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

Evaluation of two protocols for chemical restraint in guinea pigs (*Cavia porcellus*)

Gilberto Serighelli Júnior[1], Felipe Comassetto[2], Maite Zanotto Stiehl[3], Nilson Oleskovicz[4]*

Submitted: *05/08/2024*
Accepted: *21/09/2024*

[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]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
[3]Self-employed Veterinarian, Videira, State of Santa Catarina, Brazil. ORCID: 0000-0002-6247-4135.
[4]Professor, Dr., Department of Veterinary Medicine, Center for Agro-Veterinary Sciences (CAV), Santa Catarina State University (UDESC), Lages, State of Santa Catarina, Brazil. ORCID: 0000-0002-7596-730X

*Correspondence email: *gilberto.serighelli@ufpr.br*

Abstract: This study evaluated the cardiorespiratory parameters, analgesia, sedation depth and duration, and recovery quality in eight guinea pigs undergoing elective castration, using dexmedetomidine or xylazine combined with esketamine hydrochloride and morphine. In a crossover study, guinea pigs (*Cavia porcellus*) were 9 months old and 0.650 kg (±0.3 kg) of weight, received xylazine (1 mg/kg), esketamine (10 mg/kg), and morphine (0.5 mg/kg) (GXIL), or dexmedetomidine (5 µg/kg), esketamine (10 mg/kg), and morphine (0.5 mg/kg) (GDEX) intramuscularly at a two-week interval. Cardiorespiratory parameters, including respiratory rate (RR), heart rate (HR), peripheral oxygen saturation (SpO_2), and rectal temperature (RTºC), were recorded at baseline (T0) and every 5 minutes post-administration (T5 to T60). Sedation depth was assessed via ear and toe pinch response, jaw tone, righting reflex, posture, palpebral reflex, and reaction to manipulation available criteria. GXIL induced more deeper sedation, with significant differences in ear/toe pinch response (p=0.0023 and p=0.0032 respectively) and jaw tone (p=0.0011) compared to GDEX. HR and RR significantly decreased 13,6% (p=0.0038) and 32,6% (p=0.0001) in the GXIL, compared to GDEX. GDEX showed a lower overall sedation depth in the total sedation curve (p=0.0005), with a mean [minimum-maximum score] of 11[5-12] in the GXIL and 8[4-11] in the GDEX. Both protocols were effective and safe for chemical restraint in guinea pigs, as GXIL provided deeper sedation and greater cardiorespiratory depression than GDEX. However, GDEX maintained a more stable cardiorespiratory profile, suggesting greater physiological stability during sedation. Nonetheless, both treatments provided minimal cardiorespiratory alterations, demonstrating their safety for use.
Keywords: alpha-2 agonists, *Cavia porcellus*, chemical restraint, sedation.

1. Introduction

Inhalation anesthesia is the most common method for inducing and maintaining a surgical plane of anesthesia in rodents in clinical and research settings (Schmitz et al., 2016, 2017). Isoflurane, a frequently used inhalant anesthetic, presents several disadvantages when used in guinea pigs. Induction with isoflurane can cause stress or prolonged time to recumbency as the odor of the anesthetic can lead to breath-holding (Ross et al., 2000; Doerning et al., 2018). Additionally, isoflurane increases salivation, lacrimation, and bronchial secretion, leading to the accumulation of thick mucus and airway irritation, which can cause irregular breathing and respiratory compromise (Schmitz et al., 2016, 2017). In procedures lasting more than 10 minutes, hypotension, bradypnea, and significant loss of body temperature have been observed (Schmitz et al., 2016). Safe and short-duration sedation protocols are preferable for the restraint of guinea pigs, reducing stress associated with physical restraint (Dang et al., 2008; Sixtus et al., 2021; Avelino et al., 2024).

Given the limited literature related to anesthesia in this species, there is a need for studies to standardize doses and pharmacological combinations for their chemical restraint (Schwenke & Cragg, 2004; Avelino et al., 2024). This is further supported by the contraindications for anesthetic induction and the difficulty of endotracheal intubation in these animals, combined with their unique anatomy, such as the soft palate fused to the base of the tongue and the entry of the glottis through a small palatal ostium, which can lead to regurgitation and intense salivation following stimulation of the oropharynx (D'Ovidio et al., 2017). Additionally, the support blade of the laryngoscope can easily traumatize the tongue, causing severe bleeding and potential mortality (Allweiler, 2016).

Balanced chemical restraint allows for the reduction of drug doses when combined compared to single agents. Among them, alpha-2 adrenergic agonists, such as xylazine and dexmedetomidine, stand out for their analgesic and sedative properties (Schmitz et al., 2016; Dião et al., 2016). These drugs can be combined with esketamine, which acts on the N-methyl-D-aspartate (NMDA) glutamatergic pathways, inhibiting cortical excitability, effectively producing dissociative anesthesia with somatic analgesia and limited cardiorespiratory depression (Sixtus et al., 2021). Injectable anesthesia obtained with alpha-2 agonists and ketamine, either alone or in combination, has been described for diagnostic imaging in rodents (Fischetti, 2012; D'Ovidio et al., 2017). However, this combination can result in deep respiratory depression and hypothermia, with prolonged recovery (Schmitz et al., 2016). To enhance the analgesic properties of the anesthetic protocol and reduce drug doses, opioids are frequently included. Among opioids, morphine is widely used. In mammals, it provides 3 to 4 hours of analgesia, mild sedative effects, and a short latency (3 minutes) when administered intramuscularly (IM) after a single dose, with dose-dependent cardiovascular effects (West, Heard, & Caulkett, 2014).

