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Dissociative anesthesia using ketamine combined with midazolam, dexmedetomidine, or both, with or without reversal, for orchiectomy in guinea pigs (*Cavia porcellus*)

Anestesia dissociativa com cetamina combinada a midazolam, dexmedetomidina ou ambos, revertida ou não, para castração de porquinhos-da-índia (*Cavia porcellus*)

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Highlights

Dexmedetomidine produced deeper sedation than midazolam in guinea pigs.
Dexmedetomidine administration associated with a significant decrease in heart rate.
Partial flumazenil antagonism shortened the anesthesia recovery period.

Abstract

The increasing popularity of guinea pigs as pets and their high reproductive capacity have heightened demand for orchiectomy procedures. This study aimed to evaluate the effects of different anesthetic drug combinations on this species. Eighteen male *Cavia porcellus* (641 ± 135 g) were randomly assigned to three groups (n = 6) after baseline blood glucose measurement: ketamine (15 mg kg^{-1}) combined with midazolam (1 mg kg^{-1}) (M), dexmedetomidine (10 µg kg^{-1}) (D), or half-doses of each (0.5 mg kg^{-1} midazolam and 5 µg kg^{-1} dexmedetomidine) (DM). After intramuscular administration, sedation was scored using two systems that assessed posture, response to stimuli, and muscle relaxation. Orchiectomy was then performed under aseptic conditions, with all animals receiving standardized supportive care, including thermal support via a heating mattress and continuous monitoring of vital parameters. A single veterinarian performed all surgeries to ensure consistency. Anesthesia was maintained with isoflurane (1

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vol%) via face mask, adjusted by ± 0.25 vol% in response to manipulation and vital signs. Postoperatively, blood glucose was re-measured, and three animals from each group received antagonists: flumazenil (0.1 mg kg^{-1}) in group M, atipamezole (50 µg kg^{-1}) in group D, or half-doses of both drugs in group DM. Recovery was assessed using the blink reflex, time to ventral recumbency, and time to ambulation, with blood glucose levels measured again at the end of recovery. On one sedation scale, group D (score 19, range 18–19) exhibited significantly deeper sedation than group M (14.5, range 7–18), while group DM (18, range 9–19) did not differ significantly from either. Mean heart rate was highest in group M (249 ± 29) compared with groups D (184 ± 16) and DM (180 ± 21). Isoflurane concentration was lowest in group D (0.8 ± 0.2 vol%) compared with groups M (1.5 ± 0.4 vol%) and DM (1.4 ± 0.4 vol%). Antagonists reduced recovery time by 66% in group M, 30% in group D, and 48% in group DM, with significantly shorter times to ambulation observed in groups M and DM. Blood glucose levels did not differ significantly across groups. In conclusion, ketamine combined with dexmedetomidine provided deeper sedation, reduced isoflurane requirements, and, in the groups receiving dexmedetomidine, lowered heart rate compared with midazolam, whereas antagonist administration accelerated recovery in protocols containing midazolam.
Key words: Glucose. Heart rate. Post-anesthetic recovery. Respiratory rate. Sedation.

Resumo

A crescente popularidade dos porquinhos-da-índia como animais de companhia aumentou a demanda por orquiectomias, dada a sua elevada taxa reprodutiva. Assim, este estudo avaliou os efeitos de fármacos anestésicos nessa espécie. Dezoito machos de *Cavia porcellus* (641 ± 135 g) foram distribuídos em três grupos (n = 6) após mensuração inicial da glicemia: cetamina (15 mg kg^{-1}) associada a midazolam (1 mg kg^{-1}) (M), dexmedetomidina (10 µg kg^{-1}) (D) ou meias doses de cada fármaco (0.5 mg kg^{-1} de midazolam e 5 µg kg^{-1} de dexmedetomidina) (DM). Após administração intramuscular, a sedação foi pontuada por duas escalas que avaliam postura, resposta a estímulos e relaxamento muscular. Em seguida, foi realizada uma orquiectomia sob condições assépticas, e todos os animais receberam cuidados de suporte padrão, incluindo aquecimento com colchão térmico e monitorização dos sinais vitais. Todos os procedimentos cirúrgicos foram executados por um único médico-veterinário para garantir consistência. Em seguida, os animais foram anestesiados com isoflurano (1 vol %) por máscara facial, ajustado em ± 0.25 vol % com base na resposta à manipulação e nos sinais vitais. Após a cirurgia, a glicemia foi mensurada novamente e metade dos animais de cada grupo (n = 3) recebeu antagonistas: flumazenil (0.1 mg kg^{-1}) para M, atipamezol (50 µg kg^{-1}) para D e metade dessa dose para DM. A recuperação foi avaliada pelos tempos de retorno do reflexo de piscar, decúbito esternal e deambulação, enquanto os níveis de glicose no sangue foram mensurados ao final da recuperação. Em uma das escalas de sedação, D (pontuação 19, intervalo 18–19) promoveu sedação mais profunda que M (14,5, intervalo 7–18), embora nenhum dos dois diferisse de DM (18, intervalo 9–19). A frequência cardíaca média foi maior em M (249 ± 29) que em D (184 ± 16) ou DM (180 ± 21). A concentração de isoflurano foi menor em D (0.8 ± 0.2) em comparação com M (1.5 ± 0.4) ou DM (1.4 ± 0.4). Os antagonistas reduziram os tempos de recuperação em 66 % em M, 30 % em D e 48 % em DM, com um tempo de retorno à deambulação mais curto nos grupos M e DM. Em conclusão, a combinação de cetamina e dexmedetomidina proporcionou sedação mais profunda, reduziu a necessidade de isoflurano e, nos grupos que receberam dexmedetomidina,

diminuiu a frequência cardíaca em comparação ao midazolam, enquanto o uso de antagonistas acelerou a recuperação nos protocolos com midazolam.
Palavras-chave: Frequência cardíaca. Frequência respiratória. Glicose. Recuperação pós-anestésica. Sedação.

