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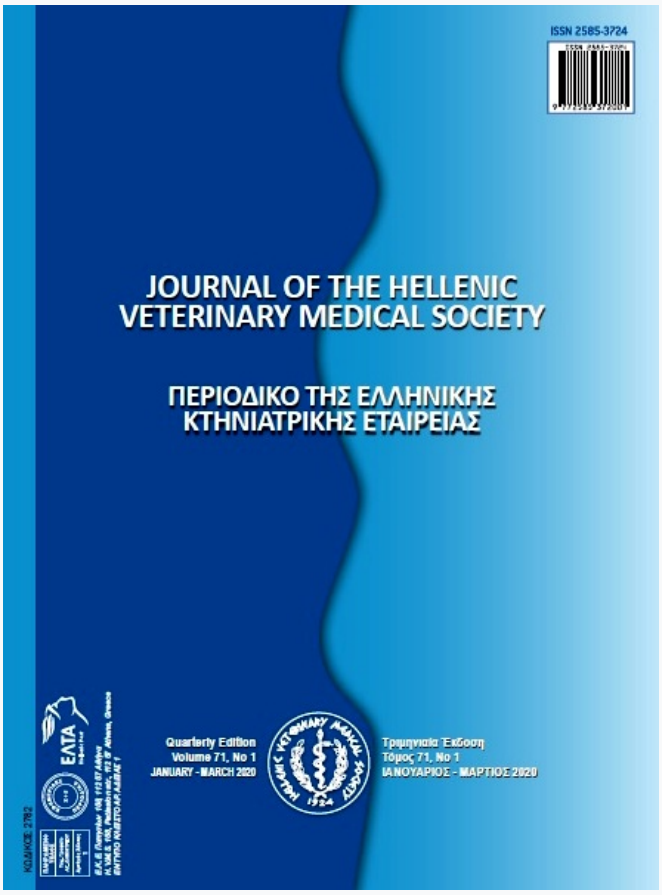

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Effect of nebulized formoterol, ipratropium bromide, and furosemide in combination with fluticasone propionate on arterial blood gases of premature calves with respiratory distress syndrome

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ABSTRACT. The purpose of this study was to assess the clinical effect of nebulized formoterol (FM), ipratropium bromide (IB) and furosemide (FS) combined with fluticasone propionate (FP) on lung function in premature calves with Respiratory Distress Syndrome (RDS). Thirty-six premature calves with RDS were randomly assigned to six different treatment groups (D1 to D6). All groups received the standard treatment, including oxygen and support treatment. Calves in D1 received only the standard treatment. The following combinations of nebulized drugs were used for the other groups: D2: FP, D3: FP+FM; D4: FP+IB; D5: FP+FS and D6: FP+IB+FM+FS. The treatment period (72 h) involved the application of FM (15 μg totally/12 h), IB (2 μg/kg/12 h), FS (1 mg/kg/12 h) and FP (15 μg/kg/12 h) for five minutes. A significant increase over time in blood pH, partial pressure of oxygen (PaO_2), oxygen saturation ($SatO_2$) and a decrease in partial pressure of carbon dioxide ($PaCO_2$) and lactate were detected in all groups that received nebulized treatment; while in the D1, a significant change was observed only for $PaCO_2$. Calves in D6 had the highest PaO_2 and lowest $PaCO_2$ values amongst all groups at the end of treatment. No statistical difference was observed between the Nebulization Groups (NG). Nebulized FM, IB and FS with FP combination in premature calves with RDS, in addition to the standard treatment showed a significant curative effect on lung function.

Keywords: arterial blood gases, nebulized drugs, respiratory, treatment, premature calves

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INTRODUCTION

Premature calves often suffer from life-threatening disorders due to incomplete development of organs, such as lungs, (Bleul, 2009) resulting in a decreased gas exchange capacity due to decreased lung volume and capillary surface area. An insufficient exchange of gas results in hypoxemia and long-lasting oxygen requirements (Ok and Birdane, 2000; Aydoğdu et al., 2016). In premature infants, surfactant deficiency and inflammation-associated fibrosis have been reported to play an important role in increasing the respiratory distress syndrome (RDS) risk (Murch et al., 1996).