Considering the difficulties in anesthetic induction and maintenance, as well as the challenges of venous access, the use of safe injectable agents for subcutaneous (SC) or IM administration is necessary for the chemical restraint of these animals (Schwenke & Cragg, 2004). The present study aimed to evaluate the cardiorespiratory parameters, analgesia, degree and duration of sedation, and recovery quality of guinea pigs subjected to sedation with dexmedetomidine or xylazine, combined with esketamine hydrochloride

and morphine. The hypothesis is that the addition of ketamine and morphine in combination with alpha-2 agonists will result in adequate sedation with lower doses than cited in the literature, with more intense depression in the xylazine group.

2. Materials and Methods

The study was approved by the Ethics Committee on Animal Use (CEUA) of the home institution (Protocol 9314220817). Eight male guinea pigs, with an average weight of 0.65 ± 0.3 kg (p = 0.3831), were clinically pre-evaluated to ensure their health. The study adopted a crossover design, where the same animals underwent both treatments, with a 14-day washout period between them. The sample size was calculated using Open Epi 3.0, with a statistical power of 80% and a confidence interval of 5%. The animals underwent a 30-day acclimation period, housed in pairs in 0.6 x 0.6-meter cages under natural light and ambient temperature. They were fed ground corn and species-specific commercial feed twice daily, along with kale leaves every night, and provided water ad libitum. All guinea pigs were attributed an American Society of Anaesthesiologists (ASA) score based on physical exams.

On the experimental design day, the animals were subjected to a 3-hour fasting period and subsequently randomized into two groups using www.randomization.com: the Dexmedetomidine Group (GDEX) (n = 8) received dexmedetomidine 5 mcg/kg (Dexdomitor 0,5 mg/mL, Zoetis Ltda., Campinas, Brazil), esketamine hydrochloride 10 mg/kg (Ketamin NP 50 mg/mL, Cristália Ltda., Itapira, Brazil), and morphine 0.5 mg/kg (Dimorf 10 mg/mL, Cristália Ltda., Itapira, Brazil); the Xylazine Group (GXIL) (n = 8) received xylazine 1 mg/kg (Xilazin 2%, Syntec Ltda., Barueri, Brazil), esketamine hydrochloride 10 mg/kg, and morphine 0,5 mg/kg. All drugs were administered intramuscularly (IM) into the left femoral quadriceps muscle. To ensure scientific rigor, this two-sequence, single-site study was conducted according to the ARRIVE guidelines. It was a blinded study, with standardization of administered volumes, diluting the treatments in 0.9% saline solution to a final volume of 0.5 mL.

pImmediately before treatment administration, baseline parameters of the animals (T0) were evaluated, including respiratory rate (RR, observed via rib cage movement), heart rate (HR, using an ECG sensor attached to the B650 monitor, GE–Datex-Ohmeda), peripheral oxygen saturation (SpO_2, with a pulse oximetry sensor connected to the B650 multiparameter monitor, GE–Datex-Ohmeda), and rectal temperature (RT, °C) using a digital thermometer inserted 1 cm into the anus and connected to the B650 module, GE–Datex-Ohmeda. Additionally, at the baseline moment (T0) and every 10 minutes thereafter, the animals were assessed for the degree of sedation (D'OVÍDIO et al., 2017) (Figure 1). This assessment included evaluations of the palpebral reflex, righting reflex, response to toe and ear pinch, posture, jaw tone, and reaction to manipulation. The evaluation followed the criteria established by D'OVÍDIO et al. (2017), where the scores obtained were summed according to the scale, resulting in a range of 0 to 16 points, with "0" representing normal guinea pig behavior and 16 indicating the highest possible sedation.

This system includes the assessment of palpebral reflex, jaw tone, and response to toe and ear-pinch, with scores of 0 (normal), 1 (decreased), and 2 (absent). Reaction to manipulation was scored as 0 (impossible to manipulate without struggling), 1 (mild struggling without sufficient strength), and 2 (no reaction). Righting reflex was scored as 0 (regains sternal recumbency immediately), 1 (regains sternal recumbency after 5-10 seconds), 2 (unsuccessfully attempts to regain sternal recumbency), and 3 (maintains dorsal recumbency without righting attempts). Posture was scored from 0 (normal) to 5 (dorsal recumbency with total relaxation). The palpebral reflex was assessed with a gentle tactile stimulation of the upper eyelid. The righting reflex was assessed by placing the GP in dorsal recumbency and observing its ability to regain sternal recumbency; the possible outcomes were present or absent. Posture was recorded immediately after this assessment. Reaction to manipulation was simulated by positioning (in dorsal recumbency) with a score ranging from 0 to 2, where 0 was indicative of normal reaction, 1 of decreased response, and 2 of absent response. Jaw tone was assessed by attempting to open the GP's mouth. Response to toe and ear-pinch was assessed by lightly pinching and applying blunt surgical forceps, for a maximum of two seconds, at the level of the distal interphalangeal junction and of the base of the ear.