Introduction

Among non-traditional companion animals, guinea pigs (*Cavia porcellus*) are increasingly presented to veterinary clinics and hospitals for a variety of procedures (Zimmerman et al., 2015; Gasparik-Küls et al., 2023). This species is highly prolific, and surgical sterilization is a common method for population control (Shomer et al., 2015). Castration is often preferred in males, since it is less invasive than ovariectomy or ovariohysterectomy in females (Kaiser et al., 2023). However, despite being a relatively simple and quick procedure, male castration still requires deep sedation with a local anesthetic block, dissociative anesthesia, or general anesthesia (Isaza & Isaza, 2020). Different combinations of techniques and drugs have been reported (Allweiler, 2016; Schmitz et al., 2017; Sixtus et al., 2021; Scarabelli & Nardini, 2019, 2020; Bennett & Lewis, 2022; Gasparik-Küls et al., 2023).

Dissociative anesthetics are commonly combined with benzodiazepines, alpha-2 agonists, and opioids, for anesthesia in guinea pigs and other rodents (Schmitz et al., 2016a,b, 2017; Sixtus et al., 2021; Bennett & Lewis, 2022). Drugs such as midazolam and dexmedetomidine offer the advantage of reversibility with specific antagonists, which shortens anesthetic recovery and accelerates the return to normal physiological functions. This is particularly important for guinea pigs, whose metabolism is faster than that of many other species (Bennett & Lewis, 2022). In rats, for example, reversal of dissociative anesthesia using ketamine and medetomidine with atipamezole significantly reduced recovery time (Cruz et al., 1998). To date, however, no guinea pig–specific studies have directly compared ketamine combined with midazolam, dexmedetomidine, or both, supplemented with isoflurane, or evaluated the reversal of part of the anesthetic protocol in a clinical setting.

Previous studies have described anesthetic protocols in guinea pigs using dexmedetomidine (0.005–0.25 mg kg^{-1}) and midazolam (0.5–2 mg kg^{-1}), in combination with alfaxalone, dissociative anesthetics, and opioids (Doerning et al., 2018; Ríos Álvarez et al., 2022; Avelino et al., 2024; Serighelli et al., 2024). Ketamine, in particular, is frequently administered alongside these adjuvant agents during guinea-pig anesthesia. Reported high doses include 75 mg kg^{-1} ketamine combined with 15 mg kg^{-1} xylazine (Schmitz et al., 2016b), while lower-dose combinations include 30 mg kg^{-1} ketamine with 2 mg kg^{-1} midazolam, 40 mg kg^{-1} ketamine with 4 mg kg^{-1} xylazine, and 15 mg kg^{-1} ketamine with 0.5 mg kg^{-1} midazolam (Sixtus et al., 2021; Wharton et al., 2024).

The present study evaluated sedation depth, isoflurane consumption, key vital parameters during orchiectomy, and recovery times in guinea pigs anesthetized with ketamine combined with dexmedetomidine, midazolam, or both, and examined the feasibility of partial antagonization of these anesthetic protocols.

Materials and Methods

Animals

The study was approved by the Ethics Committee on Animal Use of the Federal University of Pelotas (protocol no. 23110.014102/2022-84). Eighteen male Abyssinian-strain guinea pigs (*Cavia porcellus*), sharing a common genetic background due to being donated and bred in isolation from other colonies, underwent orchiectomy. This convenience sample included all available adult males. The animals weighed 641 ± 135 g (mean ± SD) and ranged between six months and two years old. They were housed on a rural property in an open-air pen (~10 m²) beneath a tree, with natural lighting and wooden shelters with lateral circular openings. The diet included commercial feed, tubers, and leafy vegetables, and all animals were clinically healthy.

Experimental protocols

Animals were randomly assigned to three groups of six animals each, as follows: M group (612 ± 134 g), receiving ketamine (100 mg mL^{-1}) at 15 mg kg^{-1} combined with midazolam (5 mg mL^{-1}) at 1 mg kg^{-1}; D group (587 ± 150 g), receiving ketamine (100 mg mL^{-1}) at 15 mg kg^{-1} combined with dexmedetomidine (500 µg mL^{-1}) at 10 µg kg^{-1}; and DM group (723 ± 94 g), receiving a combination of ketamine (100 mg mL^{-1}) at 15 mg kg^{-1}, dexmedetomidine (500 µg mL^{-1}) at 5 µg kg^{-1}, and midazolam at 0.5 mg kg^{-1} (5mg mL^{-1}). There was no statistically significant difference in body weight among the groups. Dexmedetomidine was diluted with sterile water at a ratio of 1:10 for injection using a 1 mL syringe. For each protocol, the agents were mixed in the same syringe and administered intramuscularly into the left or right semitendinosus and semimembranosus muscles, with a maximum volume of 0.3 mL per animal. Blood glucose was measured immediately before drug administration using a drop of blood from the tip of the ear (27G, 13x0.45 mm needle) and a glucometer (Accu-Chek® Active). Monitoring glycemia was important due to the guinea pigs' high metabolic rate and the potential effects of the administered drugs on this parameter.

