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Clinical and Doppler echocardiographic evaluation of rabbits sedated with dexmedetomidine in combination with midazolam and morphine

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ABSTRACT: The objective of the present study was to evaluate clinical, cardiorespiratory, and Doppler echocardiographic changes in rabbits sedated with midazolam and morphine combined with or without dexmedetomidine. This study was a blinded, randomized, controlled experiment that included 16 adult male New Zealand rabbits weighing 3.1 ± 0.3 kg. The animals were sedated using one of the following protocols: 1 mg/kg midazolam and 2 mg/kg morphine (MIDA, n = 8), or 25 mg/kg dexmedetomidine, 2 mg/kg morphine and 1 mg/kg midazolam (DEX, n = 8). Sedation latency, duration of the sedation and recovery period, sedation scores, heart rate (HR), respiratory rate (*f*), peripheral oxygen saturation (SpO2), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), and recta temperature were recorded, and Doppler echocardiography was performed. Latency periods were 7.3±1.6 min in the DEX group and 10.9 ± 5.0 min in the MIDA group (P = 0.112). Sedation duration was 122.4±14 min in the DEX group and 71.2 ± 32 min in the MIDA group (P = 0.005), whereas recovery time was 35.7±17.7 min in the DEX group and 32.5 ± 25.3 min in the MIDA group (P = 0.743). The sedation scores for the DEX group were significantly higher than those for the MIDA group throughout the monitoring period. Reductions in HR, SAP, MAP, and DAP values were observed in both groups relative to baseline values, and were significantly lower in the DEX group compared to the MIDA group. Minimal Doppler echocardiographic changes were observed. Dexmedetomidine used in combination with midazolam and morphine incremented the quality and duration of sedation in rabbits. Both protocols elicited cardiorespiratory changes that were well-tolerated, with minimal changes in myocardial function.
Key words: Doppler echocardiography, neuroleptic analgesia, sedation.

Avaliação clínica e Doppler ecocardiográfica de coelhos sedados com dexmedetomidina em combinação com midazolam e morfina

RESUMO: O objetivo do presente estudo foi avaliar alterações clínicas, cardiorrespiratórias e ecodopplercardiográficas, bem como o nível de sedação em coelhos sedados com midazolam e morfina combinadas, ou não, com dexmedetomidina. O design do estudo foi experimental controlado randomizado cego, com 16 coelhos adultos machos Nova Zelândia, pesando 3,1 ± 0,3 Kg. Os animais foram sedados aleatoriamente com um dos seguintes tratamentos: MIDA (n = 8): 1 mg/kg de midazolam e 2 mg/kg de morfina; DEX (n = 8) 25 mcg/kg de dexmedetomidina, 2 mg/kg de morfina e 1 mg/kg de midazolam; Latência, sedação e períodos de recuperação, escores de sedação, FC, *f*, SpO2, PAS, PAM, PAD, T°C, foram mensurados e ecoDopplercardiografia foi realizada. Os períodos de latência foram 7.3 ± 1.6 minutos no grupo DEX e 10.9 ± 5.0 minutos no grupo MIDA (P = 0,112). A duração da sedação foi de 122.4 ± 14 minutos no grupo DEX e 71.2 ± 32 minutos no grupo MIDA (P = 0,005). Os tempos totais de recuperação foram de 35.7 ± 17.7 minutos no grupo DEX e 32.5 ± 25.3 minutos no MIDA (P = 0,743). Os escores de sedação obtidos do grupo DEX foram estatisticamente superiores aos do grupo MIDA durante todas as observações (de 5 a 40 minutos). A redução dos valores de FC, PAS, PAM e PAD foi observada em ambos os grupos, em relação ao momento baseline, e foram significativamente menores no grupo DEX quando comparado ao MIDA. Alterações ecodopplercardiográficas mínimas foram observadas ambos os grupos. A adição de dexmedetomidina à associação midazolam/morfina aumentou o nível, a qualidade e a duração do protocolo sedativo. Ambos os protocolos causaram alterações cardiorrespiratórias bem toleradas em animais normais e com mínimas alterações na função miocárdica.
Palavras-chave: Doppler ecocardiografia, neuroleptoanalgesia, sedação.