The parametric variables were evaluated immediately before treatment administration, corresponding to time point (T0), and every 5 minutes post-administration, until the patient's recovery (T5 to T60). The evaluations were conducted in a temperature-controlled room, on a flat surface, with a thermal mattress heated to 37°C. Measurements were performed by an experienced evaluator who was blinded to the administered protocol. During the first participation in the study, each animal underwent full recovery and was returned to the acclimation cage. In the second participation, after completing the study protocol (T60) and full recovery, the animals were anesthetized again with the same protocol corresponding to each treatment and underwent elective orchiectomy surgery performed by the same veterinary surgeon. Additionally, a prophylactic dose of cephalothin at 20 mg/kg (Cephalothin 1 g, Blau Ltda, Cotia, Brazil) was administered intramuscularly (IM), followed by an intratesticular block with 2% lidocaine without epinephrine at 3 mg/kg (Xylestesin 20 mg/mL, Cristália Ltda, Itapira, Brazil). Postoperatively, meloxicam 0.5 mg/kg (Maxicam 0.2%, Ourofino Saúde Animal, Vinhedo, Brazil) and dipyrone 25 mg/kg (Dipirona 50%, Ibasa, Porto Alegre, Brazil) were administered subcutaneously (SC).

2.1. Statistical analyses

Statistical analyses were conducted using Prisma version 9.3.0 software. The Shapiro-Wilk test was employed to assess the normality of the data distribution. Parametric data were expressed as mean ± standard deviation (SD) and compared between groups using the unpaired t-test, while differences over time within the same group were analyzed using two-way repeated measures ANOVA (factors: time and group), followed by Dunnett's post-hoc test. For non-parametric data, the Kruskal-Wallis test was applied, followed by the Wilcoxon Matched-Pairs test for comparisons across different time points and the Mann-Whitney U test for comparisons between groups. Non-parametric results were presented as median, minimum, and maximum values. Statistical significance was set at $P < 0.05$.

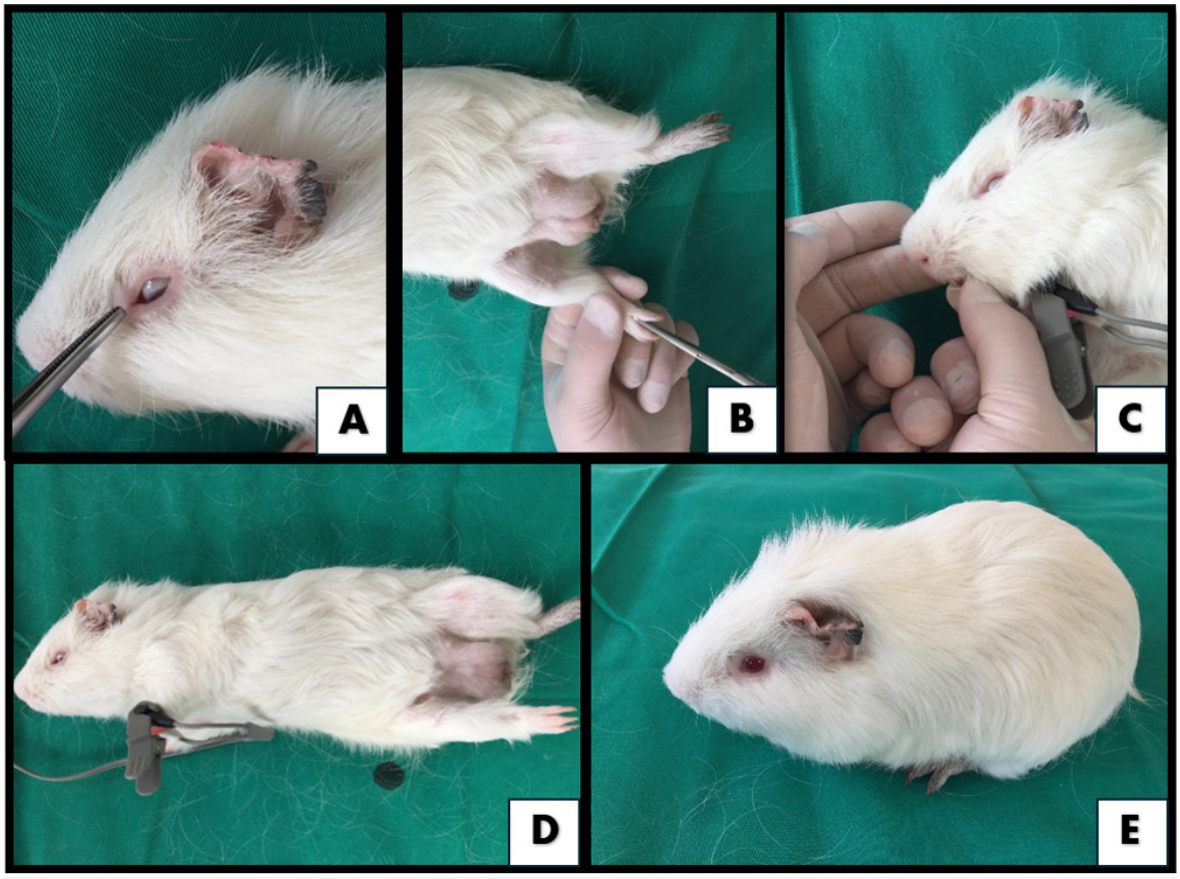

Figure 1 – Sequence images for palpebral reflex (gentle touch of the upper eyelid) (A); Response to the ear or toe pinch (application of blunt surgical forceps for no more than two seconds to the distal interphalangeal region and the base of the ear) (B); Jaw tone (resistance to mouth opening) (C); Righting reflex and Reaction to manipulation (after dorsal recumbency on a flat and firm surface) (D); Posture (based on position on the surface) (E).

3. Results

There were no significant differences in weight between the groups ($p = 0.3831$). The ages were 9 ± 1 months ($p = 0.7868$). All animals underwent physical examinations to confirm their health status and were classified as ASA I according to the American Society of Anesthesiologists.