The availability of nebulized drugs alleviates the clinical signs of RDS and improves pulmonary function (Genicot et al., 1994; Yildiz and Ok, 2017). Fluticasone propionate (FP) is one of the most commonly used inhaled steroids to reduce inflammation in pulmonary disorders and for treatment of asthma symptoms. It is also extensively used for treating respiratory problems in horses (Robinson et al., 2009) and in premature calves with RDS (Yildiz and Ok, 2017). Diuretics, such as furosemide (FS), decrease interstitial oedema and pulmonary vascular resistance and facilitate gas exchange; thereby reduce oxygen consumption (Bancalari et al., 2005). Inhaled FS has been considered to improve lung function in premature calves with RDS (Yildiz and Ok, 2017). Beta-2 adrenergic or anticholinergic aerosols are useful in treating bronchial constriction in preterm infants and they have been known to relieve from acute respiratory distress (Jarjour et al., 2006). Formoterol (FM) and ipratropium bromide (IB) are long-acting β-2 adrenergic agonists, which exert their action by relaxing the smooth muscles of the respiratory tract. The onset of action of FM is rapid and has been shown to exert long-lasting bronchodilation in children with bronchial asthma (Faulds and Hollingshead, 1991). Leemans et al., (2009) found that inhaled salbutamol and IB were effective in the treatment of respiratory disorders in cats.

Therefore, the hypothesis of this study was whether the administration of nebulized FP combined with FM, IB and FS, additionally to oxygen therapy and standard clinical care, significantly improves the condition of premature calves with RDS, just as reported in human, feline and equine neonates.

MATERIALS AND METHODS

Animals: Thirty-six premature calves of different breeds (Holstein, n=27; Simmental, n=5 and Brown Swiss, n=4) diagnosed with RDS were admitted to the Animal Hospital of the Faculty of Veterinary Medicine of Selcuk University, within 2-12 h of birth. A number from 1 to 6 was randomly assigned to these premature calves using a random number generator, as follows: D1 (n=6), D2 (n=6), D3 (n=6), D4 (n=6), D5 (n=6) and D6 (n=6). D1 was considered the standart treatment group whereas D2, D3, D4, D5 and D6 the nebulized treatment groups. Inclusion criteria were the gestational age (230 to 260 days) and RDS following a clinical examination (Yildiz and Ok, 2017). RDS criteria for premature calves included: hypoxemia ($PaO_2 < 60$ mm Hg in arterial blood); hypercapnia ($PaCO_2 > 45$ mm Hg in arterial blood); tachypnea (respiratory rate; RR ≥ 45 breaths/min) and expiration accentuated by an abdominal lift (Bleul et al., 2008; Yildiz and Ok, 2017). Peripheral oxygen saturation (SpO_2) was measured using a patient monitor device (Compact, Econet, Germany) and calves were clinically examined during the study. Calves having RDS as a result of other underlying diseases such as aspiration pneumonia or trauma were excluded from the study. Approval of the study was obtained from institutional ethics board of the Veterinary Faculty of Selcuk University (no: 2014/05).

Sample collection and blood analysis

Sodium heparin-containing plastic insulin syringes were used for the collection of arterial blood samples anaerobically from the *arteria auricularis*, as described in a previous study (Yildiz and Ok, 2017). Arterial blood pH, PaO_2, $PaCO_2$, $SatO_2$, lactate and base excess (BE) were analysed within 15 min at 0, 24, 48 and 72 h using a blood gas analyser (GEM Premier 3000, Instrumentation Laboratory, Lexington). The 0 h represents the time just before the initiation of the treatment and immediately after the collection of blood sample the nebulized drugs were administered over a course of 5-20 min depending on the combination of the drugs.