INTRODUCTION

Rabbits become easily stressed (WENGER, 2012), are commonly affected by respiratory infections, and are prone to hypothermia owing to their large body surface area in relation to their weight, in addition to their high metabolic rate (BEDIN et al., 2013; FLECKNELL, 2015). The interaction between these physiological characteristics makes this species particularly prone to complications during anesthesia induction, with high mortality rates (BRODBELT et al., 2008).

Currently, rabbits are the third most common domestic animals in the United Kingdom (GRINT & MURISON, 2008), and this trend has

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already been observed in Brazil. In clinical practice, meticulous clinical examination is necessary for a precise diagnosis. Therefore, sedative protocols for rabbits can help reduce stress and facilitate handling. Adequate muscle relaxation and analgesia facilitate radiography, ultrasonography, Doppler echocardiography, and blood sampling. The selected protocols must be safe and efficient, with a fast induction and a period of action of known length that provides enough time to complete a full clinical evaluation (MUIR & HUBBEL, 2001).

Several combinations of sedative agents have been proposed and tested in rabbits. The use of sedatives and muscle relaxants associated with analgesics is recommended, resulting in synergy between different classes and allowing the use of lower doses of each individual compound to achieve sufficient analgesia (MUIR & HUBBEL, 2001; GRUBB et al., 2020). While there is an abundance of information in the literature concerning sedative protocols, most studies refer to combinations of dissociative drugs such as ketamine and tiletamine. These combinations promote deep sedation, and even an anesthetic/surgical plane, which is not necessary in many cases and may unnecessarily prolong recovery time as well as affect its quality. The sympathomimetic effects of this class of drugs can also negatively affect the cardiovascular and respiratory systems in this species (HENKE et al., 2005; ORR et al., 2005; GRINT & MURISON, 2008; SILVA et al., 2011).

Because of the widespread adoption of this species as an experimental model and the increase in the number of individuals kept as pets, there is a unmet need for efficient and safe sedation protocols that can be implemented in clinical practice, allowing for easy handling of the animals during the required amount of time as well as for adequate recovery. Minimal cardiovascular, respiratory, and hemodynamic changes; and no significant interference with standard biomedical research procedures are also warranted. The aim of this study was to evaluate the clinical, cardiorespiratory, and echo-Doppler cardiographic changes related to the use of midazolam and morphine in combination as sedatives, as well as the potential advantages of adding dexmedetomidine to the protocol. We hypothesized that dexmedetomidine may increase sedation scores despite eliciting more pronounced cardiovascular changes, and that it may be well tolerated by healthy rabbits.

MATERIALS AND METHODS

Sixteen male New Zealand rabbits of approximately six months of age and weighing 3.1±0.3 kg, were included in this study. The patients were considered healthy based on clinical and laboratory examinations. A blinded randomized experimental design was adopted for the study. The sample size was determined according to previous calculations reported by BELLINI et al. (2014) to detect a difference of 2 points (standard deviation of 1 point) in the sedation score, with a power of 0.9 and an alpha value of 0.05, yielding a minimal sample size of seven animals per group. This study was evaluated and approved by the institutional Ethics Committee (protocol number 94/2017).

The animals had free access to water and food until they received their assigned treatments. Prior to sedation, the animals were gently held using a towel wrapped around their bodies. The fur was clipped bilaterally on the dorsal aspect of the ears and in the thoracic area. Topical anesthetic EMLA cream containing lidocaine and prilocaine (25 mg/g, AstraZeneca do Brazil, Brazil) was applied in the dorsal aspect of the ears 20 minutes before the procedure. Chlorhexidine and alcohol solutions were used prior to vascular puncture. The auricular artery was catheterized using a 22G catheter (BD Insyte, Becton Dickinson, Brazil), and systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were measured. The arterial catheter was connected to a non-compliant tubular system and a pressure transducer (TruWave; Edwards Lifesciences, USA), which were filled with heparinized saline (5 IU/mL) and pressurized at 300 mmHg. The pressure transducer was zeroed at the level of the manubrium of the sternum and calibrated using a mercury manometer. After initial manipulation, the animals were allowed to rest for 10 min.