The latency time was 3.07 (± 1.37) minutes for the GXIL group and 4.03 ± 2.34 minutes for the GDEX group ($p = 0.0985$). The mean sedation time was 72 ± 10.2 minutes for the GXIL group and 58.2 ± 24.4 minutes for the GDEX group ($p = 0.0495$), indicating a shorter latency and longer sedation duration with xylazine treatment. The results from the general linear model and repeated-measures ANOVA indicated statistically significant differences. Heart rate (HR) showed a significant reduction in the GXIL group at all evaluated time points except T5 when compared to baseline (Table 1), with an average decrease of 13.6% ($p = 0.0038$). In the GXIL group, respiratory rate (RR) significantly decreased at all evaluated time points compared to baseline (T0), with an average reduction of 32.6% ($p = 0.0001$). This significant decrease was not observed in the GDEX group. There was an initial decrease in RR in both groups compared to baseline, but subsequently, RR progressively decreased more in the GXIL group than in the GDEX group. Regarding SpO_2 (Table 1), lower values were observed in the GXIL group from T5 to T20 compared to baseline and to the GDEX group at M15 and T20. Hypothermia (RT < 37.0 °C) was not observed. Body temperature showed a significant increase at T50, T55, and T60 compared to the baseline in the GXIL group.

Sedation scores (Table 2) indicated more intense sedation in the GXIL group ($p = 0.0005$), with statistically significant differences in response to ear ($p = 0.0023$) and toe ($p = 0.0032$) pinch and jaw tone ($p = 0.0011$). The subitems posture and righting reflex (Table 2) did not show statistically significant differences between treatments ($p = 0.087$ and $p > 0.999$, respectively), indicating similar effects on these parameters across both groups. Regarding reaction to manipulation (Table 2), differences between treatments ($p = 0.0233$) were evident from M10 to T60 and compared to baseline in both treatments, with higher scores in the GXIL group. The animals in the GXIL group exhibited more resistance to handling compared to the GDEX group. Sedation in the GDEX group was milder and of shorter duration compared to the GXIL group, indicating a less profound sedative effect and quicker recovery. In total sedation scores (Figure 2), the GXIL group demonstrated deeper sedation at all time points compared to the GDEX

group (p = 0.0005). The total sedation curve for the GDEX group was consistently below that of the GXIL group, indicating a less profound sedative effect throughout the study period.

	HR *(Beat/min)*			RR *(Mov/min)*			RT (ºC)			SpO_2 *(%)*		
	GXIL	GDEX	*p*	GXIL	GDEX	*p*	GXIL	GDEX	*p*	GXIL	GDEX	*p*
T0	269±36	264±42	*0.809*	99±24	90±21	*0.461*	36±0.9	37±0.5	*0.154*	92±3	91±7	*0.419*
T5	249±22	272±37	*0.110*	76±31 A	83±21	*0.620*	37±0.7	38±1.1	*0.098*	93±6	93±5	*0.229*
T10	224±09 Aa	255±29b	*0.029*	68±18 A	74±18	*0.575*	37±0.8	37±1.0	*0.985*	91±8	91±4	*0.288*
T15	222±11 A	239±22	*0.111*	61±10 A	76±18	*0.064*	37±0.9	37±0.9	*0.718*	92±5	91±6	*0.133*
T20	222±12 A	229±14	*0.174*	60±10 A	69±9	*0.127*	37±0.9	36±1.2	*0.260*	91±6	92±4	*0.411*
T25	224±11 A	223±15	*0.842*	64±24 A	69±8	*0.681*	37±1.0	37±1.0	*0.574*	92±5	91±2	*0.097*
T30	228±08 A	226±11	*0.581*	61±12 A	71±8	*0.104*	37±1.1	37±1.0	*0.259*	91±4	87±4	*0.869*
T35	230±09 A	229±16	*0.834*	62±14 A	67±11	*0.561*	37±0.9	37±1.1	*0.260*	88±4	88±4	*0.827*
T40	236±16 A	231±15	*0.493*	67±16 A	73±20	*0.597*	37±1.0	37±0.7	*0.385*	93±3	93±3	*0.557*
T45	237±15 A	232±13	*0.559*	70±16 A	74±20	*0.654*	37±1.5	38±0.6	*0.461*	91±2	91±4	*0.485*
T50	245±25 A	234±14	*0.300*	70±17 A	75±20	*0.610*	38±1.5 A	38±1.0	*0.718*	90±4	90±4	*0.546*
T55	239±18 A	239±13	*0.956*	72±18 A	79±15	*0.344*	38±1.4 A	38±0.9	*0.861*	94±6	94±2	*0.811*
T60	243±19 A	242±10	*0.848*	70±18 A	81±7	*0.073*	38±1.2 A	38±0.9	*0.985*	90±4	89±4	*0.156*

Table 1 – Mean and standard deviation of heart rate (HR), respiratory rate (RR), rectal temperature (RT), and peripheral oxygen saturation (SpO_2) in guinea pigs (*Cavia porcellus*) chemically restrained with xylazine 1 mg/kg (GXIL) or dexmedetomidine 5 mcg/kg (GDEX), combined with esketamine 10 mg/kg, and morphine 0.5 mg/kg administered intramuscularly.

Obs. Uppercase letters in the columns indicate a significant difference compared to the baseline (T0). ANOVA test followed by Dunnett's post-hoc test, with significant differences when P < 0.05. Different lowercase letters in the rows indicate a significant difference between treatments. Paired T-test, with significant differences when p < 0.05.

