ARTICLES
Archives of Veterinary Science
UFPR
https://www.ufpr.br/

Evaluation of antinociceptive and sedative effects of methadone or morphine in combination with ketamine-midazolam in *Oryctolagus cuniculus* (*Linnaeus*, 1758; European rabbits) undergoing elective orchiectomy

Submitted: *01/10/2024*
Accepted: *03/01/202*

Gilberto Serighelli Júnior[1], Amanda Boeno Riva[2], Daniel Sérgio Cipriani[2], Kelly Mota Fernandes[2], Gabriela Borges Conterno[3], Vanessa Arnaud Rocha[3], Samuel Jorge Ronchi[4], Maria Carolina de Souza[2], Felipe Comassetto[5], Aury Nunes de Moraes[5]*

[1]M.Sc., Veterinary Sciences Graduate Program, Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Brazil, State of Paraná, Brazil. ORCID:0000-0002-0979-7939
[2]Self-employed Veterinarian, State of Santa Catarina, Brazil. ORCID: 0009-0004-2757-7288; ORCID: 0000-0001-5767-0345; ORCID: 0000-0001-6404-6401; ORCID: 0009-0004-5539-5094
[3]M.Sc. Self-employed Veterinarian, State of Santa Catarina, Brazil. ORCID: 0000-0002-1256-4677; ORCID: 0009-0008-1297-6293
[4] Dr. Self-employed Veterinarian, State of Santa Catarina, Brazil. ORCID: 0000-0003-0556-6006
[5]Professor, Dr., Department of Veterinary Medicine, Center for Agro-Veterinary Sciences (CAV), Santa Catarina State University (UDESC), Lages, State of Santa Catarina, Brazil. ORCID: 0000-0002-7355-6064; ORCID: 0000-0003-4466-6595

Author for correspondence email: Gilberto Serighelli Júnior – *gilberto.serighelli@ufpr.br*

Abstract: The objective of this study was to evaluate the effect of adding methadone or morphine to an anesthetic protocol in combination with ketamine and midazolam in 24 rabbits undergoing elective orchiectomy. The animals, weighing 3.909±0.6693 kg, were evenly distributed into three groups, receiving 15 mg/kg of ketamine and 2 mg/kg of midazolam (GCON), associated with 2 mg/kg of methadone (GMET) or morphine (GMOR), intramuscularly. Cardiorespiratory parameters, blood gas analysis, and sedation degree were recorded at baseline (T-15), 15 minutes after premedication (T0), 5 minutes after local block (T1); clamping of the first spermatic cord (T2); second (T3), and immediately after scrotorraphy (T4). Sedation degree was assessed via posture (PS), resistance to dorsal recumbency (RD), jaw tone (JT), palpebral reflex (PR), total sedation score (TS), muscle relaxation (MR), and stimuli response (RS). The inclusion of a μ-agonist opioid enhanced the degree of sedation without negatively affecting anesthetic recovery. Results showed that methadone provided deeper sedation and greater respiratory depression, while morphine had a similar but less pronounced effect. GMET exhibited a significant reduction in respiratory rate ($p<0.0001$) and an increase in PaCO2 ($p<0.0001$), indicating more pronounced respiratory depression compared to the other groups. HR remained within normal limits for GMOR and GMET, whereas GCON showed a significant increase ($p=0.0221$). No significant changes in blood pressure were observed between groups. The sedation degree was significantly higher in GMET compared to GCON ($p=0.0018$). It is concluded that opioids improved sedation degree without compromising cardiovascular stability, though oxygen therapy was required due to respiratory depression, particularly with methadone.
Keywords: anesthesiology, lagomorphs, nociception, opioids, sedation.

1. Introduction

An increasing number of exotic species are being treated as pets, among which rabbits have gained prominence and are frequently attended by veterinarians for medical evaluation, diagnosis, and treatment. As their popularity grows, there is a rising demand for high-quality medical and surgical care, often requiring sedation (AVMA, 2017; Guardhouse & Sanchez, 2022). Sedation is commonly necessary for less invasive medical evaluations or procedures, while general anesthesia is widely used for medium to highly invasive medical and surgical procedures (Nobre et al., 2022). Successful anesthetic management in rabbits requires a thorough understanding of basic anesthetic principles, awareness of limitations, and the fulfillment of requirements to ensure high standards of anesthetic care for the patient (Chapel et al., 2017; Guardhouse & Sanchez, 2022). However, anesthetic protocols that involve effective and safe analgesia for rabbits remain limited, with intraoperative nociception and prolonged recovery times being among the main causes of mortality in this species, which is believed to be approximately six to eight times higher than in dogs and cats (Nobre et al., 2022; Benato et al., 2018).

Pain is classified as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, with nociception being its sensory component. According to Nobre et al. (2022), painful and nociceptive stimuli in animals trigger a series of negative physiological changes, such as increased cardiac function and blood pressure, alterations in respiratory patterns and function, abnormal behavior, and animal stereotypies, consequently impacting their health and well-being. It is known that more than half of qualified professionals today cannot recognize pain in rabbits when it is present, and a considerable portion of them do not feel equipped to treat it even when identified (Benato et al., 2018). Therefore, the control of nociception and pain in these animals is a fundamental component of the success of surgical procedures. According to Cintra et al. (2017), among the most studied analgesic drugs, opioids stand out for being widely known and used in several species, even though they may induce a potential reduction in gastrointestinal motility, which, according to Touzot-Jourde et al. (2015) and Benato et al. (2018), does not seem to have clinical relevance in rabbits. These drugs act at the central nervous system (CNS) level by interacting with endogenous opioid receptors μ, κ, σ, and ε, triggering a synergistic effect with other drugs and providing dose-dependent neuroleptanalgesia specific to the species (Raillard et al., 2016; Comassetto et al., 2014).

The present study aimed to evaluate the antinociceptive and sedative effects, as well as the cardiovascular, respiratory, and subsequent impact on arterial blood gas parameters, of the combination of ketamine and midazolam, administered alone or in

combination with methadone or morphine in rabbits undergoing elective orchiectomy. It was hypothesized that the addition of morphine and methadone provides a greater degree of sedation compared to ketamine and midazolam alone but that the inclusion of methadone results in increased cardiorespiratory instability.