Standard treatment and oxygen therapy protocol for premature calves

Standard treatment, including oxygen therapy, supportive treatment and clinical care, was provided to each calf in all groups. Supportive treatment and clinical care protocols are described by Yildiz and Ok (2017) and oxygen therapy via mask by Bleul et al. (2008).

Nebulizer treatment protocol for premature calves

While calves in the D2 to D6 received nebulizer drugs for 5 min, calves in the control group received only 2.5 mL saline solution (every 12 h for 72 h) for 5 min via a nebulizer machine (NebuTech, GmbH, Germany). The nebulizer drug combinations were administered as follows: D2: FP; D3: FP + FM; D4: FP + IB; D5: FP + FS and D6: FP + FM + IB + FS.

Brand names of the drugs and dosages enrolled in the study as follows; FM *(VENTOFOR, Liconsa A.S, Spania)*, 15 µg totally, BID, 3 days; IB *(ATROVENt, Boehringer Ingelheim, Istanbul, Turkey)*, 2 µg / kg, BID, 3 days, FS *(DIURIL, Vetaş,* İstanbul*, Turkey)*, 1 mg/kg, BID, 3 days, FP *(FLIXOTIDE, GlaxoSmith-Kline, Australia)*, 15 µg/kg, BID, 3 days. Each drugs were diluted with 2.5 mL saline solution and were administered for 5 min using a nebulizer machine (Fig 1).

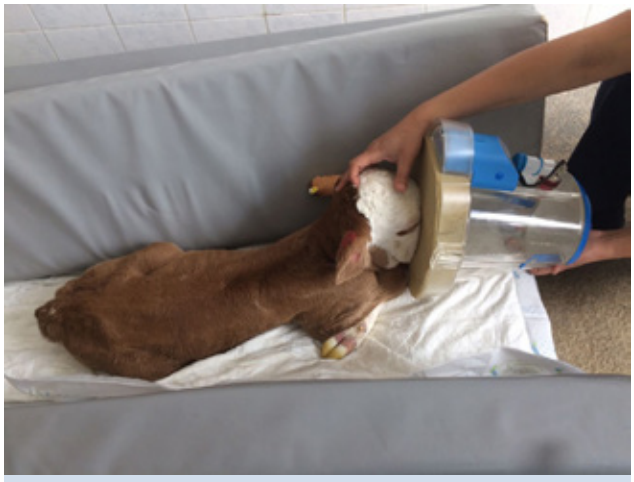

Figure 1. Nebulized drug admistration to premature calves.

Statistical analysis

Normality of data was assessed with the Kolmogorov-Smirnov test. To evaluate differences in results among the groups One-way ANOVA (Duncan's posthoc multiple comparison test) were used and the Kruskal-Wallis test was used with non-normally distributed data. The level of significance was set at $P < 0.05$. Data were analysed with a statistical software package (IBM SPSS, version 14.01 for Windows, SPSS Inc, Chicago).

RESULTS

In this study, 88.8% of premature calves with RDS (32/36) responded well to the therapy. Out of the 32 calved that survived, 28 belonged to the nebulized treatment groups (D2 to D6). Four premature calves with RDS died (D1, n=2; D4, n=1 and D6, n=1). Generally, the symptoms observed during the clinical examination of premature calves were: apnea or tachypnea, abdominal lift, weakness, cyanosis, hypothermia, wheezing and grunting sounds, lack or absence of sucking reflex, depression and increased capillary refill time . Symptoms such as tachycardia due to bronchodilator applications and hyperglycaemia due to corticosteroids were not observed. There were no differences in gestational age and admitted time to clinic after birth among the experimental groups. All data are presented as mean and standard error of the mean (mean ± SEM).

Blood gas findings

The parameters of arterial blood gas in premature calves with RDS are presented in Table 1.

In D2 to D6, there are marked increases in values including pH, pO_2, $SatO_2$ and decreases in pCO_2 and lactate levels. These values with their significance was presented separately in our group results.

Group D1: No significant differences were observed in blood gas parameters during the treatment. The gestational age at birth was 246 ± 3.79 days and admitted time to clinic after birth was 7.33 ± 1.45 h of the group.