Sedation assessment

Sedation was evaluated 10 minutes after agent administration using two scoring systems: one previously validated in guinea pigs (P scale; Table 1) and another developed for laboratory animals, specifically rats (Rattus norvegicus; R scale; Table 2). The P scale included five criteria: movement and body tone (0-5), and reaction to handling, posture, and righting reflex (0-3 each) (Sixtus et al., 2021). The R scale consisted of six criteria: spontaneous activity/position, blink reflex, resistance to mouth opening, response to noise, general appearance/attitude, and resistance to lateral recumbency, all scored from 0 to 3, except resistance to lateral recumbency (0-4) (Rondeau et al., 2020). Both scales range from 0 to 19, where 0 indicates an awake, alert, and coordinated animal, and 19 a deeply sedated, atonic animal unresponsive to stimuli. All evaluations were performed by a single observer blinded to the treatment protocol.

Table 1
Guinea-pig sedation scale (P Scale)

Score	Movement	Body tone	Reaction to handling	Posture	Righting reflex
0	Normal coordinated movement	Rigid, hunched, waking tone	Normal reaction	Normal	Regains sternal recumbency immediately
1	Coordinated movement	Relaxed but upright, muscle tone present	Decreased response (flinch/ movement/noise)	Head up, sitting	Regains sternal recumbency within 5–10 s
2	Uncoordinated movement	Drowsy, recumbent, floppy	Minimal response (slight flinch/ movement)	Head down, sternal recumbency	Attempts but fails to regain sternal recumbency
3	Infrequent uncoordinated movement	Sedate, atonic	No response	Lateral recumbency	Does not attempt to reposition
4	Infrequent weak uncoordinated movement	Dorsal recumbency, responsive to stimuli	—	—	—
5	No movement	Dorsal recumbency, unresponsive	—	—	—

Adapted from Sixtus et al. (2021).

Table 2
Rat sedation scale (R Scale)

Score	Spontaneous activity / Position	Resistance to lateral recumbency	Blink reflex	Resistance to mouth opening	Response to noise	General appearance / Attitude
0	Standing ± spontaneous exploration	Cannot be placed in lateral	Brisk	Normal response (bites wood or moves head)	Startle reaction (head + body twitch)	Awake, behaving normally
1	Ataxic / stagger when moving	Placed in lateral; returns ≤ 5 s	Slow, full corneal sweep	Reduced response (jaw moves)	Reduced startle reaction	Awake, behaving abnormally (e.g., hyperactivity)
2	Lying on belly + crawling	Remains in lateral > 5 s with tone	Slow, partial corneal sweep	Much reduced (no jaw movement; tongue stays)	Minimal startle (head twitch)	Tranquil, no movement
3	Lying down, no movement	Remains in lateral > 5 s without tone	Absent	Loss of response (tongue can be extended)	No response	Stupors, no movement
4	—	Loss of righting reflex > 5 s	—	—	—	—

Adapted from Rondeau et al. (2020).

Anesthesia and monitoring

Following sedation assessment, animals were placed in dorsal recumbency on a thermal mattress maintained at 38 ± 1 ºC and fitted with a face mask delivering 100% oxygen with isoflurane at 1 L/min in a non-rebreathing circuit. Isoflurane was initially administered at 1% using a calibrated electronic vaporizer (1415 Pinomatic, Takaoka Medical), and the expired fraction was monitored with a gas analyzer (Vamos Plus®, Dräger). Subsequently, the animals were connected to electrocardiogram (ECG) leads to record heart rate (HR) and rhythm (INcardio®, INpulse animal health), and to a pulse oximeter on a paw to measure oxygen saturation (SpO2) (R40VET®, RZ Vet). Respiratory rate (RR) was measured by direct observation of thoracic movements. Isoflurane concentration was adjusted according to the anesthetic plan until loss of the blink reflex, allowing proper placement of monitoring leads and subcutaneous scrotal and intratesticular administration of 2 mg kg^{-1} lidocaine diluted in 1 mL lactated Ringer's solution.

Intraoperative evaluation

From the onset of anesthesia, HR, SpO2, and RR were monitored through specific surgical stages: placement of surgical drapes (M1), skin incision (M2), traction, clamping, and ligation of the first testis (M3), ligation of the second testis (M4), and subcutaneous closure (M5). The evaluator responsible for recording these parameters during anesthesia was blinded to the treatment protocol. An anesthetic plane was classified as superficial when animals responded to surgical manipulation with movement or when HR or RR increased by more than 30% compared with the preceding measurement; in these cases, the isoflurane concentration was increased by 0.25%. Conversely, if a deep anesthetic plane was indicated by reduced cardiorespiratory values compared with the previous measurement, isoflurane concentration was decreased by 0.25%.