2. Materials and Methods

Twenty-four male, crossbred rabbits, approximately 10 months old, with an average weight of 3.909 (± 0.6693) kg, considered clinically healthy after evaluation based on general physical examinations and hematological tests, were used. A completely randomized design with three balanced randomization sequences (1:1:1) and a placebo control was employed. The sample size was calculated using the OpenEpi 3.0 software, with 80% statistical power and a 5% confidence interval (bilateral). The animals were housed in individual cages, under natural light and room temperature, at the Veterinary Hospital of the University of Santa Catarina State (UDESC) for a four-month acclimation period. Water was provided ad libitum, and the diet consisted of commercial feed and natural food administered twice daily. This study was approved by the UDESC Animal Ethics Committee (CEUA) under protocol no. 5214300418.

The animals underwent a 4-hour pre-procedural fasting period without water deprivation. They were then randomly assigned in a blinded manner to three groups using www.randomization.com: ketamine and midazolam group (GCON, n = 8), which received 15 mg/kg of ketamine (Cetamin 100 mg/ml, Syntec Ltda., Barueri, Brazil) and 2 mg/kg of midazolam (Dormire 5 mg/ml, Cristália Ltda., Itapira, Brazil); ketamine, midazolam, and methadone group (GMET, n = 8), which received 15 mg/kg of ketamine, 2 mg/kg of midazolam and 2 mg/kg of methadone (Mytedom, 10 mg/ml, Cristália Ltda., Itapira, Brazil); and the ketamine, midazolam, and morphine group (GMOR, n = 8), which received 15 mg/kg of ketamine, 2 mg/kg of midazolam, and 2 mg/kg of morphine (Dimorf 10 mg/ml, Cristália Ltda., Itapira, Brazil), administered intramuscularly (IM). To ensure scientific rigor, this three-sequence study was conducted at a single location, followed the ARRIVE guidelines, and was performed in a blinded fashion with standardized administration volumes, diluting the treatments in 0.9% saline solution to a final volume of 1 ml.

The animals were evaluated at the following time points: T-15 (baseline), T0 (15 minutes after protocol administration), T1 (5 minutes after local block), T2 (clamping of the first spermatic cord), T3 (clamping of the second spermatic cord), and T4 (immediately after scrotorraphy). The physiological parameters analyzed included heart rate (HR - bpm), partial hemoglobin oxygen saturation (SpO_2 - %), respiratory rate (RR - breaths per minute), systolic arterial pressure (SAP - mmHg), mean arterial pressure (MAP - mmHg), diastolic arterial pressure (DAP - mmHg), and rectal temperature (RT - °C). The blood gas parameters assessed were pH, arterial oxygen pressure (PaO_2 - mmHg), arterial carbon dioxide pressure ($PaCO_2$ - mmHg), corrected oxygen saturation (SO_2c - %), bicarbonate (HCO_3^- - mmol/L), base excess (BE - mmol/L), sodium (Na^+ - mmol/L), potassium (K^+ - mmol/L), ionized calcium (Ca++ - mmol/L), chloride (Cl- - mmol/L), and anion gap (AG - mmol/L) were measured using the Omni C hemogasometer; Roche Diagnostics, ZG, Switzerland.

Physiological parameters were monitored at all time points using a multiparameter monitor (B650 monitor, GE–Datex-Ohmeda), except for respiratory rate, which was measured by observing and counting intercostal movements. Hemogasometric analyses were performed on arterial blood samples, with cannulation of the marginal ear artery using a catheter (24 gauge, Safelet-ETFE; Nipro, SP, Brazil). The arterial catheter was connected to a pre-calibrated disposable pressure transducer (Utah/Biotrans, GE Healthcare, Chicago, USA) and a monitor (Datex-Ohmeda, B650 monitor; GE Healthcare, Chicago, USA). The accuracy of the calibration was guaranteed using a dynamic response test (square wave test). This system was connected via a low-compliance pressure tube containing heparinized saline solution from a pressurized bag set to 300 mmHg. The heparinized saline solution (10 IU/ml) was infused at a rate of 1 ml/h to prevent clot formation. Samples were collected at T-15, T0, T2, and T4 (Schroeder & Smith, 2011). The same arterial catheter was used for invasive blood pressure measurement. Venous access was established in the contralateral marginal auricular vein for fluid therapy with 0.9% NaCl solution, administered at 5 ml/kg/h (Westphal et al., 2010). Arterial and venous accesses were made before the treatment was administered.

After administering the treatment, the animals were placed in appropriate individual cages with light restriction and no handling, waiting for a 15-minute latency period. Two sedation scales were used to assess the progression of the protocols. The scale used by Bellini et al. (2014) was applied between T0 and T4, assessing posture (PS), dorsal decubitus resistance (RD), mandibular tone (JT), eyelid reflex (PR), and total sedation score (TS), with a scoring range from "0" to "11", where the highest score represented the highest level of sedation. The intraoperative scale, adapted from Comasseto et al. (2014), was applied between T2 and T4 to evaluate muscle relaxation (MR) and response to stimuli (RS), scoring from "0" to "6", where a score of "0" represented the lowest possible sedation and "6" represented the highest sedation, with no response to stimuli.

Following the initial evaluation with the scales, the animals underwent surgery in an environment heated to 21.0 ± 1.0 °C. They were positioned in dorsal recumbency and received 100% oxygen supplementation via a face mask. The procedure was always performed by the same surgeon and began with trichotomy and antisepsis of the scrotal region, followed by local anesthetic block with intratesticular and incisional administration of 2 mg/kg of 2% lidocaine without vasoconstrictor (Xylestesin 20 mg/ml, Cristália Ltda, Itapira, Brazil), using the stylet of a 24G catheter. Five minutes after the block, orchiectomy was initiated, and evaluations were carried out at T1, T2, T3, and T4.

In the postoperative period, the animals were transferred to a recovery room equipped with a veterinary heating pad to ensure proper anesthetic recovery. The parameters evaluated included time to sternal recumbency (SR - min), ambulation (AM - min), and full recovery (FR - min). Recovery quality was assessed using a simple descriptive scale adapted from Dehousser et al. (2019), classified as poor (score of zero), characterized by vocalization and agitation, or good (score of one), with no vocalization or agitation.