Baseline heart rate (HR) and peripheral oxygen saturation (SpO2) were assessed by pulse oximetry (Nellcor Sensor, Lifewindow LW8, Digicare, FL, USA) with the sensor placed on the animal's ear, while the respiratory rate (*f*) was obtained by direct observation of chest movement for one minute. Rectal temperature was measured using a digital thermometer (Geratherm Medical Latin America, São Paulo, Brazil). Systolic (SAP), diastolic (DAP), and mean arterial pressures (MAP) were obtained by invasive monitoring with an arterial pressure transducer (Tru Wave, Edwards Lifescience, USA) connected to the arterial catheter, placed at the heart level, zeroed to atmospheric pressure, and connected to a multiparameter monitor (Lifewindow LW8, Digicare, FL, USA).

After the baseline parameters were established, the animals were randomly allocated to

each experimental group, with the evaluators remaining blind to group allocation., The treatment protocol for each experimental group was as follows: MIDA (n = 8), 1 mg/kg midazolam (Dormonid, Cristália, Brazil) and 2 mg/kg morphine (Dimorf, Cristália, Brazil); or DEX (n = 8), 25 mcg/kg dexmedetomidine (Dexdomidor, Zoetis, Brazil), 2 mg/kg morphine, and 1 mg/kg midazolam. In both cases, the drugs were administered intramuscularly in the quadriceps region, and the animals were placed on an active thermal mattress immediately after being injected.

After the treatments were administered, the following parameters were evaluated: sedation latency (time elapsed from treatment administration until the moment the animal adopted lateral recumbency without head movement), sedation duration (time elapsed from the beginning of sedation until initial spontaneous head or limb movement), and recovery time (time elapsed from initial spontaneous movement until achievement of full ambulation capacity).

Sedation scores were recorded every 5 min until the animal recovered using a semi-quantitative scale described by BELLINI et al. (2014), as shown in table 1. The sedation score was measured by the same person who administered the intramuscular injections. Cardiorespiratory parameters were assessed at baseline and also every five minutes after treatment administration.

Echocardiography was performed by a veterinary cardiologist, with all measurements performed as described by SYPMANN et al. (2007) and FONTES-SOUSA et al. (2009) for the Leporidae. The animals were carefully positioned in lateral recumbency using the right and left parasternal regions from the third to sixth intercostal spaces. Two-dimensional, M-mode, pulsed, and continuous wave Doppler and color flow mapping were evaluated, with three independent measurements for each variable that were later averaged to obtain a final mean value. In the two-dimensional method, the cardiac chamber morphology was assessed. In the M-mode method, internal left ventricle (LV) dimensions, ventricular septum thickness, and left ventricular free wall thickness were measured at the end of systole and diastole. Left ventricular systolic and diastolic volumes as well as ejection fraction were calculated in according to Teichholz's formula. Doppler echocardiographic parameters were assessed before and 15 min after treatment administration. Heart rate data were collected in different ways: the heart rate shown in table 2 was obtained from the multiparametric monitor, whereas the frequency data shown in table 3 was obtained from the Doppler echocardiographic exam.

Table 1 - Semi-quantitative scale used to assess the degree of sedation in rabbits.

----------Score----------	
----------Posture----------	
0	Normal
1	Sitting with head up
2	Lying sternally, head down
3	Lying laterally
4	Lying dorsally, responding to stimuli
5	Lying dorsally, not moving when stimulated
----------Resistance to being rolled in dorsal recumbency----------	
0	Strong/normal resistance
1	Moderate resistance
2	Slight resistance
3	No resistance
----------Jaw muscle tone----------	
0	Normal
1	No resistance to mouth opened
----------Palpebral reflex----------	
0	Normal
1	Decreased
2	Absent

*BELLINI et al. (2014).