Group D2: Significant increases was seen in values of blood pH, BE and $SatO_2$ at 24, 48 and 72 h during the treatment. Additionally, a decrease in lactate and $PaCO_2$ were obtained at 24, 48 and 72 h in this group (Table 1). The birth age was mean 248 ± 3.39 day and admitted time to clinic after birth was mean 7.17 ± 1.19 hour of the group.

Group D3: A significant ($P < 0.05$) increase in the blood pH, BE and $SatO_2$ values was obtained at the 24, 48 and 72 h of the treatment. A decrease in lactate and $PaCO_2$ value at 24, 48 and 72 h was also seen in these calves. A significant ($P < 0.05$) increase in PaO_2 value at 24, 48 and 72 h was found (Table 1). The birth age was mean 247 ± 3.93 day and admitted time to clinic after birth was mean 5.83 ± 1.16 hour of the group.

Group D4: A significant ($P < 0.05$) increase in blood pH, PaO_2 and $SatO_2$ values with a decrease in lactate and $PaCO_2$ values was seen in the calves at 24, 48, and 72 h of treatment. The birth age was mean 243 ± 2.70 day and admitted time to clinic after birth was mean 6.67 ± 1.26 hour of the group.

Group D5: A significant ($P < 0.05$) increase in

blood pH, BE and $SatO_2$ values during 24, 48 and 72 h of the treatment was seen. In addition, a decrease in lactate values during 48 and 72 h and $PaCO_2$ values at the 72 h was found in this group. No significant difference was observed in PaO_2 value of premature calves with RDS (Table 1). The birth age was mean 244 ± 3.52 day and admitted time to clinic after birth was mean 6.33 ± 1.45 hour of the group.

Group D6: A significant ($P < 0.05$) increase in blood pH, BE and $SatO_2$ values during the 24, 48 and 72 h and while there were no significant difference between 0 h and 24 h, a significant increase in PaO_2 value between the 24, 48 and 72 h was seen in this group. A decrease in lactate value during the 48 and 72 h and $PaCO_2$ value during the 24, 48 and 72 h were seen in this group (Table 1). The birth age was mean 246 ± 4.16 day and admitted time to clinic after birth was mean 5.83 ± 1.92 hour of the group.

Table 1. Arterial blood gas values in the all premature calves with RDS in the present study (Mean±SEM)