Intraoperative analgesic rescue was standardized with fentanyl (2.5 µg/kg, IV) (Fentanest, 0.05 mg/ml, Cristália Ltda., Itapira, Brazil) if there was a 20% increase in at least two of the three main parameters (HR, RR, and SAP) relative to T0 (Cubas et al., 2020; Raillard et al., 2016). In cases of bradycardia (HR < 120 bpm), atropine (Atropina 0.25mg/ml, B-Braun Ltda., Canoas, Brazil) (0.1 mg/kg, IV) was administered (Cubas et al., 2020). For hypotension (SAP < 90 mmHg or MAP < 60 mmHg), a continuous infusion of dobutamine (Cloridrato de dobutamina 250 mg/20ml, Hipolabor Ltda., Belo Horizonte, Brazil) (5 µg/kg/min, IV) was initiated (Sun et al., 2015). In the event of apnea, supraglottic airway devices were used for intubation and assisted ventilation until resolution (Kazakos et al., 2007). Any described anesthetic complications resulted in the animal's exclusion from the study. Prophylactic antibiotic therapy consisted of enrofloxacin (Chemitril 2.5%, Cristália Ltda, São Paulo, Brazil) 5 mg/kg, IM, BID, for 7 days, along with meloxicam (Maxicam 0.2%, Ourofino Saúde Animal, Vinhedo, Brazil) 0.2 mg/kg, SC, SID and dipyrone (Dipirona 50%, Ibasa, Porto Alegre, Brazil) 25 mg/kg, SC, BID for 3 days (Cubas et al., 2020; Lupu et al., 2022).

2.1. Statistical analyses

Statistical analyses were performed using the Prisma software, version 9.3.0. All data were subjected to the Shapiro-Wilk normality test. Parametric data were analyzed using one-way ANOVA, followed by Tukey's test for multiple group comparisons. For multiple comparisons between time points within the same group, repeated measures ANOVA was used, followed by Dunnett's test. Parametric results were expressed as mean and standard deviation. Non-parametric data were analyzed using the Kruskal-Wallis test, followed by Dunn's test for multiple comparisons, with the results expressed as median (minimum and maximum values). A significance level of $p < 0.05$ was adopted.

3. Results

There were no significant differences in weight between the groups, with an average of 3.909 (± 0.6693) kg ($p = 0.1348$). The ages were 10 ± 2 months ($p = 0.3492$). All the animals underwent physical examinations to confirm their health and were classified as ASA I, according to the American Society of Anesthesiologists. The heart rate (HR) of the animals remained within normal ranges (Table 1), according to the values obtained by Chapel et al. (2017) and Cubas et al. (2020), for all groups and at all times. GMOR and GMET showed no statistical differences between the moments within the same group compared to T-15, whereas GCON showed a significant increase at all time points analyzed, with an average increase of 27.45% ($p = 0.0221$). From T2, HR in GMET was statistically lower than in GCON by up to 19.8% ($p = 0.0383$), while GMOR showed no significant differences compared to the other groups.

	Groups	Time points					
		T-15	T0	T1	T2	T3	T4
HR (bpm)	GCON	213.4 ± 31	277.3 ± 23A	267.4 ± 28A	277.8 ± 21Aa	273.6 ± 39Aa	263.8 ± 33Aa
	GMOR	239.4 ± 30	265.1 ± 36	251.6 ± 23	245.1 ± 26ab	245.1 ± 24ab	237.9 ± 22ab
	GMET	250.1 ± 35	281.4 ± 15	233.1 ± 46	239.1 ± 36b	219.5 ± 36b	217.9 ± 36b
RR (mpm)	GCON	131.8 ± 56	60.3 ± 42A	50.3 ± 16Aa	62.0 ± 16Aa	65.3 ± 33Aa	71.1 ± 19Aa
	GMOR	168.5 ± 41	66.5 ± 44A	32.5 ± 13Ab	37.5 ± 26Aab	35.5 ± 15Ab	47.5 ± 40Ab
	GMET	168.8 ±62	47.0 ± 28A	16.8 ± 09Ab	19.5 ± 16Ab	15.8 ± 11Ab	23.3 ± 15Ab
SpO_2 (%)	GCON	92.6 ± 02	88.0 ± 03Aa	98.9 ± 01Aa	97.0 ± 03	97.1 ± 03A	98.3 ± 03A
	GMOR	92.6 ± 03	88.4 ± 05a	96.8 ± 04a	95.4 ± 06	97.3 ± 02A	96.1 ± 05
	GMET	93 ± 04	78.4 ± 09Ab	86.9 ± 09b	95.5 ± 04	95.9 ± 04	97.3 ± 03
SAP (mmHg)	GCON	107.1 ± 17	103.5 ± 11	100.6 ± 10	103.4 ± 11	104.8 ± 13	100.3 ± 10
	GMOR	99.0 ± 13	102.5 ± 18	88.4 ± 16	91.4 ± 15	95.4 ± 14	94.1 ± 14
	GMET	104 ± 10	92.6 ± 12	103.3 ± 20	106.3 ± 19	98.5 ± 08	97.6 ± 14
DAP (mmHg)	GCON	90.0 ± 14	88.8 ± 08	86.9 ± 10	92.6 ± 08	91.4 ±12	86.5 ± 12
	GMOR	76.9 ± 14	79.8 ± 16	70.0 ± 15	72.9 ± 18	77.0 ± 15	72.9 ± 12
	GMET	85.1 ± 07	73.4 ± 12	84.3 ± 19	83.4 ± 19	79.8 ± 09	73.8 ± 15
MAP (mmHg)	GCON	95.0 ± 10	95.1 ± 08	91.1 ± 11	97.6 ± 09	96.6 ± 12	90.6 ± 08
	GMOR	83.5 ± 12	88.0 ± 17	78.1 ± 13	79.5 ± 17	84.1 ± 15	81.1 ± 12
	GMET	93.1 ± 07	80.1 ± 11	93.3 ± 20	93.9 ± 20	88.0 ± 08	81.3 ± 14
RT (ºC)	GCON	38.7 ± 0.8	38.9 ± 0.9	39.2 ± 0.5	39.1 ± 0.5	39.0 ± 0.6	39.0 ± 0.6
	GMOR	39.2 ± 0.6	39.2 ± 0.6	39.3 ± 0.5	39.1 ± 0.6	38.8 ± 0.7	38.6 ± 0.7
	GMET	38.7 ± 0.5	39.2 ± 0.8	39.0 ± 0.2	38.8 ± 0.3	38.6 ± 0.3	38.4 ± 0.5

Table 1 – Mean values and standard deviations of the cardiorespiratory parameters analyzed (heart rate - HR; partial oxygen saturation of hemoglobin - SpO_2; respiratory rate - RR; systolic arterial pressure - SAP, mean arterial pressure - MAP, and diastolic arterial pressure - DAP; and rectal temperature - RT) in rabbits subjected to an anesthetic protocol composed of ketamine and midazolam (GCON); ketamine, midazolam, and morphine (GMOR); or ketamine, midazolam, and methadone (GMET), for elective orchiectomy (T-15 - baseline; T0 - 15

minutes after protocol administration; T1 - 5 minutes after local block; T2 - clamping of the first spermatic cord; T3 - clamping of the second spermatic cord; and T4 - immediately after scrotorraphy).
Obs: Uppercase letters within rows for the same variable indicate a difference in relation to the baseline moment (T-15). Lowercase letters within rows for the same variable in the same column indicate a difference between groups at the same time point.