		T0	T10	T20	T30	T40	T50	T60
Response to the ear pinch (0-2)	GX	0 [0-0]	1 [0-1] Ab	1 [0-1]	1 [0-1] Ab	1 [0-1] Ab	1 [0-1] A	1 [0-1]
	GD	0 [0-0]	0 [0-1] a	0 [0-1] A	0 [0-1] a	0 [0-1] a	0 [0-1]	0 [0-1]
Response to the toe pinch (0-2)	GX	0 [0-0]	0 [0-1]	0 [0-1]	0 [0-1] Ab	0 [0-1]	0 [0-1]	0 [0-0]
	GD	0 [0-0]	0 [0-0]	0 [0-1]	0 [0-1] a	0 [0-1]	0 [0-1]	0 [0-0]
Jaw Tone (0-2)	GX	0 [0-0]	2 [0-2] A	2 [0-2] Ab	2 [0-2] Ab	1 [0-1] Ab	0 [0-1] A	0 [0-1]
	GD	0 [0-0]	0 [0-1]	0 [0-2] Aa	2 [0-2] Aa	0 [0-1] a	0 [0-1]	0 [0-0]
Righting reflex (0-3)	GX	0 [0-0]	3 [2-3] A	3 [2-3] A	3 [3-3] A	3 [2-3] A	3 [2-3] A	3 [1-3] A
	GD	0 [0-0]	3 [2-3] A	3 [1-3] A	3 [3-3] A	3 [2-3] A	3 [2-3] A	2 [1-3] A
Posture (0-5)	GX	0 [0-0]	4 [2-4] A	4 [0-4] A	4 [2-4] A	4 [1-4] A	4 [0-4] A	4 [0-4] A
	GD	0 [0-0]	4 [3-4] A	4 [2-4] A	4 [3-4] A	4 [3-4] A	4 [3-4] A	4 [0-4] A
Reaction to manipulation (0-2)	GX	0 [0-0]	2 [1-2] Ab	2 [1-2] Ab	2 [1-2] Ab	2 [1-2] Ab	2 [1-2] Ab	2 [0-2] Ab
	GD	0 [0-0]	1 [0-2] Aa	2 [0-2] Aa	2 [0-2] A	2 [0-2] A	1 [0-2] Aa	0 [0-1] Aa
Palpebral reflex	GX	Present	Present	Present	Present	Present	Present	Present
	GD	Present	Present	Present	Present	Present	Present	Present
TOTAL	**GX**	**0 [0-0]**	**12 [5-13]**	**12 [3-13]**	**12 [6-13]**	**11 [4-12]**	**11 [3-12]**	**9 [1-11]**
	GD	**0 [0-0]**	**8 [5-11]**	**9 [3-13]**	**11 [6-13]**	**9 [5-12]**	**8 [5-12]**	**6 [1-8]**

Table 2 – Median and minimum and maximum scores for sedation, obtained in eight guinea pigs (*Cavia porcellus*) chemically restrained with xylazine 1 mg/kg (GXIL) or dexmedetomidine 5 mcg/kg (GDEX), combined with esketamine 10 mg/kg and morphine 0.5 mg/kg, administered intramuscularly, according to the sedation scale of D'Ovídio et al. (2017).

Obs. Palpebral reflex (gentle touch of the upper eyelid); Response to the ear or toe pinch (application of blunt surgical forceps for no more than two seconds to the distal interphalangeal region and the base of the ear); Jaw tone (resistance to mouth opening); Righting reflex (after dorsal recumbency on a flat and firm surface); Posture (based on position on the surface); Reaction to manipulation (reaction). Uppercase letters in the column indicate values significantly different from T0 (Baseline) according to Kruskal-Wallis followed Wilcoxon Matched-Pairs test ($P < 0.05$). Different lowercase letters indicate values significantly different between groups, according to followed followed by Mann-Whitney test ($P < 0.05$).

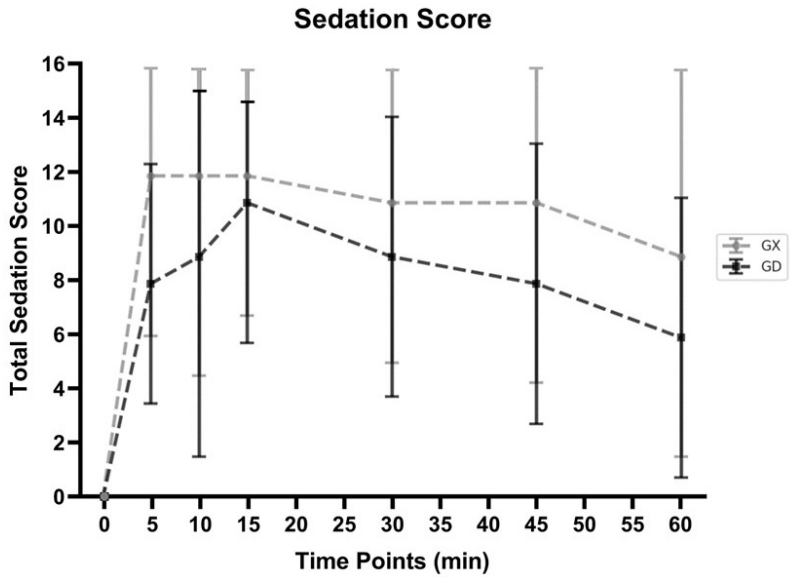

Figure 2 – Total scores for sedation, obtained in eight guinea pigs (*Cavia porcellus*) chemically restrained with xylazine 1 mg/kg (GXIL) or dexmedetomidine 5 mcg/kg (GDEX), combined with esketamine 10 mg/kg and morphine 0.5 mg/kg.