Parameters	Groups	0. hour	24. hour	48. hour	72. hour
pH	D1	7.11±0.11^{b}	7.26±0.09^{aB}	7.31±0.10^{a}	7.38±0.12^{a}
	D2	7.29±0.04^{b}	7.45±0.03^{aA}	7.47±0.04^{a}	7.47±0.02^{a}
	D3	7.31±0.02^{b}	7.44±0.02^{aA}	7.46±0.01^{a}	7.48±0.01^{a}
	D4	7.24±0.08^{b}	7.44±0.02^{aA}	7.47±0.03^{a}	7.47±0.01^{a}
	D5	7.09±0.1^{b}	7.41±0.03^{aA}	7.43±0.03^{a}	7.48±0.02^{a}
	D6	7.30±0.04^{c}	7.41±0.01^{bA}	7.46±0.01^{ab}	7.50±0.01^{a}
$PaCO_2$ mmHg	D1	61.6±7.48	55.3±8.29^{A}	53.8±6.58^{A}	50.6±7.77
	D2	54.3±5.38^{a}	39.3±1.61^{bB}	39.0±3.86^{bB}	42.3±2.74^{b}
	D3	50.6±3.9^{a}	42.1±2.23^{bB}	39.0±1.93^{bB}	39.8±1.62^{b}
	D4	57.3±6.98^{a}	44.0±2.34b^{AB}	42.2±1.83^{bB}	42.4±2.77^{b}
	D5	58.1±7.35^{a}	45.6±3.01^{abAB}	46.0±3.06^{abAB}	42.1±3.15^{b}
	D6	49.1±0.79^{a}	41.5±1.52^{bB}	39.0±1.95^{bB}	37.8±0.80^{b}
PaO_2 mmHg	D1	33.5±5.14	36.3±5.67	39.6±7.2	43.5±7.32^{B}
	D2	37.6±4.62	50.6±4.93	52.8±5.06	50.3±4.72^{AB}
	D3	36.3±1.91^{c}	45.5±4.01^{b}	54.5±3.06^{a}	55.3±1.76^{aAB}
	D4	33.8±4.16^{b}	46.8±5.9^{ab}	45.8±7.08^{ab}	56.6±5.26^{aAB}
	D5	38.0±5.67	39.8±6.11	43.3±5.71	48.5±5.57^{AB}
	D6	36.5±3.84^{c}	44.0±2.91^{c}	55.4±2.38^{b}	60.2±3.17^{aA}
BE	D1	-9.75±4.62^{B}	-3.30±4.33^{B}	0.27±4.88	4.10±6.04
	D2	-1.48±1.41^{bAB}	3.40±1.47^{aA}	4.13±1.52^{a}	6.95±1.19^{a}
	D3	-1.2±1.22^{bAB}	4.20±1.53^{aA}	4.18±0.96^{a}	6.07±1.91^{a}
	D4	5.52±2.50^{A}	5.17±1.12^{A}	6.52±1.84	6.70±1.98
	D5	-5.4±3.92^{bB}	3.40±1.41^{aA}	5.18±2.21^{a}	6.75±1.77^{a}
	D6	-3.6±2.33^{bAB}	1.18±0.61^{aAB}	3.88±1.44^{a}	5.90±1.33^{a}
SatO2 %	D1	41.3±11.7	54.0±11.8^{B}	60.1±13.64^{B}	69.1±12.5^{B}
	D2	61.3±10.4	83.5±6.45^{A}	84.8±6.64^{A}	85.0±4.63^{AB}
	D3	62.6±3.28^{b}	83.0±5.01^{aA}	89.1±1.62^{aA}	90.5±0.72^{aA}
	D4	52.3±10.2^{b}	80.1±4.77^{aA}	78.0±8.79^{aAB}	89.8±2.03^{aA}
	D5	38.0±13.1^{b}	68.1±7.89^{aAB}	75.1±5.63^{aAB}	83.5±4.16^{aAB}
	D6	59.0±7.47^{b}	76.0±5.54^{aAB}	89.8±1.16^{aA}	90.2±1.56^{aA}
Lactate mmol/L	D1	7.95±2.55^{AB}	5.65±1.96^{AB}	4.88±2.08	4.03±2.23
	D2	3.67±0.47^{aB}	1.55±0.41^{bC}	1.30±0.31^{b}	0.75±0.07^{b}
	D3	4.23±0.61^{aAB}	1.47±0.10^{bC}	1.18±0.28^{b}	0.78±0.10^{b}
	D4	5.72±0.89^{aAB}	2.82±0.46^{bBC}	1.44±0.19^{ab}	0.92±0.15^{b}
	D5	8.9±2.27^{aA}	4.08±1.19^{abABC}	3.48±2.03^{b}	2.58±1.16^{b}
	D6	7.95±1.07^{aAB}	6.38±1.32^{aA}	2.94±0.79^{b}	2.62±1.05^{b}

Different letters in the same row (a, b), in the same column (A, B) are statistically significant ($P < 0,05$), pH: concentration of hydrogen ions, $PaCO_2$: partial pressure of arterial carbon dioxide, PaO_2 partial pressure of arterial oxygen, $SatO_2$%: oxygen saturation, SEM: Standart error of mean.

pH: The pH value was significantly ($P < 0.05$) increased in all groups at 24 h. However, on comparing with D1, it was found that the nebulizer groups showed a statistically higher ($P < 0.05$) pH value at the 24 h. Yet, no statistical difference was observed between the nebulizer groups (Table 1).

