders, and prolonged malnutrition (Trefz et al 2017).

Sepsis is associated with altered macrovascular and microvascular hemodynamics and local perfusion, thrombosis, vascular endothelial dysfunction, increase vascular permeability and fluid leakage into the interstitium (De Backer et al 2002, De Backer et al 2014). Persistent fluid leak (persistent preload defect) outside the vascular bed and vasomotor dysregulation can lead to the hypovolemia in septic patients (Ilman 1984, Antonucci et al 2014). Previous study in septic calves showed that severe dehydration at time of admission may be led to the erythrocytosis and increases hematocrit concentration in calves with severe sepsis compare to healthy calves (Naseri et al 2019). In our opinion, increased levels of and RBC and HCT in hypotensive calves may be due to alteration of permeability, fluid loss and dehydration.

In sepsis, damage to the organs is the most important cause of mortality. Studies in dogs and calves have been shown that sepsis-related organ dysfunction can lead to the poor outcome and intensive care unit's mortality (Kenney et al 2010, Basoglu et al 2018). Respiratory and cardiovascular systems are the most commonly involved organs during organ dysfunction. Even though, respiratory dysfunction manifested as the acute respiratory distress syndrome (ARDS) and hypoxemia (Ranieri et al 2012), cardiovascular dysfunction clinically manifested by hypotension and/or elevated serum lactate levels. Also, lactate is produced in cases of hypoxia and poor tissue perfusion and is used as an indirect marker of tissue hypoxia (Pirrone et al 2012, Yildiz et al 2017). In a study conducted on premature infants, it was found that lactate increases the mortality rate due to its effects on blood pH (Nadeem et al 2010). Our finding indicated that determinants of hypoxemia (low venous pO_2 and SO_2 , hyperlactatemia) have major role to prediction of mortality in septic calves. Also, ROC analysis established that levels of lactate has sensitivity and specificity close to 100% for establish mortality at optimum cut-off value of 6.70 mmol/L. However, implanted studies by Naseri et al (2019) in septic calves and Ince et al (2019) in septic puppies were unsuccessful to show applicability of measured lactate to differentiate between survivor and non-survivors, some investigators demonstrated that lactate levels have prognostic values for prediction mortality in veterinary medicine. Yildiz et al (2017) demonstrated that blood lactate levels >7.5 mmol/L and $pCO_2 > 63.5$ mm Hg can use as prognostic index in calves with ARDS. They concluded that hypoxia play key role in hyperlactatemia, hypercapnia, and decreased levels of oxygen (pO_2) in non-survivor premature calves. However, in the present study the levels of pCO_2 were not different between survivor and non-survivors calves, the negative correlation between lactate concentration and venous pO_2 and SO_2 can support the evidence of tissue hypoxia, cellular metabolic abnormality, and severity of disease in non-survivor septic calves.





Conclusion

In conclusion, mortality rate in hypotensive calves is higher than non-hypotensive calves. Hypoglycemia, volume depletion and clinical appearance were worsened in hypotensive calves. Our findings indicated that tissue hypoxia is the strongest determinant of mortality in septic calves. Blood lactate levels at cut-off point of 6.7 mmol/L is the best prognostic indicator to differentiate between survivor and non-survivor calves with sepsis.

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Conflict of Interest

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During this study, any pharmaceutical company which has a direct connection with the research subject, a company that provides and / or manufactures medical instruments, equipment and materials or any commercial company may have a negative impact on the decision to be made during the evaluation process of the study or no moral support.

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Ethical Approval

This study was carried out with the permission of the Selcuk University Veterinary Faculty Experimental Animals Production and Research Center Ethics Board (Decision No: SUVDA-MEK 2020/52) report.

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