There were no statistical differences between time points or between groups in systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) for GCON, GMOR, and GMET (Table 1). Rectal temperature (RT) also showed no statistical differences between groups or within groups across time points (Table 1).

A significant decrease in respiratory rate (RR) was observed in all groups from T0 compared to T-15 (Table 1). GMET showed a significant average reduction of 85.50% in RR relative to T-15 ($p < 0.0001$). Similarly, GMOR and GCON exhibited average reductions of 73.95% ($p < 0.0001$) and 53.11% ($p < 0.0001$), respectively, relative to T-15. In terms of oxygen saturation (SpO2), GMET differed significantly from GCON and GMOR at T0 and T1, while GMOR did not present significant differences compared to GCON at any of the evaluated time points.

The pH variable showed a significant decrease from T-15 in the GMOR group, with reductions observed at T2 ($p = 0.0247$) and T4 ($p = 0.039$) (Table 2). $PaCO_2$ significantly increased in GCON at T2, while GMOR and GMET showed increases from T0, with an average increase of 13.18% in GCON ($p = 0.089$), 46.71% in GMOR ($p < 0.0001$), and 89.89% in GMET ($p < 0.0001$). PaO2 showed a significant decrease in all groups compared to T-15, with GMET demonstrating statistical differences at T0. Corrected oxygen saturation (SO2c) also showed a significant decrease in GMET compared to GCON and GMOR at T0. Na+, K+, Ca++, and Cl- levels remained within normal values for the species.

	Groups	Time points			
		T-15	T0	T2	T4
pH	GCON	7.32 ± 0.17	7.38 ± 0.14	7.36 ± 0.09	7.38 ± 0.07
	GMOR	7.44 ± 0.07	7.43 ± 0.03	7.33 ± 0.03A	7.36 ± 0.05A
	GMET	7.40 ± 0.05	7.39 ± 0.03	7.27 ± 0.11	7.24 ± 0.19
PaO_2 (mmHg)	GCON	75.1 ± 10	62.1 ± 04Aa	389.8 ± 69A	405.1 ± 47A
	GMOR	78.2 ± 07	58.4 ± 05Aa	414.0 ± 56A	344.1 ± 177A
	GMET	81.6 ± 05	48.5 ± 07Ab	329.6 ± 100A	294.0 ± 134A
$PaCO_2$ (mmHg)	GCON	35.1 ± 09a	38.9 ± 03A	43.7 ± 04Aa	41.2 ± 04a
	GMOR	28.9 ± 03ab	37.3 ± 03A	53.6 ± 05Aab	49.8 ± 05Aab
	GMET	27.7 ± 02b	39.7 ± 04A	67.3 ± 23Ab	75.7 ± 40Ab
SO_2c (%)	GCON	90.8 ± 10.3	90.0 ± 5.5a	99.9 ± 0.1	99.9 ± 0.1
	GMOR	95.8 ± 0.9	90.6 ± 2.3Aa	99.9 ± 0.1A	98.7 ± 2.4A
	GMET	95.6 ± 0.8	82.2 ± 6.1Ab	98.7 ± 2.2A	98.9 ± 1.9A
HCO_3^- (mmol L^{-1})	GCON	18.61 ± 6.33	23.61 ± 6.74A	24.46 ± 3.92Aa	24.20 ± 4.20A
	GMOR	19.61 ± 3.85	24.44 ± 2.57A	27.93 ± 3.37Aab	28.06 ± 3.41A
	GMET	16.95 ± 2.51	23.40 ± 2.14A	29.03 ± 2.47Ab	28.80 ± 4.81A
BEc (mmol L^{-1})	GCON	- 7.28 ± 8.56	- 1.48 ± 8.85A	- 0.93 ± 5.20	- 0.90 ± 5.22A
	GMOR	- 4.46 ± 4.80	0.20 ± 2.95A	2.06 ± 3.76A	2.75 ± 3.93A
	GMET	- 7.84 ± 3.26	- 1.58 ± 2.36A	1.95 ± 1.90A	1.41 ± 4.84A
Na^+ (mmol L^{-1})	GCON	142.60 ± 3.49	142.30 ± 2.10	141.70 ± 1.52a	141.30 ± 1.60a
	GMOR	144.70 ± 2.51	144.40 ± 2.85	145.00 ± 2.31b	144.90 ± 1.56b
	GMET	144.60 ± 2.18	144.20 ± 1.70	145.40 ± 2.40b	145.00 ± 1.70b
K^+ (mmol L^{-1})	GCON	4.07 ± 0.44a	3.60 ± 0.31A	3.50 ± 0.48Aa	3.24 ± 0.35A
	GMOR	3.55 ± 0.23b	3.35 ± 0.37	3.14 ± 0.16Ab	3.19 ± 0.22A
	GMET	3.70 ± 0.22b	3.58 ± 0.24	3.32 ± 0.21Aab	3.30 ± 0.33A
Ca^{++} (mmol L^{-1})	GCON	1.28 ± 0.14	1.25 ± 0.20	1.25 ± 0.19	1.18 ± 0.06
	GMOR	1.16 ± 0.11	1.22 ± 0.22	1.32 ± 0.10A	1.29 ± 0.12A
	GMET	1.17 ± 0.13	1.13 ± 0.14	1.15 ± 0.17	1.19 ± 0.10
Cl^- (mmol L^{-1})	GCON	104.80 ± 3.38	103.60 ± 2.83	103.10 ± 1.43	103.30 ± 1.86
	GMOR	107.80 ± 2.15	106.00 ± 3.14	105.10 ± 1.83A	105.20 ± 1.56A
	GMET	107.30 ± 1.83	105.60 ± 1.77A	105.20 ± 2.04A	104.70 ± 1.67A
AG (mmol L^{-1})	GCON	23.26 ± 5.52	18.66 ± 6.19A	17.66 ± 3.02A	17.01 ± 2.90A
	GMOR	20.79 ± 3.94	17.29 ± 1.69A	15.13 ± 1.97A	14.85 ± 1.93A
	GMET	24.01 ± 3.34	18.88 ± 1.67A	14.60 ± 2.32A	14.85 ± 3.84A