4. Discussion

The results of this study confirm the initial hypothesis that the combination of xylazine or dexmedetomidine with esketamine and morphine would promote effective sedation with minimal adverse effects in guinea pigs (GP). Notably, sedation with xylazine was found to be more pronounced and deeper compared to dexmedetomidine. The literature commonly cites doses for dexmedetomidine at 0.25 mg/kg IM or IP, xylazine at 5 mg/kg IP or IM, and ketamine at 40–100 mg/kg IM or IP, or a combination of ketamine with dexmedetomidine at 40 mg/kg and 0.25 mg/kg IP, respectively (Flecknell, 2023). The combination of dexmedetomidine with ketamine at the above doses is frequently used to achieve a surgical plane of anesthesia but can result in variable effects such as apnea and significant cardiorespiratory depression (Souza et al., 2012; Avelino, 2024).

Dexmedetomidine provides sedative, muscle relaxant, and analgesic effects in dogs and cats, and while sedation in guinea pigs can be variable, dexmedetomidine is known to potentiate the effects of other anesthetic agents (Flecknell, 2015). The greater depth of sedation observed with the GXIL combination in this study can be attributed to the pharmacological doses used and the selectivity of the adrenergic receptors involved. Studies indicate that dexmedetomidine is approximately 10 times more potent than xylazine, a difference primarily due to its higher selectivity for α2-adrenoreceptors (Souza et al., 2012; Flecknell, 2015). The activation of vascular α1-adrenoreceptors contributes to vasoconstriction and increased systemic vascular resistance, while the activation of central α1-adrenoreceptors is associated with increased arousal, restlessness, vigilance, and locomotor activity in animals. In theory, α2-adrenoreceptor agonists that are less selective for α2-adrenoreceptors are more likely to cause adverse effects such as paradoxical excitement and locomotor activity. Among commonly used α2-adrenoreceptor agonists, xylazine has the lowest α2:α1 adrenoreceptor binding ratio (Souza et al., 2012). As observed in the present study, the GXIL group exhibited more intense but tolerated respiratory and cardiovascular depression within a sedation regimen.

The deeper sedation observed with xylazine in this study aligns with the known pharmacological profile of α2-adrenoreceptor agonists, which are known to provide potent sedation and analgesia. The combination of these agents with ketamine and morphine enhances their sedative and analgesic effects while potentially reducing the required doses and minimizing adverse effects. This balanced approach to chemical restraint is crucial in veterinary practice, especially for species like guinea pigs, where physical restraint can be stressful and challenging (Flecknell, 2015). Furthermore, considering receptor affinity (dexmedetomidine has an α2:α1 affinity ratio of 1620:1 compared to xylazine's 160:1) (Souza et al., 2012), one might expect dexmedetomidine-induced sedation to be more potent. However, when using lower doses, the observed sedation may be equivalent to or less intense than that provided by xylazine. The literature reports dexmedetomidine doses ranging from 15 to 50 µg/kg for sedation in rodents, providing mild to moderate sedation when administered intramuscularly or subcutaneously (Izer et al., 2014). Thus, the pharmacological

combinations used in this study achieved moderate sedation with both protocols, even at lower doses than commonly cited in the literature, contributing to a reduction in adverse effects.

Moderate sedation was confirmed by the subitems of righting reflex and posture (Table 2), with animals showing no attempts to right themselves but still responding to stimuli. This indicates a good sedative effect, crucial for procedures requiring immobility without intense depression (Álvarez et al. 2022). The effects of sedation are also influenced by esketamine and morphine. Ketamine and its enantiomer, esketamine, are potent analgesics acting on NMDA receptors, with esketamine being 1.5 times more potent due to its composition solely of the S (+) enantiomer, unlike ketamine, which contains the less potent R (-) enantiomer (Lener, 2017). This composition enhances esketamine's sedative, hypnotic, and analgesic effects by inhibiting cortical excitability (Sixtus et al., 2021). Esketamine is widely used for anesthetizing laboratory rodents, typically in combination with sedatives or sedative-analgesics, as it does not induce adequate anesthesia on its own. When combined with agents like medetomidine or dexmedetomidine, anesthesia depth is more consistent (Flecknell, 2015). In this study, esketamine was combined with dexmedetomidine (GDEX) or xylazine (GXIL) in guinea pigs undergoing elective castration. Our findings align with Schmitz et al. (2016), who reported a shorter chemical restraint time with higher doses of ketamine and xylazine, yet the inclusion of morphine in our protocols likely extended the sedation duration, as morphine is known to prolong sedation and enhance analgesia (West, Heard & Caulkett, 2014). Additionally, the deeper sedation observed in the GXIL group may be attributed to the potent sedative effects of xylazine, an α2-adrenergic agonist, in combination with the opioid analgesic properties of morphine.

Morphine enhances sedation and analgesic effects by acting on opioid receptors, producing sedation and analgesia lasting up to four hours (West, Heard, & Caulkett, 2014). In guinea pigs, the recommended dosage ranges from 2 to 5 mg/kg, administered either intramuscularly (IM) or subcutaneously (SC), with analgesic effects lasting for up to four hours (Flecknell, 2015). Despite the growing emphasis on animal welfare and the refinement of animal models, it is concerning that less than 25% of laboratory rodents undergoing surgical procedures receive any form of analgesic treatment (Stokes et al., 2009). In a literature review on analgesic administration in laboratory rodents used in experimental research, Stokes et al. (2009) concluded that, unfortunately, analgesic administration remains suboptimal. Except for studies combining a systemic analgesic with an anesthetic regimen that includes an analgesic component, few studies reported the use of multimodal analgesia in laboratory animals.