Statistical analyses were performed using SigmaPlot for Windows (v. 12.0, SPSS Inc., Chicago, USA). The Shapiro–Wilk test was performed to evaluate the distribution of the analyzed data. Intragroup differences were analyzed by one-way analysis of variance with repeated measures (ANOVA-RM), followed by Dunnett's test. Comparisons between groups were performed using the t-test, whereas non-parametric data such as sedation scores were evaluated using the Mann-Whitney rank sum test. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

All animals from both experimental groups completed the experimental stage without noticeable adverse effects during sedation or during the recovery period. Sedation latencies were 7.3 ± 1.6 min in the DEX group and 10.9±5.0 minu in the MIDA group, with no statistically significant differences ($P = 0.112$). Sedation duration was longer in the DEX group (122.4 ± 14 min) compared to the MIDA group (71.2 ± 32 min; $P = 0.005$). The recovery time was 35.7 ± 17.7 min in the DEX group and 32.5

Table 2 - Heart rate (HR), respiratory rate (*f*), systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic pressure (DAP), body temperature (TºC), and peripheral oxygen saturation (SpO_2) observed in rabbits sedated with 25 mcg/kg dexmedetomidine, 2 mg/kg morphine and 1 mg/kg midazolam (DEX, n=8), or with 2 mg/kg morphine and 1 mg/kg midazolam (MIDA, n=8).

Parameters	Group	Time (min)								
		Baseline	5	10	15	20	25	30	35	40
HR	DEX	191 ±31	175 ±42	165* ±38	165*† ±34	161* ±34	160* ±28	156* ±25	153* ±26	145* ±23
(bpm)	MIDA	213 ±29	207 ±14	209 ±30	187*† ±32	173* ±25	167* ±30	162* ±42	154* ±46	147* ±24
f	DEX	162 ±20	100*† ±54	64*† ±43	55*† ±35	47*† ±47	47*† ±52	40*† ±36	44*† ±46	50* ±48
(mpm)	MIDA	168 ±20	171† ±28	163† ±27	156† ±51	141† ±57	132† ±46	108*† ±44	98*† ±55	87*† ±45
SAP	DEX	90 ±8	87† ±3	79*† ±6	77*† ±7	77*† ±9	75*† ±8	76*† ±7	75*† ±5	76*† ±4
(mmHg)	MIDA	95 ±6	96† ±7	89† ±7	90† ±10	91† ±10	87† ±4	88† ±5	87† ±5	82*† ±6
MAP	DEX	79 ±10	72 ±4	66*† ±5	62*† ±8	62*† ±10	59*† ±9	60*† ±6	60*† ±5	66* ±9
(mmHg)	MIDA	85 ±4	78 ±12	76† ±5	79† ±10	77† ±11	73† ±5	76† ±5	76† ±5	70 ±6
DAP	DEX	72 ±11	64† ±6	58*† ±5	55*† ±8	55*† ±9	51*† ±7	52*† ±5	53*† ±4	54* ±4
(mmHg)	MIDA	78 ±5	80† ±9	69*† ±12	69† ±9	70† ±8	64*† ±5	66*† ±6	67† ±6	61* ±7
TºC	DEX	39.3 ±0.4	38.9 ±0.5	38.9 ±0.4	38.9 ±0.5	38.8 ±0.4	38.6* ±0.4	38.6* ±0.4	38.6* ±0.4	38.3* ±0.4
(ºCelcius)	MIDA	39.5 ±0.4	39.3 ±0.3	39.2 ±0.3	38.9* ±0.4	38.6* ±0.7	38.7* ±0.7	38.5* ±0.8	38.3* ±1.1	38.3* ±0.8
SpO2	DEX	95 ±3	95 ±2	90 ±5	90*† ±4	88* ±5	87 ±9	91 ±5	92 ±4	92 ±3
(%)	MIDA	96 ±1	96 ±1	95 ±2	94† ±3	95 ±2	96 ±3	95 ±3	95 ±2	95 ±4

Values are shown as mean and standard deviation. Heart rate data was collected with a multi-parameter monitor.
* on the line indicates significant difference against Baseline values. † on columns, indicates significant difference between groups.