Table 2 – Mean values and standard deviations of the arterial blood gas parameters analyzed (hydrogen potential - pH; arterial oxygen pressure - PaO_2; arterial carbon dioxide pressure - $PaCO_2$; corrected oxygen saturation - SO_2c; bicarbonate - HCO_3^- corrected base excess - BE; sodium - Na^+; potassium - K^+; ionized calcium - Ca^{++}; chloride - Cl^-, and anion gap - AG) in rabbits subjected to an anesthetic protocol composed of ketamine and midazolam (GCON); ketamine, midazolam, and morphine (GMOR); or ketamine, midazolam, and methadone (GMET) for elective orchiectomy (T-15 - baseline; T0 - 15 minutes after protocol administration; T2 - clamping of the first spermatic cord, and T4 - immediately after scrotorraphy).
Obs: Uppercase letters within rows for the same variable indicate a difference in relation to the baseline moment (T-15), after analysis by Repeated Measures One-way ANOVA followed by Dunnett's test ($p < 0.05$). Lowercase letters within rows for the same variable in the same column indicate a difference between groups at the same time point.

The assessment of sedation quality, based on the specific scale for rabbits proposed by Bellini et al. (2014), is presented in Table 3. GMET showed significantly superior sedation compared to GCON, with statistical differences in the variables posture (PS) at T3 ($p = 0.0031$) and T4 ($p = 0.0053$), resistance to dorsal recumbency (RD) at T3 ($p = 0.083$), and palpebral reflex (PR) at T3 ($p = 0.01602$). GMET's total score (TS) was also significantly higher than GCON at T2 and T3 ($p = 0.0018$). GMOR did not show significant differences compared to GCON or GMET for the analyzed variables and time points. Both GMET and GMOR reached scores close to the maximum on the scale (11 points), suggesting a higher degree of sedation, while GCON scored a lower score, indicating a lower degree and quality of sedation (Figure 1).

	Groups	Time points				
		T0	T1	T2	T3	T4
PS	GCON	4 [3-5]	4.5 [3-5]	4 [3-5]	4 [3-5]a	4 [3-5]a
	GMOR	4.5 [4-5]	4.5 [4-5]	5 [5-5]	5 [4-5]	4.5 [4-5]
	GMET	4.5 [4-5]	5 [5-5]	5 [5-5]	5 [5-5]b	5 [5-5]b
RD	GCON	2 [1-3]	2.5 [1-3]	2 [1-3]	2 [1-3]a	2 [1-3]
	GMOR	3 [2-3]	3 [3-3]	3 [3-3]	3 [2-3]	3 [2-3]
	GMET	3 [2-3]	3 [3-3]	3 [3-3]	3 [3-3]b	3 [3-3]
JT	GCON	0 [0-1]	0.5 [0-1]	0 [0-1]	0 [0-1]	0 [0-1]
	GMOR	0 [0-0]	0.5 [0-1]	0.5 [0-1]	0.5 [0-1]	0.5 [0-1]
	GMET	0 [0-1]	1 [0-1]	1 [0-1]	1 [0-1]	1 [0-1]
PR	GCON	1 [0-2]	1 [0-2]	1 [0-2]	1 [0-2]a	1 [0-1]
	GMOR	1 [0-1]	1 [1-2]	1 [1-2]	1 [1-2]	1 [1-2]
	GMET	1 [0-2]	2 [1-2]	2 [1-2]	2 [2-2]b	2 [1-2]
TS	GCON	7.5 [5-11]	9.5 [4-11]	8 [4-9]a	7 [4-9]a	8.5 [5-13]a
	GMOR	8 [6-9]	9 [9-11]	9.5 [9-11]	9.5 [7-11]	9 [7-11]
	GMET	8.5 [7-10]	11 [9-11]	11 [10-11]b	11 [10-11]b	11 [9-11]b
MR	GCON	-	-	1 [1-3]a	1 [0-2]a	1,5 [1-3]
	GMOR	-	-	3 [3-3]b	3 [2-3]b	3 [2-3]
	GMET	-	-	3 [3-3]b	3 [3-3]b	3 [3-3]
RS	GCON	-	-	1.5 [1-2]a	0.5 [0-2]a	1.5 [1-2]a
	GMOR	-	-	3 [3-3]b	2.5 [2-3]b	2.5 [1-3]b
	GMET	-	-	3 [3-3]b	3 [3-3]b	3 [3-3]b

Table 3 – Total score sedation (median) followed by the maximum and minimum values of the scores obtained using the sedation scale proposed by Bellini et al. (2014) (posture - PS; resistance to dorsal recumbency - RD; jaw tone - JT; palpebral reflex - PR, and total sedation score - TS) and using the sedation scale adapted from Comasseto et al. (2014) (muscle relaxation - MR and response to stimuli - RS) in rabbits subjected to an anesthetic protocol composed of ketamine and midazolam (GCON); ketamine, midazolam, and morphine (GMOR); or ketamine, midazolam, and methadone (GMET). For elective orchiectomy (T0 - 15 minutes after protocol administration; T1 - 5 minutes after local block; T2 - clamping of the first spermatic cord; T3 - clamping of the second spermatic cord, and T4 - immediately after scrotorraphy).
Obs: Lowercase letters within rows for the same variable in the same column indicate a difference between groups at the same time point.

The sedation quality score, according to the scale adapted from Comasseto et al. (2014), is shown in Table 4. GMOR and GMET showed significant improvements in sedation quality compared to GCON at T2 ($p = 0.0260$ and $p = 0.0260$) for the variables muscle relaxation (MR) and response to stimuli (RS) ($p < 0.0001$ and $p < 0.0001$, respectively), with no statistical differences between GMOR and GMET. GMET and GMOR were significantly superior to GCON at T3 ($p = 0.0011$ and $p = 0.0015$) for MR and ($p = 0.001$ and $p = 0.0018$) for RS, and at T4 only for RS ($p = 0.0192$ and $p = 0.0025$).

Latency time showed no significant differences between GCON, GMOR, and GMET. Similarly, in the postoperative period, the scores obtained using the simple descriptive scale adapted from Dehousser et al. (2019) resulted in medians of 1 [1-1] for all groups,

indicating satisfactory anesthetic recovery across all treatments. Regarding recovery times, a numerical increase was observed with the use of methadone, compared to the control group (GCON), the time to sternal recumbency (SR) increased by 85.9%, time to ambulation (AM) by 63.8%, and time to full recovery (FR) by 7.1%. In contrast, the use of morphine led to reductions in SR (8.5%), DM (6.0%), and FR (9.7%) times compared to GCON.

