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Intramuscular alfaxalone with or without buprenorphine or hydromorphone provides sedation with minimal adverse effects in healthy rabbits (*Oryctolagus cuniculus*) in a randomized blinded controlled trial

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OBJECTIVE
To evaluate the effects of alfaxalone administered IM with or without buprenorphine or hydromorphone in healthy rabbits (*Oryctolagus cuniculus*).

ANIMALS
24 male rabbits undergoing elective orchiectomy between August 21, 2021, and November 6, 2021.

PROCEDURES
In this controlled clinical trial, rabbits were randomly assigned to receive alfaxalone (4 mg/kg, IM) alone (group A; n = 8) or with buprenorphine (0.03 mg/kg, IM; group BA; 8) or hydromorphone (0.1 mg/kg, IM; group HA; 8). Vital signs and sedation scores were recorded immediately prior to (T0) and 10 minutes after (T1) treatment. Ease of IV catheter placement and pain scores were also evaluated. All rabbits received ketamine (2.5 mg/kg, IV), midazolam (0.13 mg/kg, IV), and meloxicam (0.5 mg/kg, SC) before orchiectomy but after IM treatments. Results were compared across groups with ANOVA or Fisher exact tests and across time with paired *t* tests.

RESULTS
Sedation score, median time to recumbency, and ease of catheter placement did not differ among groups. Supraglottic airway device placement was possible for 1 rabbit in group A, 1 in group BA, and 2 in group HA. Mean respiratory rate at T1 versus T0 was significantly decreased for groups BA (63.8 vs 128.6 breaths/min) and HA (66.7 vs 123.2 breaths/min). Mean postoperative pain scores were significantly lower for rabbits in group HA (0.58), compared with those in groups A (2.25) and BA (2.06).

CLINICAL RELEVANCE
All 3 treatments provided reliable sedation; however, alfaxalone (4 mg/kg, IM) combined with hydromorphone (0.1 mg/kg, IM) may be a better choice for painful procedures.

Rabbits (*Oryctolagus cuniculus*) are at a higher risk of anesthetic-related death (overall risk of 1.39% [114/8,209]) when compared to other domestic species such as dogs (0.17% [163/98,036]) and cats (0.24% [189/79,178]).[1] In a retrospective study[2] of 210 rabbits, a 4.8% (10/210) mortality rate within 72 hours after anesthesia/sedation was reported. Possible contributing factors for the higher mortality rate include undiagnosed cardiovascular or respiratory diseases such as *Pasteurella multocida*, challenges with IV catheterization and endotracheal intubation due to size, anatomic differences (eg, small oropharyngeal cavity and glottis), and physiologic responses to stress.[1–4] Rabbits are prey animals and become stressed when approached or manipulated for examinations and procedures. Administration of sedatives and anesthetics is often required, and the IM route is ideal due to less handling and associated risks and stress of IV administration and catheter placement.

Sedation or anesthesia in rabbits with injectable drugs such as ketamine and medetomidine may result in adverse effects.[5,6] Ketamine administered IM is reported to be painful, and rabbits anesthetized with medetomidine-based protocols have a higher risk of developing laryngospasm and bradycardia.[6,7] Therefore, drugs such as alfaxalone have gained popularity as an alternative for sedation and immobilization of rabbits. Alfaxalone is a neurosteroid anesthetic agent commonly administered IV for induction of anesthesia, but it can also be safely administered IM

(extralabel). It has a rapid onset but short duration of action and appears to cause minimal to mild cardiovascular effects. Studies[8–11] evaluating the safety of IM administration of alfaxalone at doses of 4 to 7 mg/kg reported smooth and rapid onset of sedation with minimal adverse effects. Due to alfaxalone's lack of analgesic properties, agents such as α_2-adrenoceptor agonists and opioids are often added to drug protocols to provide appropriate analgesia for animals undergoing invasive or painful procedures.

Buprenorphine and hydromorphone are effective opioid analgesics commonly administered in veterinary medicine.[12,13] These drugs exert minimal but dose-dependent effects on the cardiopulmonary system.[14] In rabbits, preemptive administration of buprenorphine was found to blunt the reflex response to painful somatic and colorectal visceral pain.[3,15] Buprenorphine is a partial μ-opioid receptor agonist with a high affinity for the μ receptor but only partial activity.[16] In rabbits, onset of thermal analgesia following 0.015 mg/kg IV and 0.03 mg/kg SC buprenorphine is 15 to 30 minutes with a duration of action of 8 to 10 hours.[12,17] Effective analgesia is more likely to be achieved following IM or IV administration due to higher blood plasma levels obtained with these routes.[18] Hydromorphone is a full μ-opioid receptor agonist used to treat moderate to severe pain. Its administration provides analgesia but may also result in mild to moderate sedation in dogs and cats.[19] Reported analgesic onset times in mammals are within 15 to 30 minutes, and onset of sedation may be evident within 5 minutes of IM or IV administration.[13,20] Commonly recommended IV and IM doses of hydromorphone in rabbits range from 0.1 to 0.5 mg/kg.[21,22] Based on a recent literature review, there are no studies comparing the physiologic and sedative effects of alfaxalone administered IM with or without buprenorphine or hydromorphone.