Reversal and postoperative evaluations

At the end of surgery, isoflurane administration was discontinued and blood glucose levels were measured. Subsequently, three animals in each group received intramuscular antagonists to dexmedetomidine, midazolam, or both. The M group received flumazenil (0.1 mg mL^{-1} 0.1 mg kg^{-1}), the D group atipamezole (5000 µg mL-1, 50 µg kg^{-1}), and the DM group received half of the individual doses administered to the M and D groups. From this point onward, recovery was monitored by recording the time (in minutes) to return of the blink reflex, maintenance of ventral recumbency, and initiation of ambulation, even if ataxic or uncoordinated. Blood glucose was reassessed after ambulation began. Postoperative analgesia consisted of orally administered meloxicam (0.5 mg/kg) and dipyrone (500 mg per mL), at a dosage of one drop (25 mg) every 8 hours for 3 days.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0 software (San Diego, California, USA). Data distribution was tested with the Shapiro-Wilk test. Normally distributed data were analyzed by one-way ANOVA with Tukey's post hoc test to compare groups at each time point, while non-normal data were evaluated with the Kruskal-Wallis test followed by Dunn's post hoc test. Recovery times were compared with either an unpaired t-test (non-parametric data) or the Mann-Whitney test (parametric data). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Sedation was assessed using two scoring systems. On the guinea pig-specific scale (P scale), no statistically significant intergroup differences were observed. However, on the rat-specific scale (R scale), sedation scores were significantly higher in group D than in group M ($p = 0.007$) (Figure 1).

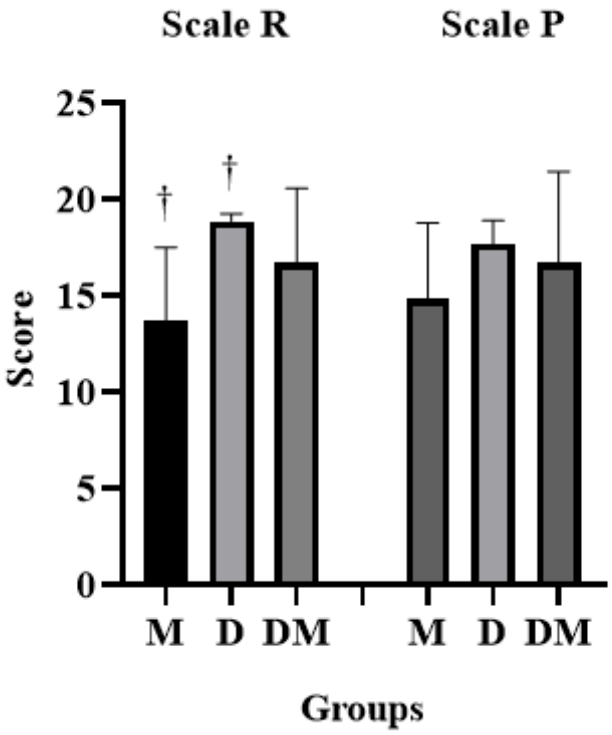

Figure 1. Sedation scores in guinea pigs following administration of ketamine with midazolam (M), ketamine with dexmedetomidine (D), or ketamine with dexmedetomidine and midazolam (DM). The R scale corresponds to the sedation scale described for rats, whereas the P scale corresponds to the sedation scale developed specifically for guinea pigs (Rondeau et al., 2020; Sixtus et al., 2021). Statistically significant intergroup differences are indicated by (†, P ≤ 0.01).
Scale R e Scale P = R scale e P Scale.

During anesthesia, HR values in groups D and DM were comparable but significantly lower than in group M at most surgical stages (M2-M5) (P < 0.05) (Table 3). At M1, there was no significant difference between groups M and D (p = 0.09), but group DM differed from group M (p = 0.03). At this stage (M1), isoflurane concentration was lowest in group D (0.8 ± 0.2 vol%) and highest in group DM (1.8 ± 0.2 vol%) (p = 0.0002). At the end of surgery (M4 and M5), isoflurane concentrations were significantly higher in group M (1.5 ± 0.4 and 1.6 ± 0.3 vol%) compared with group D (0.7 ± 0.3 and 0.8 ± 0.2 vol%) (p = 0.007 and 0.01, respectively), but did not differ significantly from group DM. When mean isoflurane requirements (vol%) were calculated, group D required significantly less isoflurane (0.8 ± 0.2 vol%) than both groups M (1.4 ± 0.4 vol%) and DM (1.3 ± 0.4 vol%) (p = 0.0004 and 0.001, respectively).

Table 3
Cardiorespiratory parameters - heart rate (HR, beats/min), respiratory rate (RR, breaths/min), peripheral oxygen saturation (SpO_2, %), and isoflurane concentration (vol%, as delivered by a calibrated vaporizer with a face mask). Parameters were measured in guinea pigs anesthetized with isoflurane following administration of ketamine and midazolam (M), ketamine and dexmedetomidine (D), or ketamine, midazolam, and dexmedetomidine (DM), during orchiectomy