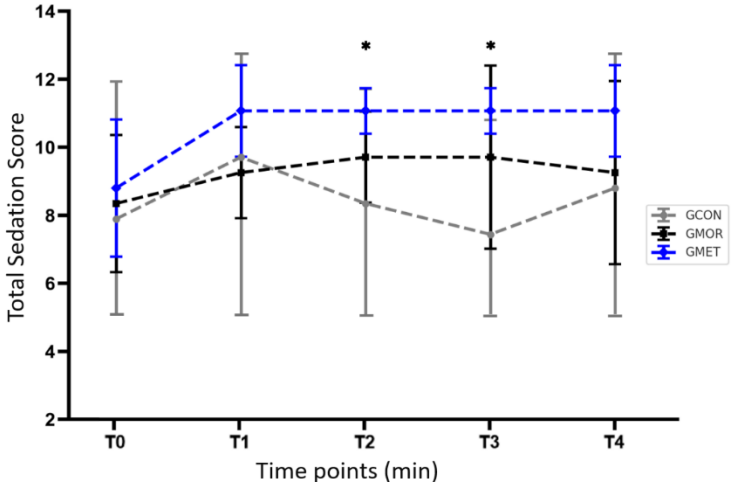

Figure 1 – Total sedation score (median) followed by the respective standard deviations, maximum and minimum values of the scores obtained by summing the subitems of the sedation scale proposed by Bellini et al. (2014) (posture - PS; resistance to dorsal recumbency - RD; jaw tone - JT; palpebral reflex - PR) in rabbits subjected to an anesthetic protocol composed of ketamine and midazolam (GCON); ketamine, midazolam, and morphine (GMOR); or ketamine, midazolam, and methadone (GMET) for elective orchiectomy (T0 - 15 minutes after protocol administration; T1 - 5 minutes after local block; T2 - clamping of the first spermatic cord; T3 - clamping of the second spermatic cord; and T4 - immediately after scrotorraphy).
Obs: * Indicates a difference between GMET and GCON at the same time point.

4. Discussion

The results of this study confirm the initial hypothesis by demonstrating that the heart rate (HR) of the animals was significantly influenced by the administration of μ-agonist opioids, with more pronounced effects for methadone compared to morphine, as observed by Benato et al. (2018) and Silva et al. (2011). The reduction in HR in the GMET and GMOR groups can be attributed to the stimulation of central vagal tone and negative chronotropy through the increased central nervous system vagal efferent impulses induced by opioids, along with the positive chronotropic and inotropic effects of ketamine, which counterbalanced the negative effects of the opioids. Methadone, in particular, caused a more pronounced and prolonged decrease in HR, as noted by Cintra et al. (2017) and Maiante et al. (2009), which can be explained by its additional affinity for NMDA and alpha-2 adrenergic receptors, as well as its higher analgesic potency compared to morphine (Bitti et al., 2017). The prolongation of the Q-T interval associated with methadone, as described by Govoni et al. (2020), may also explain this greater reduction in HR compared to morphine, in addition to its effect on the release of vasopressin, which elevates blood pressure and consequently reduces HR (Bitti et al., 2017). The significant increase in HR observed in the control group (GCON), which did not receive opioids, probably reflects the sympathomimetic action of ketamine, as pain was ruled out as a cause (Cubas et al., 2020; Raillard et al., 2016). In contrast, in the GMOR and GMET groups, the use of opioids stimulated the parasympathetic pathways, resulting in the maintenance of HR (Govoni et al., 2020; Benato et al., 2018).

The stability of blood pressure parameters (SAP, DAP, and MAP) between the groups reinforces that the combination of ketamine and midazolam was effective in maintaining blood pressure within normal limits, even with the concomitant use of opioids. This effect was facilitated by the absence of inhalant agents, which often cause vasodilation and reduce systemic vascular resistance (SVR) (Uccello et al., 2020; Silva et al., 2011). Although there were no statistically significant differences, SAP and MAP values in the GMET group were slightly higher compared to the GMOR group, which may be related to the effect of vasopressin release, increasing systemic vascular resistance (SVR) and, consequently, blood pressure (Bitti et al., 2017). However, intervention with agents such as atropine or dobutamine was not required, suggesting that the protocol used was safe from a cardiovascular perspective.

Rectal temperature (RT) remained stable across groups, corroborating the findings of Albozachri et al. (2019) and Canpolat et al. (2016), who indicate that low-invasiveness, short-duration surgical procedures, combined with adequate thermal support, minimize the effects of μ-agonist opioids on body temperature regulation. The CNS depressant effect at the hypothalamic level caused by therapeutic doses of morphine or methadone does not trigger significant hypothermia, as seen in the present study, where the values remained within normal ranges for all groups and at all time points (Cubas et al., 2020).

Respiratory depression caused by opioids was evident in the reductions in respiratory rate (RR) and oxygen saturation (SpO_2), with GMET showing the greatest decreases, confirming the more pronounced effects of methadone on the respiratory system, as described by Hunter et al. (1968). The more significant respiratory depression with methadone can be attributed to its ability to increase the CO_2 response threshold in the respiratory center (Nobre et al., 2022). Oxygen therapy starting at T2 appears to have

been a significant variable in stabilizing SpO_2, suggesting that the initial impact of respiratory depression was mitigated by oxygen supplementation. These findings, along with blood gas analysis, showed that the use of opioids, particularly methadone, resulted in hypercapnia and respiratory acidosis, as evidenced by increases in $PaCO_2$ and decreases in pH (normal values for the species range between 7.35 and 7.54) (Horr et al., 2019; Hunter et al., 1968). Metabolic compensation was observed through increased HCO_3^- levels, indicating a buffering compensatory response to attempt to stabilize pH (Caldwell et al., 2022; Aidar et al., 2020). These findings confirm that μ-agonist opioids, especially methadone, induce significant respiratory depression in rabbits, as described by Nobre et al. (2022).

The main cause of the observed electrolyte variations is believed to be respiratory acidosis, which in this study is indirectly correlated with opioid use. Studies such as those by Reece et al. (2017) have observed variations in the levels of the main measurable cations, as well as Cl^- and HCO_3^-, in individuals being treated with opioids, which has been attributed mainly to the acidemia observed in such patients and, to a lesser extent, to the ability of these drugs to indirectly affect the Ca^{++} dependent co-transport of Na^+, K^+, and Cl^- at the central nervous system level. However, it cannot be definitively stated that this is a valid variable to explain the electrolyte alterations found in this study. About the Cl^- values observed, another explanation is the exchange of this anion with intracellular HCO_3^-, related to hypercapnia. This change is compatible with the increase in both arterial blood gas variables, HCO_3^- and $PaCO_2$, as well as the decrease in Cl^- levels at T0 (Caldwell et al., 2022). (Caldwell et al., 2022).