The shorter sedation duration observed in the GDEX group (Figure 2), along with the more inconsistent onset of sedation, may be attributed to the pharmacokinetics of the administered drugs. Xylazine and dexmedetomidine achieve peak plasma concentrations at approximately 15 and 30 minutes, respectively, following intramuscular administration (Afonso & Reis, 2012; Comassetto et al., 2023). The delay in reaching peak plasma concentration could account for the initially less pronounced sedation seen in the GDEX group. Furthermore, the relatively lower dose of dexmedetomidine, considering its potency, in combination with the rapid metabolism characteristic of small mammals, likely contributes to the expedited metabolism of the drugs administered (D'Ovidio et al., 2017).

The decision to use lower doses was guided by the principle of multimodal anesthesia, which involves administering a combination of pharmacological agents at reduced doses. This approach enhances cardiorespiratory stability, ensures adequate sedation, and increases the overall safety of anesthetic procedures (Dião et al., 2016). The efficacy of this strategy was demonstrated by the satisfactory sedation scores observed in both treatment groups, likely resulting from the synergistic interaction between the opioid and esketamine. Esketamine, in particular, contributes significantly to the enhanced sedation, hypnosis, and analgesia observed, as the S (+) enantiomer present in this formulation is 1.5 times more potent than its racemic counterpart, as mentioned above (Lener, 2017).

Xylazine administration resulted in a noticeable decrease in heart rate (HR). This reduction can be attributed to the specific doses used, the pharmacological class of the agents, and their affinity for adrenergic receptors. In the GXIL group, the lower selectivity of xylazine for α2-adrenoceptors likely exacerbated the reduction in heart rate, which can be explained by the activation of vagal baroreceptors leading to reflex bradycardia (Comassetto et al., 2023). Comparable findings were reported by Schmitz et al. (2016), who observed a 38% reduction in heart rate from baseline approximately 12.3 minutes after intraperitoneal administration of a combination of ketamine (75 mg/kg) and xylazine (15 mg/kg), or medetomidine (0.2 mg/kg), midazolam (1 mg/kg), and fentanyl (0.025 mg/kg) in male guinea pigs.

A decrease in respiratory rate (RR) was observed in the GXIL group, likely due to the more profound level of sedation achieved in this group, leading to a progressive reduction in respiratory rate. The differential selectivity of α2-adrenoreceptor agonists also plays a role in this effect, as dexmedetomidine-induced respiratory depression appears to be less pronounced than that induced by xylazine (Afonso & Reis, 2012). Supporting this, Molina et al. (2015) conducted a study on Wistar rats (*Rattus norvegicus*) using ketamine (75 mg/kg) in combination with the α2-adrenergic agonists xylazine (2.5 mg/kg) or medetomidine (0.5 µg/kg). They reported significant neurovegetative sympathetic depression, manifesting as a dose-dependent reduction in respiratory rate in the xylazine-treated group, with consideration for oxygen supplementation during the anesthetic procedure.

Body temperature exhibited a significant increase at T50, T55, and T60 compared to baseline (T0) in the GXIL group. This temperature rise is likely attributed to the ambient room temperature and the use of a thermal mattress beneath the animals to mitigate heat loss to the environment. Maintaining normothermia during anesthesia is particularly critical in guinea pigs due to their large body surface area relative to their size, which predisposes them to rapid temperature loss. Therefore, the implementation of additional thermal support, such as a thermal mattress and carefully regulated surgical room temperature, is strongly recommended during anesthesia (West et al., 2014). Concerning SpO_2 (Table 1), although the values did not show significant differences, some instances displayed lower-than-expected readings. This may be associated with the vasoconstrictive effects of α2-adrenoreceptor

agonists, which can interfere with the infrared detection capabilities of the oximetry sensor (Dião et al., 2016; Comassetto et al., 2023).

This study had some limitations. Blood pressure measurement was not feasible due to the clinical nature of the study, and there are no validated cuffs or ranges for non-invasive blood pressure measurements in these patients. Additionally, most oscillometric monitors cannot detect blood pressure in guinea pigs. Further studies are needed to highlight the impact of these protocols on the blood pressure of GPs. Finally, the population included in our study was healthy, and the results cannot be extrapolated to diseased patients.

5. Conclusion

This study demonstrated the effectiveness of both chemical restraint protocols in guinea pigs. However, the combination of dexmedetomidine, esketamine, and morphine showed lower sedative quality compared to the combination with xylazine, as well as shorter sedation duration. Nonetheless, both treatments provided minimal cardiorespiratory alterations, demonstrating their safety for use.

Acknowledgments: Coordination for the Improvement of Higher Education Personnel (CAPES) and UDESC-Lages for providing the facilities for conducting the study.

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 9314220817).