Table 3 - Heart rate (HR), aorta diameter (dAo), left atrial to aortic root ratio (LA/Ao), thickness of interventricular septum at end-diastole (IVSd), left ventricle internal diameter at end-diastole (LVIDd), thickness of left ventricular free wall at end-diastole (LVFWd), left ventricle internal diameter in systole (LVIDs), ejection fraction (EF), fractional shortening (FS), left ventricular outflow tract velocity time integral (LVOT), S-wave, E-wave and A-wave deceleration times (DT:S; DT:E; DT:A), observed in rabbits sedated with 25 mcg/kg of dexmedetomidine associated with 2 mg/kg of morphine and 1 mg/kg of midazolam (DEX, n=8), or sedated with 2mg/kg of morphine associated with a 1 mg/kg of midazolam (MIDA, n=8).

Parameters	Groups	----------Time (minutes)----------	
		Baseline	15
HR - Doppler	DEX	197 ± 26	196 ± 42
(bpm)	MIDA	199 ± 18	216 ± 29*
dAo	DEX	6 ± 0.3	5.7 ± 0.3*
(mm)	MIDA	5.8 ± 0.3	5.7 ± 0.3
LA/Ao	DEX	1.4 ± 0.1	1.3 ± 0.1
	MIDA	1.5 ± 0.2	1.4 ± 0.2
IVSd	DEX	2.3 ± 0.3	2.4 ± 0.2
(mm)	MIDA	2.3 ± 0.4	2.4 ± 0.2
LVIDd	DEX	13.6 ± 0.9	12.6 ± 1.4
(mm)	MIDA	14.4 ± 1.1	13.5 ± 1.1
LVFWd	DEX	2.1 ± 0.4	2.3 ± 0.2
(mm)	MIDA	2.3 ± 0.5	2.3 ± 0.4
LVIDs	DEX	9.8 ± 0.8	8.8 ± 1.3
(mm)	MIDA	10.2 ± 1	9 ± 0.7*
EF	DEX	58.3 ± 3.8	60.3 ± 10
(%)	MIDA	60 ± 6.7	65.8 ± 3.4*
FS	DEX	28.1 ± 2.2	29.6 ± 6
(%)	MIDA	29.4 ± 4.4	33.1 ± 2.4*
LVOT	DEX	7.2 ± 1.1	6.7 ± 1.2
	MIDA	7.2 ± 1	7.3 ± 0.9
DT:S	DEX	9.9 ± 1.3	9.0 ± 1.3
	MIDA	10.5 ± 2	9.9 ± 1.6
DT:E	DEX	11.3 ± 1.3	11 ± 1.9
	MIDA	11.4 ± 1	12 ± 2.3
DT:A	DEX	8.6 ± 1.9	6.3 ± 1.1*†
	MIDA	8.2 ± 1	8.6 ± 0.9†

Values are shown as mean and standard deviation. Collection of heart rate data using echocardiography

* indicates a significant difference compared to baseline value.

† indicates a significant difference between experimental groups.

± 25.3 min in the MIDA group, with no significant difference (P = 0.743). The sedation scores obtained for the DEX group were significantly higher than those obtained for the MIDA group at all assessed time points, as shown in figure 1.

When compared with baseline values, the HR was lower at 10 and at 15 min after drug administration in the DEX and the MIDA groups, respectively. Mean HR values were lower in the DEX group than in the MIDA group at 15 min (Table 2). A decrease in *f* compared to baseline was observed in the DEX group starting at five minutes after sedation, with a maximum decrease (up to 75%) at 30 min after sedation (Table 2). In the MIDA group, a decrease in *f* was only observed at the last evaluation, with a maximum reduction of 48% at 40 min. The *f* values in the DEX group were lower than those in the MIDA group at all evaluated time points after treatment was applied.