The aim of this study was to evaluate the effects of alfaxalone (4 mg/kg, IM) alone or with buprenorphine (0.03 mg/kg, IM) or hydromorphone (0.1 mg/kg, IM) in healthy rabbits. We hypothesized that IM alfaxalone combined with hydromorphone would result in higher sedation scores than alfaxalone with buprenorphine and alfaxalone alone.

Materials and Methods

Animals

Twenty-four sexually intact male rescue-owned rabbits undergoing elective orchiectomy at Midwestern University Companion Animal Hospital between August 21, 2021, and November 6, 2021, were included in this study. The estimated age categories were juvenile (4 to 6 months) and adult (7 months to 1 year). Enrollment criteria included an American Society of Anesthesiologists health status classification of 1 or 2 based on history and physical examination. A power analysis based on the Kruskal-Wallis H test determined that 8 rabbits/group were required to detect a 1-point difference with SD of 0.5 and achieve 80% power with a significance level of .05. This study was approved by Midwestern University's IACUC.

Experimental treatment

Upon arrival, body weight was obtained, then rabbits were housed individually and allowed to acclimatize for 2 hours before the study started. Baseline physiologic variables after acclimation (T0) were recorded and included respiratory rate (RR), heart rate (HR), rectal temperature and indirect mean blood pressure (BP), and indirect systolic BP (Vet20 oscillometric BP monitor; SunTech Medical Inc). The width of the BP cuff was 30% to 40% of limb circumference and placed on the left anterior forelimb proximal to the carpus. Pain was also scored using the rabbit grimace scale (RbtGS),[23] and sedation was assessed using a semiquantitative rating scale. The sedation scale consisted of 6 categories, and their scores were summed for a total score ranging from 0 to 15 **(Appendix)**. Investigators (RSC and RT) examining and scoring the rabbits were blinded to the treatment group. Rabbits were left undisturbed for an additional 30 minutes after physical examination was performed and baseline data obtained. Food but not water was also removed 30 minutes prior to the IM treatment.

Rabbits were randomly assigned to 1 of 3 treatment groups using a random-number generator (Excel RAND function; Microsoft Corp) to receive alfaxalone (Alfaxan; 4 mg/kg, IM) alone (group A [control group]; n = 8) or with either buprenorphine (Buprenorphine HCl; 0.03 mg/kg, IM; group BA; 8) or hydromorphone (HYDROmorphone HCl; 0.1 mg/kg, IM; group HA; 8). Drugs were mixed immediately prior to injection and administered with a 22-gauge needle into the epaxial muscles. Extralabel use of alfaxalone was performed with client consent and complied with provisions of AMDUCA and 21 CFR §530. Combining the assigned sedatives into 1 syringe for administration was done so that each rabbit received only 1 IM injection. After receiving the assigned IM treatment, each rabbit was placed in their individual cage and left undisturbed for another 10 minutes. Changes in behavior, muscle tone, and body position were monitored and recorded. After 10 minutes of treatment administration (T1), pain and sedation scores followed by physiologic variables (RR, HR, BP, and rectal temperature) were recorded. After data were collected, a Doppler probe (Parks Medical Products Inc) was placed over the femoral artery and pulse rate was monitored during IV catheter and supraglottic airway device placement.

Following hair clipping and aseptic skin preparation, placement of a 24-gauge IV catheter was attempted in the left cephalic vein. If not successful, placement was attempted in the left auricular vein with a maximum of 2 attempts on each site. Assessment of ease of IV catheter placement was based on a semiquantitative rating scale, and scores were 1 to 5, as follows: no resistance, minimal restraint, and catheterized on first attempt (1); slight resistance, moderate restraint, and successful on first or second attempt on the same site (2); moderate resistance, moderate restraint, and successful on first attempt on the second site (3); moderate resistance, considerable restraint, and successful on second at-

tempt on the second site (4); and marked resistance, marked restraint, and unable to place the IV catheter (5). Time between IM drug treatment to first attempt of IV catheter placement was also recorded.

After IV catheter placement, oral insertion of a supraglottic airway device (V-Gel; Docsinnovent) was attempted. If insertion of the airway device was not possible, half of the induction drugs were administered: ketamine (Zetamine; 2.5 mg/kg, IV) and midazolam (0.13 mg/kg, IV). Once patency of the selected supraglottic airway device was confirmed via capnography, rabbits were connected to a nonrebreathing system and general anesthesia was performed with isoflurane delivered in oxygen.