$PaCO_2$: The values of $PaCO_2$ showed a statistical decrease ($P < 0.05$) at 24 and 48 h in D3, D4 and D6 groups as compared to D1 group. D6 group showed the lowest mean value of $PaCO_2$ amongst all groups at the end of the treatment. However, no statistical difference was observed between the nebulizer groups (Table 1).

PaO_2: On comparing D1 with nebulizer treatment groups, it was seen that all the nebulizer treatment groups except D6 did not show any statistical differences at the 72 h. D6 showed the highest value ($P < 0.05$) of PaO_2 amongst all groups at 72 h (Table 1). Yet, no statistical difference was seen amongst the nebulizer groups (Table 1).

BE: On comparing D1 group with nebulizer treatment groups, D2, D3, D4 and D5 showed statistical differences ($P < 0.05$) at the 24 h. But no statistical difference was determined amongst the nebulizer groups (Table 1).

$SatO_2$: On comparing D1 group with nebulizer treatment groups, statistical differences ($P < 0.05$) were seen in D2, D3, D4 at the 24 h, in D2, D3, D6 at the 48 h, and in D3, D4, D6 at the 72 h. However, no statistical difference was observed amongst the nebulizer groups (Table 1).

Lactate: On comparing D1 group with nebulizer treatment groups, a statistically significant difference was observed in lactate value at 24 h in D2, D3 and D6. Though there was no statistical difference between the groups at the end of the treatment, the highest mean value of lactate was found in D1 (Table 1).

Amongst the calves that did not survive, high values of lactate (> 8.5 mmol/L) and $PaCO_2$ (> 75 mmHg) and low levels of PaO_2 (< 40 mmHg) and $SatO_2$ (< 52%) were observed before death. The clinical symptoms of the non-survive premature calves were continuous abdominal respiration, high RR (> 70/min), absence of sucking reflex, abdominal distension related with feeding and low body temperature (34-36.5 °C). Parameters used for monitoring and clinical observations, including rectal temperature respiratory rate and peripheral oxygen saturation (SpO_2) are presented in Table 2.

Table 2. Monitoring and clinical parameters of the premature calves with RDS in the present study (Mean±SEM)

Paramaters	Groups	0. hour	24. hour	48. hour	72. hour
Temperature (°C)	D1	35.5 ± 0.75^{bAB}	38.3 ± 0.41^{aAB}	38.3 ± 0.34^{aAB}	38.4 ± 0.20^{a}
	D2	36.1 ± 1.01^{bAB}	38.3 ± 0.14^{aAB}	38.6 ± 0.35^{aAB}	38.7 ± 0.23^{a}
	D3	37.7 ± 0.50^{A}	38.7 ± 0.21^{A}	38.5 ± 0.16^{AB}	38.3 ± 0.25
	D4	36.0 ± 1.32^{b}	38.0 ± 0.30^{abAB}	38.8 ± 0.20^{aA}	38.7 ± 0.30^{a}
	D5	34.6 ± 1.03^{bB}	37.6 ± 0.42^{aAB}	38.1 ± 0.14^{aAB}	38.6 ± 0.29^{a}
	D6	36.2 ± 0.78^{bAB}	37.4 ± 0.62^{abB}	38.0 ± 0.15^{aB}	38.5 ± 0.15^{a}
Respiratory rate (minute)	D1	51.3±9.22	64.6±13.8	58.3±13.6	56.0±14.9
	D2	47.5±10.3	58.8±12.3	65.6±15.3	35.0±5.26
	D3	49.6±6.4	45.2±9.33	44.8±4.59	37.2±2.58
	D4	54.3±7.51	52.3±7.47	44.4±7.55	37.5±5.66
	D5	49.8±6.73	58.0±13.2	50.3±7.23	35.0±5.99
	D6	68.0 ± 8.10^{a}	49.5 ± 7.51^{b}	44.0 ± 7.23^{b}	36.0 ± 4.47^{b}
SpO_2 (%)	D1	74.0 ± 6.87^{b}	85.0 ± 5.97^{ab}	89.5 ± 4.97^{abAB}	87.5 ± 1.76^{a}
	D2	79.4±8.40	91.6±2.48	95.3 ± 1.76^{A}	95.5±1.44
	D3	83.1 ± 3.15^{b}	92.6 ± 1.15^{a}	94.8 ± 0.83^{aA}	93.5 ± 0.99^{a}
	D4	75.1 ± 4.19^{b}	85.3 ± 2.85^{a}	88.8 ± 0.86^{aAB}	90.6 ± 3.11^{a}
	D5	70.5 ± 4.92^{b}	85.8 ± 4.16^{a}	84.3 ± 3.49^{aB}	90.3 ± 3.12^{a}
	D6	83.5±3.38	87.5±1.54	88.8 ± 1.59^{AB}	90.8±3.26