Parameters	Group	M1	M2	M3	M4	M5
HR (RPM)	M	234 ± 35^{a}	247 ± 34^{a}	248 ± 30^{a}	263 ± 26^{a}	250 ± 17^{a}
	D	190 ± 22^{ab}	185 ± 15^{b}	184 ± 13^{b}	184 ± 14^{b}	177 ± 16^{b}
	DM	185 ± 30^{b}	171 ± 8^{b}	178 ± 22^{b}	179 ± 21^{b}	188 ± 22^{b}
RR (BPM)	M	53 ± 24	50 ± 25	36 ± 5	45 ± 17	51 ± 19
	D	46 ± 14	46 ± 21	36 ± 10	48 ± 23	41 ± 14
	DM	28 ± 15	30 ± 7	24 ± 7	34 ± 8	34 ± 11
SpO_2 (%)	M	99 ± 1	98 ± 2	99 ± 1	98 ± 3	98 ± 3
	D	98 ± 2	99 ± 1	99 ± 1	99 ± 1	98 ± 2
	DM	100	100	99 ± 1	99 ± 1	100
Isoflurane (%)	M	1.3 ± 0.4^{ab}	1.5 ± 0.4	1.2 ± 0.3	1.5 ± 0.4^{a}	1.6 ± 0.3^{a}
	D	0.8 ± 0.2^{a}	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.3^{b}	0.8 ± 0.2^{b}
	DM	1.8 ± 0.2^{b}	1.4 ± 0.3	1.4 ± 0.3	1.2 ± 0.5^{ab}	0.9 ± 0.3^{ab}

Values sharing identical letters, or lacking letters, do not differ significantly ($P > 0.05$).
Different letters indicate significant differences ($P \leq 0.05$).

In the recovery phase, administration of antagonists to dexmedetomidine, midazolam, or both, did not significantly alter the time to return of the blink reflex between reversed and non-reversed animals, although numerical variation was observed (Figure 2). However, time to ventral recumbency was shorter in animals reversed with midazolam alone (M) or dexmedetomidine (D) ($p = 0.01$ and 0.04, respectively). Similarly, ambulation, even when ataxic, occurred sooner in animals reversed with midazolam alone (M) and with the combined antagonists atipamezole and flumazenil (DM) ($p = 0.01$ and 0.04, respectively). By contrast, animals subjected to dexmedetomidine reversal (group D) showed only a non-significant trend towards faster ambulation ($p = 0.09$).

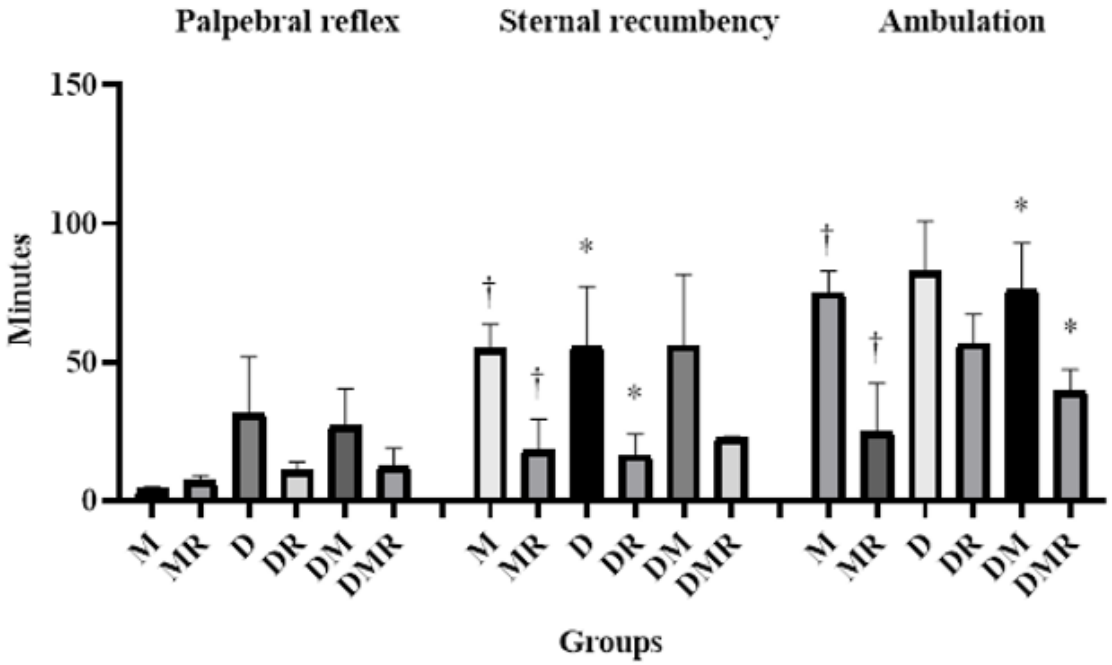

Figure 2. Recovery times (minutes) for the return of the blink reflex, maintenance of sternal recumbency, and onset of ambulation in guinea pigs anesthetized with ketamine and midazolam, ketamine and dexmedetomidine, or a combination of both. After inhalational anesthesia with isoflurane and orchiectomy, half of the animals in each group received flumazenil, atipamezole, or both for drug reversal. Statistically significant intergroup differences are indicated by (*, P ≤ 0.05; †, P ≤ 0.01).
Midazolam group without reversal (M)
Midazolam group with reversal (MR)
Dexmedetomidine group without reversal (D)
Dexmedetomidine group with reversal (DR)
Dexmedetomidine and Midazolam group without reversal (DM)
Dexmedetomidine and Midazolam group with reversal (DMR).

Blood glucose was measured at three time points: pre-anesthesia, post-anesthesia, and at the end of recovery. No statistically significant differences were observed between or within groups. However, blood glucose values in groups D and M exhibited a non-significant trend following recovery (p = 0.07) (Table 4).