Concerning sedation deeper, similar data are reported by Touzot-Jourde et al. (2015), demonstrating the superior effect of methadone regarding the quality of sedation in anesthetic protocols involving its use compared to other pure μ-agonist opioids in rabbits. This occurs because methadone has an additional effect on NMDA and alpha-2 adrenergic receptors present in the central nervous system and peripheral ganglionic branches, triggering an additive and synergistic effect with other anesthetic drugs compared to pure μ-agonist opioids, which lack such additional depressant effects as seen with morphine (Cintra et al., 2017; Maiante et al., 2009). Similarly, when evaluating muscle relaxation quality and response to stimuli using the scale adapted from Comasseto et al. (2014), the addition of a μ-agonist opioid to the anesthetic protocol in rabbits showed a synergistic drug effect, as evidenced in other species, enhancing sedation and improving its overall quality (Albozachri et al., 2019; Canpolat et al., 2016; Comasseto et al., 2014).

Finally, the latency time for the onset of sedation was significantly shorter with methadone, which can be attributed to its higher liposolubility and rapid passage through the blood-brain barrier (Nobre et al., 2022). This, combined with its longer-lasting analgesic effect, explains the 25.8% reduction in latency time with methadone compared to morphine. In the postoperative period, the good anesthetic recovery observed in all groups can be attributed to the local anesthetic block with lidocaine, which provided adequate analgesia during the procedure and in the immediate postoperative period, as described by Aksoy et al. (2009). Concerning recovery times, it suggests that methadone, at an equivalent dose, exerts a more pronounced depressant effect than morphine in rabbits, as evidenced by the longer recovery times. Morphine, on the other hand, appears to be a more advantageous option for anesthetic recovery, reducing the times for adopting sternal recumbency ambulation and achieving full recovery. These findings support the notion that, despite the deeper sedation provided by methadone, morphine may offer a faster and smoother recovery, which is clinically relevant when selecting an anesthetic protocol for this species.

A limitation of this study is the subjective nature of some sedation assessments, although this was mitigated by the use of consistent evaluation by the same blinded assessors. Additionally, the use of healthy, experimental rabbits limits the generalizability of the findings, as the effects of opioids may vary significantly in hemodynamically unstable or clinically compromised animals. Further research is necessary to assess the effects of these treatments under different clinical conditions and in animals with varying health statuses.

5. Conclusion

The inclusion of μ-agonist opioids in the anesthetic protocol with ketamine and midazolam demonstrated a significant improvement in the quality of sedation in rabbits without compromising anesthetic recovery or the main cardiovascular parameters, such as heart rate, systolic, diastolic, and mean arterial pressure, as well as rectal temperature and electrolyte balance. Specifically, methadone caused more pronounced respiratory depression than morphine, resulting in hypoxemia and respiratory acidosis, as evidenced by significant alterations in respiratory rate, SpO_2, $PaCO_2$, PaO_2, and SO_2c. Therefore, 100% oxygen supplementation during the anesthetic period is necessary for this species and should ideally begin as soon as the animal allows for the proper fixation of the oxygenation mask or other oxygen therapy devices.

Acknowledgments: The authors wish to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and UDESC-Lages for providing the facilities for conducting the study and Ms. Vanessa Regina Olszewski for reviewing the final translated document.

Informative Notes: The study was approved by the Ethics Committee on Animal Use (CEUA) of the home institution, Animal Experimentation Ethics Committee – CEUA/UDESC (Protocol 5214300418).

6. References

Aidar ESA, et al. Hemodynamic and ventilatory parameters in rabbits anesthetized with isoflurane submitted to pressure controlled ventilation with or without PEEP. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 72, n. 3, p. 843-852, 2020. (DOI: 10.1590/1678-4162-11283)

Aksoy M, et al. Ultrasonographic examination of the scrotal content in the rabbit. Reproduction in Domestic Animals,

v. 44, p. 156-160, 2009. (DOI: 10.1111/j.1439-0531.2007.01055.x)

Albozachri JMK, et al. A comparison study of nefopam ketamine, tramadol ketamine and xylazine ketamine anesthesia in rabbit. Research Journal of Pharmacy and Technology, v. 12, n. 5, p. 2439-2442, 2019. (DOI: 10.5958/0974-360X.2019.00409.8)

AVMA. In: AVMA pet ownership and demographics sourcebook. Schaumburg, IL: American Veterinary Medical Association; 2017.

Bellini L, et al. Evaluation of sedation and clinical effects of midazolam with ketamine or dexmedetomidine in pet rabbits. Veterinary Record, v. 175, n. 15, p. 372-375, 2014. (DOI: 10.1136/vr.102595)

Benato L, et al. Pain and analgesia in pet rabbits within the veterinary environment: a review. Veterinary Anaesthesia and Analgesia, v. 46, n. 2, p. 151-162, 2018. (DOI: 10.1016/j.vaa.2018.10.007)

Bitti FS, Campagnol D, Rangel JP, Nunes Junior JS, Loureiro B, Monteiro ER. Effects of three methadone doses combined with acepromazine on sedation and some cardiopulmonary variables in dogs. Veterinary Anaesthesia and Analgesia, 44(2), 237–245, 2017. (DOI: 10.1016/j.vaa.2016.04.003)

Botman J, et al. Postanaesthetic effects of ketamine–midazolam and ketamine–medetomidine on gastrointestinal transit time in rabbits anaesthetised with isoflurane. Veterinary Record, v. 186, n. 8, p. 249-255, 2020. (DOI: 10.1136/vr.105491)

Caldwell HG, et al. Trans-cerebral HCO3- and PCO2 exchange during acute respiratory acidosis and exercise-induced metabolic acidosis in humans. Journal of Cerebral Blood Flow and Metabolism, v. 42, n. 4, p. 559-571, 2022. (DOI: 10.1177/0271678X211065924)

Canpolat I, et al. The efficacy of intranasal administration of dexmedetomidine, ketamine and morphine combination to rabbit. International Journal of Development Research, v. 6, n. 7, p. 8634-8636, 2016.