Different letters in the same row (a, b), in the same column (A, B) are statistically significant ($P < 0,05$), SpO_2 (%): Peripheral oxygen saturation, SEM: Standart error of mean.

DISCUSSION

Although numerous medical treatment strategies in human medicine, such as antenatal steroids, surfactant replacement, nitric oxide administration, and mechanic ventilation strategies (Sahni and Phelps, 2011), there has been no shown economically useful intervention to treat the RDS of premature calves, except for a study (Yildiz and Ok, 2017) to date.

A reliable method for the evaluation of pulmonary function (Bleul et al., 2007) is to determine arterial blood pCO_2 and pO_2. PaO_2 of less than 60 mmHg is considered to be indicative of RDS in infants (Verder et al., 1999). In healthy calves after birth, PaO_2 levels were showed to vary between 47 to 58 mmHg. In the calves with RDS one after birth, PaO_2 levels were found to change between 29 ± 12 and 38 ± 8.8 (Bleul, 2009). Yildiz and Ok (2017) reported that the mean value of PaO_2 levels in the premature calves with RDS at admitted to the clinic was found to be between 28 ± 3.5 and 36 ± 5.4 mmHg. In present study, the value of PaO_2 at 0 h was found to be between 33 ± 5.1 and 38 ± 5.6 mmHg (Table 1) and these animals posed difficulty in respiration (Table 2), which can be considered pathognomonic for all treatment groups. The mean value of PaO_2 was observed to be above 50 mmHg at the 72 h in all nebulizer groups, except D5. In addition, the mean respiratory rate reverted back to the normal level (< 45 RR/min) in the nebulizer treatment groups (Table 2). The mean value of PaO_2 in D6 increased to mean 60 ± 3.1 mmHg, and that of RR/min decreased to 36 ± 4.4 at the 72 h (Table 1-2). However, only the mean value of PaO_2 in D6 reached above the specified critical value (60 mmHg) (Bleul, 2009). This is indicating that nebulized treatment has a positive effect in pulmonary function. The level of PaO_2 in D1 at the 72 h was found to be 43.5 ± 7.32 mmHg (Table 1), while the mean respiratory rate continued to be 56 RR/min (Table 2). The level of PaO_2 below 45 mmHg is likely to be pathognomonic for RDS with significant clinical signs such as high respiratory rate (> 45 RR/min) (Bleul, 2009; Yildiz and Ok, 2017). Oxygen therapy is recommended for use in any newborn where $SatO_2$ is less than 90% or PaO_2 is less than 60 mmHg. The low levels of arterial $SatO_2$ (50.6 ± 7.7) in D1 indicate that the treatment without nebulized drugs may be insufficient to raise the level of $SatO_2$ up to the recommended values (Palmer, 2005; Yildiz and Ok, 2017). On the other hand, a combination of inhaler FP along with FM, IB and FS in addition to the standard treatment increases the value of $SatO_2$ adequately to the recommended levels (Palmer, 2005; Yildiz and Ok, 2017) in premature calves with RDS. Inefficient vital capacity of premature calves impairs the elimination of carbon dioxide from lungs, increasing its level in blood resulting in respiratory acidosis (Bleul et al., 2007) and the increase in the level of $PaCO_2$ above 45 mmHg indicates to RDS (Bleul et al., 2008). Yildiz and Ok (2017) reported that the levels of $PaCO_2$ (50 to 74 mmHg) in premature calves with RDS significantly decreased by the inhaler therapy. In our study, the levels of $PaCO_2$ were determined that higher than 49 mmHg in all premature groups at 0 h (Table 1). The $PaCO_2$ values decreased in all groups during the treatment period (Table 1). The results of this study are consistent with the previous studies (Bleul et al., 2007; Yildiz and Ok, 2017). The studies (Bleul, 2009; Güzelbekteş et al., 2012; Yildiz and Ok, 2017) suggested that blood pCO2 and lactate levels are important biomarkers for the detection of tissue hypoxia in premature calves. The concentration of plasma lactate greater than 4 mmol/L is a predictor for death within 24 h in cattle with pneumonia (Coghe et al., 2000). In a study with premature calves (Yildiz and Ok, 2017) has been reported that the mean lactate levels before the treatment were between 3.9 and 8.4 mmol/L and they also reported that in premature calves which were treated with nebulized drug combination with oxygen decreased the lactate to the normal level (< 2 mmol/L) however, in the premature calves that were treated only with oxygen therapy, the mean lactate level did not fall below the critical level (4 mmol/L). In the present study, mean lactate concentration at 0 h ranged between 3.67 and 7.95 mmol/L for all groups (Table 1). A significant ($P < 0.05$) decrease in the blood lactate levels below the critical value was seen at the 72 h in the nebulizer treatment groups (Table 1). However, the lactate levels of premature calves who did not receive nebulized treatment (D1 group) showed no significant decrease during the course of the treatment and did not fall below the critical level even at the 72 h (Table 1). Yildiz and Ok (2017) reported that high lactate (> 10 mmol/l) and $PaCO_2$ (> 74 mmHg) levels may be an indicator for high risk of death in premature calves with RDS. And also, in premature calves with RDS, Yildiz et al. (2017) reported that the cut-off values for lactate and pCO_2 were 7.5 mmol/L and 63.5 mmHg, respectively and positive correlation was found between mortality and increasing lactate and pCO_2 levels. In this study, premature calves which have lactate level higher than 8.5 mmol/L were non-survived like previous studies