A decrease in the values of SAP, MAP, and DAP occurred 10 min after sedation in the DEX

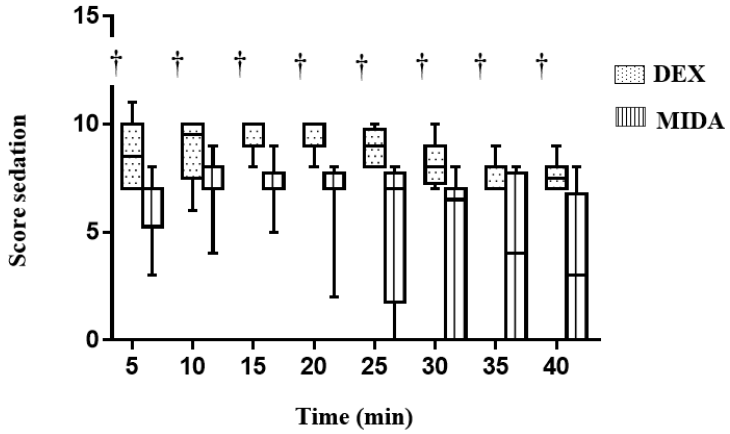

Figure 1 - Sedation scores in rabbits administered 25 mcg/kg dexmedetomidine, 2 mg/kg morphine and 1 mg/kg midazolam (DEX, n=8), or 2 mg/kg morphine and 1 mg/kg midazolam (MIDA, n=8).

The values are presented as block plots. Each box contains 50% of the data points. The bold horizontal line represents the median and the bars represent the score variation. † indicates a significant difference between experimental groups.

group, and they remained lower than the baseline values until the end of the evaluation. A reduction in the MIDA group was also observed for SAP at 40 min and for DAP at 10, 25, 30, and 40 min. The DEX group exhibited a lower SAP compared to the MIDA group from 5 min to 40 min after the start of treatment. The DEX group also exhibited lower MAP and DAP values compared to the MIDA group from 10 min to 35 min, and from 5 min to 35 min after the start of treatment, respectively (Table 2).

The rectal temperature was reduced from 20 to 40 min and from 15 to 40 min after the start of treatment in the DEX and MIDA groups, respectively, with no significant difference between the groups (Table 2). At 15 min and 20 min, SpO2 was lower in the DEX group compared to baseline, whereas no significant difference was observed in the MIDA group (Table 2). There was a reduction in dAo and A-wave deceleration time (DT:A) compared to baseline in the DEX group. In the MIDA group, left ventricle internal diameter in systole (LVIDs), ejection fraction (EF), fractional shortening (FS), and DTA were significantly different from baseline values (Table 3). Fifteen minutes after sedation, DTA was significantly lower in the DEX group than in the MIDA group (Table 3).

Both protocols resulted in satisfactory sedation levels; however, deeper and longer-lasting sedation was observed in the DEX group than in the MIDA group. Midazolam sedation doses used in rabbits can vary from 0.25 to 1 mg/kg (HENKE et al. 2005; LICHTENBERGER & KO., 2007). Therefore, in the present study, a higher sedation level was achieved without the need for additional doses, in contrast with the observation by BELLINI et al. (2014) that the use of 0.2 mg/kg midazolam combined with 30 mg/kg ketamine or 25 mcg/kg of dexmedetomidine requires supplementary doses to achieve the desired sedative effect. When used alone, the dexmedetomidine dose used in this study did not cause signs of sedation in rabbits (ZORNOW, 1991). We believe that deeper and more homogenous sedation was achieved in the DEX group due to the synergism between alpha-2 adrenergic receptor agonists, and benzodiazepines and opioids (BOL et al., 2000; HENKE et al., 2005). Our results corroborate those reported by BELLINI et al. (2014), who used 25 g/kg dexmedetomidine with 0.2 mg/kg midazolam. The combination of midazolam with dexmedetomidine and morphine improved the degree of sedation of the animals owing to its muscle relaxation effect, as observed by the sedation scores, and the increased ease in the handling of the sedated animals.