Table 4
Blood glucose levels (mg/dL) in guinea pigs anesthetized with ketamine and midazolam (M), ketamine and dexmedetomidine (D), or ketamine, midazolam, and dexmedetomidine (DM), following isoflurane inhalational anesthesia and orchiectomy. Measurements were taken at three time points: pre-anesthesia, post-anesthesia, and post-recovery

Group	Pre-anesthesia	Post-anesthesia	Post-recovery
M	101 ± 17	133 ± 29	125 ± 27
D	98 ± 8	126 ± 37	85 ± 16
DM	95 ± 13	113 ± 19	99 ± 25

No significant differences were detected among groups (P > 0.05).

The present study used relatively low doses of ketamine, dexmedetomidine, and midazolam, consistent with previously reported protocols (Fox et al., 2016; Schmitz et al., 2017; Serighelli et al., 2024; Wharton et al., 2024). The exact ages of the animals could not be determined because they were housed in mixed-sex enclosures containing both juveniles and adults. Despite this variation, mean body weight did not differ significantly between groups, and none of the animals displayed clinical signs such as reduced food intake, poor coat condition, or lethargy, either before or after the procedure. All recovered uneventfully from anesthesia and surgery, with successful wound closure and without requiring postoperative antibiotics or additional interventions.

Assessment of sedation depth highlighted differences between the scales applied. The R scale proved more sensitive, since it incorporated parameters such as spontaneous activity, posture, righting reflex, blink reflex, response to mouth manipulation, response to noise, and general condition and behavior (Rondeau et al., 2020). By contrast, the P scale evaluated movement, body tone, reaction to handling, posture, and righting reflex (Sixtus et al., 2021). The greater sensitivity of the R scale likely reflects its evaluation of mouth movement and tongue manipulation. Moreover, previous studies of guinea pigs anesthetized with xylazine or dexmedetomidine combined with esketamine and morphine reported persistence of the blink reflex (Serighelli et al., 2024), whereas in the present study this reflex was consistently reduced or absent, probably due to differences in drug choice and dosing.

The design of the scales themselves also helps explain these results. The P scale was originally applied following the administration of single drugs, whereas the R scale was tested after drug combinations (Rondeau et al., 2020; Sixtus et al., 2021). In those studies, ketamine alone achieved the highest peak cumulative sedative-depth score (16.7 ± 0.6 out of 19), followed by alfaxalone (13.9 ± 2.2), with diazepam (9.3 ± 2.1) and midazolam (7.6 ± 2.7) producing lower scores respectively (Sixtus et al., 2021). Rondeau et al. (2020), also showed that alfaxalone + hydromorphone and ketamine + midazolam + hydromorphone yielded comparable median total sedation

scores at 5 minutes (10.9 [8.09–13.70] versus 7.9 [5.13–10.60]) and 15 minutes (9.1 [6.26–11.90] versus 11.3 [8.59–14.00]), but only the ketamine combination maintained a high score at 20 minutes (11.8 [9.05–14.50]), whereas the alfaxalone combination declined to 7.2 [4.34–9.99], reflecting the shorter duration of alfaxalone. In the present study, dexmedetomidine consistently produced deeper sedation, both quantitatively and clinically, reinforcing findings in dogs and cats that alpha-2 agonists are more effective sedatives than benzodiazepines (Murrell & Hellebrekers, 2005).

Conversely, in rodents, especially guinea pigs, this distinction is less consistently reported, partly because of the limited number of studies evaluating the sedative efficacy of benzodiazepines and alpha-2 agonists in these species, as well as the heterogeneity of dosing regimens and assessment methods (Avelino et al., 2024). This variability was also observed in the present study, where one scale detected differences in sedation depth, whereas the other did not. Nevertheless, additional evidence supports this finding. For example, nasal administration of butorphanol combined with either dexmedetomidine or midazolam in rabbits produced greater sedation with dexmedetomidine (Okur et al., 2023).

Rodents generally require higher anesthetic dosages than other species. In guinea pigs, this is largely due to their increased metabolic rate compared to other mammals (Shomer et al., 2015). Required doses also depend on the anesthetic protocol used and the invasiveness of the procedure, with more invasive interventions demanding higher dosages, broader drug combinations, and more complex techniques (Allweiler, 2016; Scarabelli & Nardini, 2019, 2020; Bennett & Lewis, 2022). For instance, Sixtus et al. (2021) reported that midazolam, when administered alone, produced the lowest total sedation scores in guinea pigs when compared with diazepam, alfaxalone, or ketamine, although their study did not assess alpha-2 agonists.

In a study aimed at achieving a surgical plane of anesthesia in guinea pigs, there was no significant difference in sedation between midazolam and dexmedetomidine when each was combined with alfaxalone and fentanyl. However, during the pilot phase, where dexmedetomidine or midazolam was combined with the same dose of alfaxalone but without fentanyl, dexmedetomidine resulted in deeper sedation than midazolam (Avelino et al., 2024). These findings, consistent with those of the present study, align with reports in rats and rabbits showing superior sedative efficacy of alpha-2 agonists over benzodiazepines (Boehm et al., 2010; Bellini et al., 2014; Kawano et al., 2015).

Another aspect that warrants discussion is the freezing response exhibited by guinea pigs. In the present study, this behavior was unlikely to have influenced the results. The dissociative effects of ketamine rendered the animals unresponsive to environmental stimuli, as reflected by the medium-to-high sedation scores recorded across all groups. Although the R scale has not been formally validated in guinea pigs, rats and guinea pigs share several behavioral patterns, and the parameters assessed capture the same core indicators of sedation. Consequently, the R scale proved more sensitive in detecting subtle differences in sedation depth.