(Yildiz et al., 2017; Yildiz and Ok, 2017). Premature calves had high CO_2 and low base excess levels along with decreased pH at admission to hospital (Yildiz et al., 2017). Yildiz and Ok (2017) found that the pH values of arterial blood in premature calves that received nebulized treatment were within the normal range (7.35-7.45) at the 72 h of the study except in those premature calves who did not receive nebulized treatment (pH < 7.35). In D1, the pH value remained below normal, possibly due to the continued CO_2 retention in blood (Bleul et al., 2007), since the mean value of $PaCO_2$ was found to be more than 50 mmHg at the 24 and 48 h of the treatment (Table 1).

The findings of this study show that nebulized therapy is beneficial for pulmonary functions in premature calves. These effects were most likely associated with nebulized FP combination with FM, IB, and FS. FP is known to reduce the release of inflammatory mediators in the lungs resulting from anoxic conditions (Robinson et al., 2009). FM and IB, the inhaled bronchodilators, have been reported to be effective in improving lung function and decreasing the respiratory symptoms (Duvivier et al., 1999; Chhabra et al., 2006) and also preventing neutrophilic infiltration and pulmonary oedema (Zhang et al., 2010). Inhaler diuretics like furosemide improve pulmonary compliance (Broadstone et al., 1991).

CONCLUSIONS

In conclusion, despite numerous medical advances, no single intervention will prevent or treat the RDS of premature calves. The use of nebulized drugs provides short-term improvement in lung mechanics, still these results do not support to use of these drugs instead of surfactant. But the drug combinations can be used in veterinary practice due to their ease of applicability and low cost for to reduce the serious damage due to the RDS in the first few days. The therapeutic effect of nebulized FP combination along with FM, IB, and FS on pulmonary function is a promising treatment for premature calves with RDS.

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CONFLICT OF INTEREST

None declared.

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