6. References

Afonso J, Reis F. Dexmedetomidina: Papel atual em anestesia e cuidados intensivos. Revista Brasileira de Anestesiologia, 62:(1); 118-133, 2012. (DOI: 10.1590/S0034-70942012000100015)

Allweiler SI. How to improve anestesia and analgesia in small mammals. Vet Clin Exot Anim, 19, 361-377, 2016. (DOI: 10.1016/j.cvex.2016.01.012)

Álvarez ER, Solé LV, Mateo AGC, Comparison of subcutaneous sedation with alfaxalone or alfaxalone-midazolam in pet guinea pigs (*Cavia porcellus*) of three different age groups. Journal of the American Veterinary Medical Association, 260:(9); 1024-1030, 2022. (DOI:10.2460/javma.21.02.0104)

Avelino JA, Walsh CA, Wharton KN, et al. Comparison of Three Anesthetic Drug Combinations for Use in Inducing Surgical Anesthesia in Female Guinea Pigs (*Cavia porcellus*). American Association for Laboratory Animal Science. 23:(64), 2024. (DOI: 10.30802/AALAS-JAALAS-23-000064)

Bar-Ilan A, Marder J. Acid-base status in unanesthetized unstrained Guinea Pigs. Pfliigers Arch. 384; 93-97, 1980. (DOI: 10.1007/BF00589520)

Brendle M, Robison R, Malone DC. Cost-effectiveness of esketamine nasal spray compared to intravenous ketamine for patients with treatment-resistant depression in the US utilizing clinical trial efficacy and real-world effectiveness estimates, Journal of Affective Disorders, 319; 388-396, 2022. (DOI: 10.1016/j.jad.2022.09.083)

Burnside WM, Flecknell PA, Cameron AI, et al. A comparison of medetomidine and its active enantiomer dexmedetomidine when administered with ketamine in mice. BMC Vet Res, 9:(48), 2013. (DOI:10.1186/1746-6148-9-48)

Comassetto F, Serighelli GJ, Stiehl MZ, et al. Efeito da dexmedetomidina sobre o requerimento de propofol para a indução anestésica em cães, Archives Veterinary Science, 28:(2); 2023. (DOI: 10.5380/avs.v28i2.86825)

Dang V, Bao S, Ault A, et al. Efficacy and safety of five injectable anesthetic regimens for chronic blood collection from the anterior vena cava of Guinea pigs. J Am Assoc Lab Anim Sci, 47:(6); 56 – 60, 2008. (PMCID: PMC2687127)

Dião HX, Jiang S, Gao YP, et al. Comparison of the effects of propofol and emulsified isoflurane alone or combined with dexmedetomidine on induction of anesthesia in dogs. Veterinary Anaesthesia and Analgesia, 43:(2); 145 -152, 2016. (DOI: 10.1111/vaa.12287)

D'Ovidio D, Marino F, Noviello E, et al. Sedative effects of intramuscular alfaxalone in pet guinea pigs (*Cavia porcellus*). Veterinary Anaesthesia and Analgesia, 45:(2); 183-189, 2017. (DOI:10.1016/j.vaa.2017.08.004.)

Flecknell P. Laboratory Animal Anaesthesia and Analgesia, 5th edn. London, UK: Academic Press, 2023.

Izer JM, Whitcomb TL, Wilson RP, Atipamezole reverses ketamine-dexmedetomidine anesthesia without altering the antinociceptive effects of butorphanol and buprenorphine in female C57BL/6J mice. J. Am. Assoc. Lab. Anim. Sci, v:(6); 675-83, 2014. (PMID: 25650975)

Kästner SB, Von Rechenberg B, Keller K, et al. Comparison of medetomidine and dexmedetomidine as premedication in isoflurane anaesthesia for orthopedic surgery in domestic Sheep. Journal of Veterinary Medicine, 48:(4); 231–241, 2001. (DOI: 10.1046/j.1439-0442.2001.00354.x)

Lener MS, Kadriu B, Zarate CAJ. Ketamine and Beyond: Investigations into the Potential of Glutamatergic Agents to Treat Depression. Drugs, 77:(4); 381-401, 2017. (DOI: 10.1007/s40265-017-0702-8)

Molina AM, Moyano MR, Serrano-Rodriguez JM, et al. Analyses of anesthesia with ketamine combined with different sedatives in rats. Veterinární medicína, 60:(7); 2015. (DOI: 10.17221/8384-VETMED)

Schmitz S, Tacke S, Guth B, et al. Comparison of Physiological Parameters and Anaesthesia Specific Observations during Isoflurane, Ketamine-Xylazine or Medetomidine-Midazolam-Fentanyl Anaesthesia in Male Guinea Pigs. PLOS ONE, 11:(9); 2016. (DOI: 10.1371/journal.pone.0161258)

Schwenke DO, Cragg PA. Comparison of the depressive effects of four anesthetic regimens on ventilatory and cardiovascular variables in the guinea pig. Comp Med, 54:(1); 77-85, 2004. (PMID: 15027622)

Sixtus RP, Pacharinsak C, Gray CL, et al. Differential effects of four intramuscular sedatives on cardiorespiratory stability in juvenile guinea pigs (*Cavia porcellus*). PLoS One, 16:(11), 2021. (DOI: 10.1371/journal.pone.0259559)

Souza JFF, Monteiro ER, Campagnol D, et al. Evaluation of nociception, sedation, and cardiorespiratory effects of a constant rate infusion of xylazine alone or in combination with lidocaine in horses. Journal of Equine Veterinary Science, 32(6); 1-7, 2012. (DOI: 10.1016/j.jevs.2011.11.010)

Stokes EL, Flecknell PA, Richardson CA. Reported analgesic and anesthetic administration to rodents undergoing experimental surgical procedures. Lab Anim, 43(2): 149–154, 2009. (DOI: 10.1258/la.2008.008020)

West G, Heard D, Caulkett N, Zoo animal & Wildlife Immobilization and anesthesia. Wiley. 2. ed. 2014. Iowa: Blackwell, 2014.

Zhang Y, Cui F, Ma JH, et al. Mini-dose esketamine–dexmedetomidine combination to supplement analgesia for patients after scoliosis correction surgery: a double-blind randomized trial, British Journal of Anaesthesia, 131:(2); 385-396, 2023. (DOI: 10.1016/j.bja.2023.05.001).