Heart rate decreased with dexmedetomidine administration, consistent with previous findings in guinea pigs anesthetized with alfaxalone and fentanyl combined with either midazolam or dexmedetomidine (Avelino et al., 2024). This effect is likely due to dexmedetomidine's direct suppression of sympathetic activity and indirect elevation of blood pressure through peripheral vasoconstriction, which triggers a reflex increase in parasympathetic activity (Bennett & Lewis, 2022). Despite the HR reduction observed with dexmedetomidine in the present study, no arrhythmias occurred during the procedure. By contrast, midazolam and other benzodiazepines generally do not significantly affect cardiovascular function at recommended doses (Allweiler, 2016).

Blood pressure measurements obtained via the oscillometric method were deemed unreliable when compared with validated invasive and non-invasive techniques in conscious and anesthetized guinea pigs (Schmitz et al., 2016a,b, 2017; Sixtus et al., 2021; Avelino et al., 2024). Challenges in accurately measuring blood pressure in this species arise due to their limb morphology, smaller body size relative to dogs and cats, and the lack of species-adapted equipment. Nonetheless, dexmedetomidine likely exerts a vasopressor effect in guinea pigs, similar to its actions in other mammals.

Respiratory rate and SpO_2 did not differ significantly among groups and remained within reference ranges for the species, with no apnea episodes and SpO_2 consistently above 96%. Nevertheless, hypercapnia and respiratory depression cannot be entirely ruled out. Oral secretions accumulated in some animals across all groups, consistent with previous reports (Cantwell, 2001; Lennox & Capello, 2008), and were promptly removed using Halstead mosquito hemostatic forceps wrapped in gauze. This buildup likely from brief pre-anesthetic fasting, since food and water were withheld for only a short period before the procedure, combined with ketamine and isoflurane effects on mucous membranes (Schmitz et al., 2016b; Serighelli et al., 2024).

When evaluating the effects of anesthetic protocols on isoflurane requirements, it is plausible that the higher dexmedetomidine dosage may have contributed to the reduced isoflane concentrations observed in this study. Similar findings have been reported in rabbit castration, where ketamine combined with medetomidine, a racemic mixture containing dexmedetomidine, significantly decreased the isoflurane requirement from 2.1 ± 0.6 % to 1.2 ± 0.8 % compared to ketamine with midazolam (Grint & Murison, 2008). Notably, in this study, isoflurane was delivered via a face mask covering the nasal and oral planes rather than intubation, and inspired isoflurane concentrations for each animal were titrated to maintain adequate anesthetic depth for stable surgical positioning and sensor placement.

Atipamezole and flumazenil doses were based on previous studies and the clinical experience of the investigators. In the DM group, each antagonist was administered at half the standard dose to match the reduced dexmedetomidine and midazolam doses, avoiding potential overdosing. Sedation duration did not differ with or without reversal, consistent with pharmacokinetic data from dogs, which showed similar plasma clearance rates for intramuscular midazolam (0.2 mg kg^{-1}) and dexmedetomidine (10 µg

kg^{-1}) (Schwartz et al., 2013; Aarnes et al., 2023). A similar observation was made in rabbits, where pharmacokinetic studies on dexmedetomidine (20 µg kg^{-1} IV) found an elimination half-life of 80 minutes, comparable to the 97-minute half-life of 1-hydroxymidazolam, the active metabolite of midazolam (1.2 mg kg^{-1} IV) (Bailey et al., 2017; Rousseau-Blass et al., 2021).

A pharmacokinetic study in guinea pigs (Wang et al., 2021) examined oral doses of midazolam (5, 10, and 25 mg kg^{-1}), measuring plasma concentrations of the drug and 1-hydroxymidazolam, its active metabolite. The authors reported that the average elimination half-life of midazolam and its active metabolite were 72 and 144 minutes; 84 and 216 minutes; and 174 and 330 minutes, respectively, for the escalating doses. However, no comparable data exist for dexmedetomidine in guinea pigs.

Pharmacodynamic studies in rabbits demonstrated that intravenous midazolam at 0.35 mg kg^{-1} produced a 40-minute duration of action, whereas dexmedetomidine at a dose of 35 µg kg^{-1} yielded a shorter 30-minute effect (Bienert et al., 2014). Conversely, combining butorphanol (0.4 mg kg^{-1}) with dexmedetomidine (0.1 mg kg^{-1}) produced deeper sedation than butorphanol with midazolam (2 mg kg^{-1}), even though sedation lasted longer with midazolam (Okur et al., 2023). This prolonged effect is likely related to residual 1-hydroxymidazolam. However, in the present study, the use of multiple agents and the length of surgery likely masked the extended sedative action of midazolam.

No significant difference in the blink reflex was observed between animals receiving reversal drugs and those that did not. This outcome likely reflects high interindividual variability, which increased standard deviations and reduced statistical power, compounded by the small sample size in this study, which may have limited the ability to detect differences between the two groups. The timing of blink reflex return may depend more on recovery from isoflurane anesthesia than on the reversal and clearance of injectable drugs. Moreover, the blink reflex in guinea pigs is subtler than in larger species, complicating its accurate assessment. While it may indicate that the animal has regained consciousness, it should not be interpreted in isolation.