The mean latencies for both protocols tested in this study were similar to those reported in other studies that used combinations of alpha-2 adrenergic receptor agonists, opioids, and/or benzodiazepines (HENKE et al., 2005; BELLINI et al., 2014). On the other hand, great variability was observed between individual animals in both groups, in contrast to the shorter and more predictable

latency periods reported for protocols involving the use of dissociative drugs (AVSAROGLU et al., 2003). Although we initially expected that the DEX group would have a shorter latency period due to the combination of dexmedetomidine with other drugs, the inter-individual variations and the stress levels before treatment administration could have contributed to elicit a delay in the sedative effect (RAEKALLIO et al., 2002) and to the subsequent the absence of significant differences between the two groups. The duration of sedation was 48% higher in the DEX group than in the MIDA group, demonstrating the additive effect of the alpha-2 adrenergic receptor agonist (BOL et al., 2000).

There was a reduction in HR in the DEX group from 10 min onwards, which was possibly related to the chronotropic negative effects of alpha-2 adrenergic receptor agonists (GRANHOLM et al., 2007) and has previously been observed in rabbits (HENKE et al., 2005; BELLINI et al., 2014). The HR of the MIDA group was higher than that of the DEX group, but after 15 min became statistically lower when compared to baseline values, probably due to the increase vagal tone derived from opioid administration (HENCKE et al., 2005).

The combined use of different sedatives and/or analgesic drugs allows for an increase in the level, quality, and duration of sedation, although they can be associated with important physiological changes, especially in the respiratory system (FLECKNELL, 2015). A reduction in the value of *f* of up to 48% was noted in the MIDA group, and of up to 75% in the DEX group. The combination of midazolam with different opioids, such as butorphanol and buprenorphine, results in a decrease in *f*, which is linked to hypoxemia (SCHROEDER & SMITH, 2011). The combined use of opioids and other drugs can cause clinically relevant respiratory depression, especially when they are administered in the absence of pain (LINCHTENBERGER & KO, 2007; BARTER, 2014). The *f* values were lower in the DEX group than in the MIDA group between 5 min and 35 min after administration. This corroborates the results of HENCKE et al. (2005), who reported that periods of apnea followed the combined use of alpha-2 adrenergic receptor agonists, opioids, and benzodiazepines; and oxygen supplementation was required. BELLINI et al. (2014) demonstrated that dexmedetomidine combined with midazolam promoted lower *f* values when compared to the combination of midazolam and ketamine; however, there were no changes in the oxygen partial pressure (PaO2). These findings partially differ from those observed in the present study, in which *f* values were also lower in the DEX group at baseline but SpO2 values were lower after 15 min and 20 min, with the mean values at the other evaluation timepoints below 95% .

In the DEX group, hypoperfusion may have been caused by dexmedetomidine administration (SAZUKA et al., 2012), leading to interference with the pulse oximeter and subsequent underestimation of the assessed values. Although effects triggered by the pharmacological combinations used in the study may have caused a decrease in the mean SpO2 below the physiological values typical of the species, and the *f* values were statistically lower than the baseline values, the fact that rabbits are particularly susceptible to stress cannot be ignored, as evidenced by the high *f* values registered at baseline. Moreover, the decrease observed over time can be linked to the sedative effect of the protocols being tested, corroborating the results of SCHROEDER & SMITH (2011), as well as those from ORR et al. (2005). Unfortunately, arterial blood gas analysis, which would show in detail and confirm the actual ventilatory changes triggered by the administered treatments, could not be performed.

The arterial blood pressure values measured in the MIDA group were lower than the baseline values throughout the evaluation period, although they were still within the acceptable range for sedated animals. The effect of alpha-2 adrenergic agents on arterial pressure values in rabbits is unclear, because different studies have reported initial elevation followed by a decrease reaching hypotension levels (HENCKE et al., 2005), elevation and sustained high values (BELLINI et al., 2014), or minimal changes (SAZUKA et al.; 2012). Dexmedetomidine at a dose of 25 mcg/kg in combination with 2 mg/kg morphine and 1 mg/kg midazolam has been shown to be capable of eliciting a sustained decrease in the values of arterial pressure, which may result from inhibitory mechanisms of the sympathetic tonus (BEKKER & STURAITIS, 2005) and reduction in the cardiac output (SAZUKA et al., 2012).