Chapel JM, et al. Electrocardiographic reference values for healthy Netherland dwarf rabbits and the influence of body position, age and gender. World Rabbit Science, v. 25, n. 4, p. 399-406, 2017. (DOI: 10.4995/wrs.2017.7424)

Cintra PP, et al. Cardiorespiratory and electrocardiographic effects of methadone or morphine in the perioperative period in anesthetized dogs with continuous rate infusion of propofol and submitted to ovariohysterectomy. Semina: Ciências Agrárias, v. 38, p. 209-220, 2017. (DOI: 10.5433/1679-0359.2017v38n1p209)

Comassetto F, et al. Analgesic and sedative evaluation of two protocols in swine underwent orchiectomy. Acta Scientiae Veterinariae, v. 42, n. 1212, p. 1-8, 2014.

Dehuisser V, et al. Alfaxalone total intravenous anaesthesia in dogs: pharmacokinetics, cardiovascular data and recovery characteristics. Veterinary Anaesthesia and Analgesia, v. 46, n. 5, p. 605-612, 2019. (DOI: 10.1016/j.vaa.2019.04.014)

Eatwell K, et al. Use of arterial blood gas analysis as a superior method for evaluating respiratory function in pet rabbits (Oryctolagus cuniculus). Veterinary Record, v. 173, n. 7, p. 150-172, 2013. (DOI: 10.1136/vr.101218)

Fodor GH, et al. Dopamine ameliorates bronchoconstriction induced by histaminergic and cholinergic pathways in rabbits. Respiratory Physiology & Neurobiology, v. 259, p. 156-161, 2019. (DOI: 10.1016/j.resp.2018.10.006)

Gardhouse S, Sanchez A. Rabbit sedation and anesthesia. Veterinary Clinics of North America: Exotic Animal Practice, v. 25, n. 1, p. 181-210, 2022. ISSN 1094-9194. ISBN 9780323896764. (DOI: 10.1016/j.cvex.2021.08.012)

Govoni S, et al. Brain and heart dynamics. Switzerland: Springer, 2020.

Horr M, et al. Effects of inspired oxygen fractions in rabbits anesthetized with isoflurane or sevoflurane, maintained on spontaneous ventilation. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 71, n. 3, p. 944-952, 2019. (DOI: 10.1590/1678-4162-10472)

Hunter AR, et al. The respiratory depressant effects of barbiturates and narcotic analgesics in the unanaesthetized rabbit. British Journal of Anaesthesia, v. 40, n. 12, p. 927-935, 1968. (DOI: 10.1093/bja/40.12.927)

Jepson L. Rabbits. In: Jepson L. Exotic animal medicine: a quick reference guide. Missouri: Elsevier, 2016. v. 2, p. 42-87.

Kazakos GM, et al. Use of the laryngeal mask airway in rabbits: placement and efficacy. Lab Animal, v. 36, n. 4, p. 29-34, 2007. (DOI: 10.1038/laban0407-29)

Lupu G, et al. Pain management and analgesics used in small mammals during post-operative period with an emphasis on metamizole (dipyrone) as an alternative medication. Molecules, v. 27, n. 21, p. 1-10, 2022. (DOI: 10.3390/molecules27217434)

Maiante AA, et al. Comparison of the cardio-respiratory effects of methadone and morphine in conscious dogs. Journal of Veterinary Pharmacology and Therapeutics, v. 32, n. 4, p. 317-328, 2009. (DOI: 10.1111/j.1365-2885.2008.01042.x)

Malik A. Pain in rabbits: a review for veterinary nurses part 2: management of pain in hospital. Veterinary Nursing Journal, v. 36, n. 4, p. 132-138, 2021. (DOI: 10.1080/17415349.2020.1840471)

Navarrete-Calvo R, et al. Cardiorespiratory, anaesthetic and recovery effects of morphine combined with medetomidine and alfaxalone in rabbits. Veterinary Record, v. 174, n. 4, p. 95-99, 2014. (DOI: 10.1136/vr.101293)

Nobre CV, et al. Efficacy and safety of morphine and methadone as analgesics in a balanced anaesthetic protocol in rabbits. Revista de Investigaciones Veterinarias del Perú, v. 33, n. 4, p. 1-11, 2022. (DOI: 10.15381/rivep.v33i4.23358)

Pessoa CA. Lagomorpha (Coelho, Lebre e Tapiti). In: Cubas ZS, et al. Tratado de animais selvagens: medicina veterinária. Brazil: Roca, 2020. v. 2, p. 1209-1239.

Raillard M, et al. Comparison of medetomidine-morphine and medetomidine-methadone for sedation, isoflurane requirement and postoperative analgesia in dogs undergoing laparoscopy. Veterinary Anaesthesia and Analgesia, v. 44, p. 17-27, 2017. (DOI: 10.1111/vaa.12394)

Reece AS, et al. Serum ionic dysequilibria in clinical opioid dependence: cross-sectional and longitudinal studies. Human and Experimental Toxicology, v. 36, n. 8, p. 776-784, 2017. (DOI: 10.1177/0960327116666649)

Schroeder CA, Smith LJ. Respiratory rates and arterial blood-gas tensions in healthy rabbits given buprenorphine, butorphanol, midazolam, or their combinations. Journal of the American Association for Laboratory Animal Science, v. 50, n. 2, p. 205-211, 2011.

Silva EF, et al. Echocardiographic evaluation in rabbits: association of midazolam and ketamine. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 63, n. 6, p. 1399-1404, 2011.

Sun CZ, et al. Effect of dobutamine on lung aquaporin 5 in endotoxine shock-induced acute lung injury rabbit. Journal of Thoracic Disease, v. 7, n. 8, p. 1467-1477, 2015. (DOI: 10.3978/j.issn.2072-1439.2015.08.22)

Touzot-Jourde G, et al. Comparison of methadone and morphine sedation and analgesia in the NZW rabbit. Journal of Veterinary Pharmacology and Therapeutics, v. 38, p. 73-74, 2015. (DOI: 10.1111/jvp.12246)

Uccello O, et al. Cardiovascular effects of increasing dosages of norepinephrine in healthy isoflurane-anesthetized New Zealand White rabbits. Veterinary Anaesthesia and Analgesia, v. 47, n. 6, p. 781-788, 2020. (DOI: 10.1016/j.vaa.2020.07.001)

Westphal GA, et al. Vasodilation increases pulse pressure variation, mimicking hypovolemic status in rabbits. Clinics, v. 65, n. 2, p. 189-194, 2010. (DOI: 10.1590/S1807-59322010000200011)

Yanmaz LE, et al. Effects of intramuscular and intranasal administration of midazolam-dexmedetomidine on sedation and some cardiopulmonary variables in New Zealand white rabbits. Veterinary Anaesthesia and Analgesia, v. 49, p. 113-117, 2022. (DOI: 10.1016/j.vaa.2021.10.003)