Recovery of the righting reflex differed between animals that received antagonists and those that did not, reflecting residual effects of injectable drugs. Although antagonists were only administered after isoflurane was discontinued, the cumulative time from sedative administration through preparation and surgery likely influenced recovery. In the DM group, no significant difference was observed, likely due to high variability among animals without antagonism. Conversely, time to regain ambulation declined by 66.67% in the M group, 30.49% in the D group, and 48.68% in the DM group. These results indicate that pharmacological antagonism of dexmedetomidine and midazolam is clinically relevant for facilitating rapid recovery from anesthesia, particularly in shorter procedures.

While no previous studies have used an identical experimental model, considering variations in dosage, procedural length, parameters, and species, the results observed have clear clinical relevance. Recovery was consistently faster both with complete antagonism of administered drugs

and with partial antagonism of the anesthetic protocol. In this study, partial antagonism reduced mean ambulation times to 25 ± 18 minutes in the M group, 57 ± 11 minutes in the D group, and 39 ± 8 minutes in the DM group, compared with 75 ± 18 minutes, 82 ± 18 minutes, and 76 ± 17 minutes, respectively, in animals without antagonists.

Other studies in guinea pigs have highlighted the benefits of antagonizing anesthetic drugs (Schmitz et al., 2016a,b, 2017). For instance, Schmitz et al. (2016b) compared complete antagonism of medetomidine (0.2 mg kg^{-1}), midazolam (1 mg kg^{-1}), and fentanyl (0.025 mg kg^{-1}), using atipamezole (1 mg kg^{-1}), flumazenil (0.1 mg kg^{-1}), and naloxone (0.03 mg kg^{-1}), with the selective reversal of ketamine (75 mg kg^{-1}) and xylazine (15 mg kg^{-1}), using only atipamezole (0.15 mg kg^{-1}). Complete antagonism provided a faster recovery (7 minutes), whereas selective reversal prolonged sedation (59 minutes) (Schmitz et al., 2016b). This effect was attributed to the high ketamine dose, which has no antagonist and was five times higher than that used in the present study.

An additional consideration is that dexmedetomidine, unlike midazolam, also has analgesic properties (Allweiler, 2016). Consequently, administration of an antagonist reverses both its sedative and analgesic effects. This raises ethical concerns when antagonists are used after invasive procedures, as reported in clinical case studies of total intravenous anesthesia with propofol in guinea pigs (Gasparik-Küls et al., 2023). Thus, during invasive procedures, it may be preferable not to antagonize dexmedetomidine in order to preserve analgesia. However, because midazolam has no analgesic effect, its antagonism can safely reverse anesthetic adjuvant and muscle relaxant properties without compromising analgesia. Evidence in rats also suggests that midazolam enhances dexmedetomidine-induced analgesia (Boehm et al., 2010). Thus, antagonizing midazolam in combination protocols with dexmedetomidine may inadvertently reduce overall analgesic effects during more invasive procedures.

In this study, blood glucose concentrations were not significantly affected, likely due to the relatively low dosages compared with those typically reported for rodents. Physiological ranges for guinea pigs vary widely across studies (80–110 mg dL^{-1}, 89–297 mg dL^{-1}, and 83–352 mg dL), reflecting methodological and equipment differences (Rabe, 2011; Shomer et al., 2015; Schmitz et al., 2016b). Despite these variations, mean blood glucose concentrations in the present study remained within published ranges. However, after recovery of ambulation, animals in the D group (dexmedetomidine) exhibited mean values at or below the lower limit of some reference ranges, indicating a late decline in blood glucose concentration.

Hyperglycemia following administration of alpha-2 agonists has been previously described in guinea pigs (Schmitz et al., 2016b, 2017; Kint et al., 2020). The biphasic glucose response in the D group may reflect a counter-regulatory mechanism: dexmedetomidine initially suppresses insulin secretion, increasing blood glucose, followed by a compensatory rise in insulin as the drug's effect on the pancreas diminishes, promoting cellular glucose uptake. This same effect was observed in rats anesthetized with ketamine and medetomidine (Connell et al.,

2022). By contrast, the M group (midazolam) maintained stable blood glucose levels, likely due to ketamine's sympathomimetic metabolic effect, which increases blood glucose. Midazolam has also been associated with hyperglycemia in rabbits (Atalan et al., 2019).

The main limitation of this study was the small sample size, since animals were obtained from a non-governmental organization and all available individuals underwent castration. Additionally, accurately measuring blood pressure non-invasively posed a significant challenge. The absence of validated, species-specific equipment for guinea pigs further required the use of monitors designed for dogs and cats, which were unsuitable for the small body size of the animals weighing less than one kilogram. Moreover, the inability to perform endotracheal intubation prevented more accurate monitoring of respiration and end-tidal carbon dioxide pressure. Therefore, access to guinea pig-specific non-invasive blood pressure measurement devices and endotracheal intubation equipment could strengthen physiological monitoring and provide a more comprehensive evaluation of anesthetic effects.

Conclusions

Under the conditions of the present study, dexmedetomidine combined with ketamine produced deeper sedation than midazolam combined with ketamine. Moreover, dexmedetomidine administration decreased HR. Antagonists appeared beneficial only in midazolam-treated groups or in short-duration procedures. The combination of dexmedetomidine and midazolam provided no clinical benefit at the tested doses.

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