Although there was a significant reduction in rectal temperature in both groups, the values remained within the normal range for the species. Body temperature reduction is a common effect of rapid occurrence in rabbits (BELLINI et al., 2014), but the reduction was of small magnitude in this case due to the use of an active thermal mattress and a constant room temperature (24 °C)

The Doppler echocardiographic values obtained at baseline were within the reference range for male New Zealand rabbits (FONTES-SOUSA et al., 2006; FONTES-SOUSA et al., 2009). Numerical

comparisons showed that the values for some of the variables assessed after treatment differed from the results obtained in the scientific literature when other sedative/anesthetic protocols were used (STYPMANN et al., 2007; FONTES-SOUSA et al., 2009; SILVA et al., 2011), demonstrating that different pharmacological combinations can result in differential changes in myocardial function.

During the performance of the Doppler echocardiography examination, some animals from the MIDA group presented some resistance when being restrained and positioned in lateral recumbency during the examination, resulting in a significant elevation of the Doppler-HR. This sympathetic stimulation was possibly a consequence of stress experienced by the patients during the restraining and positioning procedure resulting in turn from insufficient sedation and relaxation, and it could have contributed to the increase in contraction force and ejection volume, which explains the lower LVIDs and the higher EF and FS values observed in the MIDA group. Similar results due to pharmacological sympathomimetic effects have been reported with the use of ketamine in rabbits (STYPMANN et al., 2007; SILVA et al., 2011).

The mean dAo values at baseline were lower than those reported in other studies (FONTES-SOUSA et al., 2009; SILVA et al., 2011). Moreover, dAo was lower in the DEX group, which can be attributed to a reduction in cardiac output (SAZUKA et al., 2012). YAMAMOTO et al. (1993) indicated that the importance of the heart rate on the late diastolic filling velocity resides not only in the shortening or prolongation of the period of diastolic filling but also in the changes associated with hemodynamics, such as atrial contractility, left ventricular compliance, and heart rate, which may influence the A wave speed. Although significant changes due to the treatments were observed, all values were within the normal range for conscious New Zealand rabbits (FONTES-SOUSA et al., 2006).

Rabbits are commonly anesthetized for physical examinations or short surgical procedures, are frequently used as experimental models, and are susceptible to painful procedures without appropriate pain management (BARTER & KWIATKOWSKI, 2013; NAVARRETE-CALVO et al., 2013). Thus, the use of safe anesthetic protocols that allow for adequate sedation and provide sufficient analgesia is necessary to improve the well-being of these animals.

The lack of blood gas analysis in the animals is an important limitation of this study. Indirect evaluation of oxygenation using pulse oximetry does not reflect the efficiency of pulmonary gas exchange with sufficient accuracy, and it was therefore not possible to determine neither the effective degree of respiratory depression experienced by the animals nor the best approach to reverse this depression. Another limitation of this study is that only healthy animals and not those with previous disorders were recruited. Because of the intense depression caused by dexmedetomidine, cardiorespiratory monitoring is crucial to evaluate the need for oxygen supplementation in animals with respiratory depression and hypoxemia.

CONCLUSION

The combination of dexmedetomidine with midazolam and morphine at the proposed doses resulted in better quality and longer sedation times. Both protocols triggered cardiorespiratory changes that were well-tolerated by healthy animals; however, rabbits may experience hypotension, and oxygen supplementation is indicated when dexmedetomidine is being administered. The Doppler echocardiographic changes were similar to those obtained using other sedation protocols or reported as reference for the species. Thus, both treatment protocols tested in the present study can be readily applied in research as well as in routine clinical practice.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The present study was evaluated and approved by the institutional Ethics Committee under protocol number 94/2017.

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