All rabbits received meloxicam (0.5 mg/kg, SC) prior to surgery. If ketamine and midazolam administered IV were not already administered to allow for successful placement of a supraglottic airway device, the same doses, 2.5 mg of ketamine/kg and 0.13 mg of midazolam/kg were given IV prior to skin incision. All rabbits received a total of 2.5 mg of ketamine/kg IV and 0.13 mg of midazolam/kg IV. Rabbits received intraoperative IV fluid therapy (lactated Ringer solution; 10 mL/kg/h). Measurements of HR, RR, end-tidal carbon dioxide, pulse oximetry, indirect BP, and rectal temperature were recorded every 5 minutes, as were the isoflurane vaporizer setting and oxygen flow rate. Heat support was provided perioperatively as needed. Intraoperative interventions that were required, if any, were also recorded. A closed castration was performed by making a scrotal skin incision over each testicle. Spermatic cords were ligated with 3-0 poliglecaprone 25 and 2 ligatures (strangle and transfixation). Scrotal sites were apposed with skin glue. In the postoperative period, HR, RR, and rectal temperature were assessed every 30 minutes until the animal was fully recovered. Recovery and pain scores were also recorded postoperatively. The recovery scale consisted of 4 categories: smooth recovery, regains functions and rights self with minimal disturbances (1); adequate recovery, rights self with mild excitement, shivering, or ataxia (2); fair recovery, moderate excitement and ataxia, mild paddling or kicking, few attempts to right self (3); or poor recovery, paddling, vocalizing, or kicking, some restraint necessary (4). Rescue analgesia was administered to animals with an RbtGS ≥ 3/10.

Statistical analysis

Statistical analysis was performed using R (version 4.1.0; R Foundation for Statistical Computing). Data were reported as mean, SD, and range. Baseline, demographic, and outcome variables were compared between the treatment groups with 1-way ANOVA. Physiologic variables at time 0 and 10 minutes were compared with a paired *t* test. Normality was assessed visually with histograms and Q-Q plots. Ordinal scores, such as sedation, IV catheter, and pain, were analyzed by the Kruskal-Wallis test. The significance level was set at .05.

Results

No significant differences were found in body weight (P = .21), estimated age categories (P = .24) and baseline (T0) physiologic variables between treatment groups. After 10 minutes following IM treatment (T1), mean HR of rabbits in group BA was significantly higher when compared to baseline (P = .03) but mean BP remained unchanged and within normal ranges. No differences in mean HR were found in groups A and HA **(Table 1)**. Mean RR was significantly lower when compared to baseline in groups BA (P = .007) and HA (P = .02) but not in A (P = .06). No apnea occurred in rabbits in any treatment group. No significant differences in mean rectal temperature and mean BP between T0 and T1 were found. The only adverse event observed after

Table 1—Comparisons (paired *t* test) of results for physiologic variables for 24 rescue-owned healthy male rabbits immediately before (T0) versus 10 minutes after (T1) receiving IM administration of either alfaxalone (4 mg/kg) alone (group A; n = 8), alfaxalone (4 mg/kg) combined with buprenorphine (0.03 mg/kg; group BA; 8), or alfaxalone (4 mg/kg) combined with hydromorphone (0.1 mg/kg; group HA; 8) before receiving ketamine (2.5 mg/kg, IV), midazolam (0.13 mg/kg, IV), and meloxicam (0.5 mg/kg, SC) for elective orchiectomy during a randomized controlled clinical trial between August 21, 2021, and November 6, 2021.

Variable	Group	Mean ± SD (range) at T0	Mean ± SD (range) at T1	*P* value[a]
HR (beats/min)	A	205.5 ± 52.4 (123–270)	221.9 ± 39.1 (165–300)	.71
	BA	196.1 ± 40.9 (136–267)	238.3 ± 17.5 (220–270)	.03[a]
	HA	202.8 ± 41.6 (128–260)	210.3 ± 44.3 (120–260)	.52
RR (breaths/min)	A	110.1 ± 27.1 (88–160)	65.4 ± 40.7 (28–150)	.06
	BA	128.6 ± 36.8 (88–170)	63.8 ± 35.6 (32–130)	< .01[a]
	HA	123.2 ± 35.4 (64–200)	66.7 ± 38.5 (32–150)	.02[a]
Rectal temperature (°C)	A	38.1 ± 2.3 (35.6–39.4)	38.4 ± 1.0 (37.6–38.9)	.73
	BA	38.6 ± 0.8 (37.7–39.2)	38.3 ± 1.0 (37.4–39.2)	.52
	HA	38.7 ± 1.0 (37.7–39.3)	38.7 ± 0.4 (38.4–39)	.88
SBP (mm Hg)	A	128.0 ± 27.6 (71–152)	117.4 ± 21.2 (80–150)	.49
	BA	110.3 ± 23.2 (81–138)	128.4 ± 25.3 (85–167)	.22
	HA	148.0 ± 32.2 (122–201)	120.4 ± 15.1 (96–135)	.10
MBP (mm Hg)	A	110.0 ± 16.8 (90–137)	104.3 ± 37.5 (57–173)	.76
	BA	104.0 ± 46.9 (66–203)	103.6 ± 24.4 (69–137)	.98
	HA	118.0 ± 30.1 (88–164)	93.4 ± 12.5 (79–112)	.09

[a]Values of P < .05 indicate a significant difference between results for T0 versus T1.
HR = Heart rate. MAP = Indirect mean blood pressure. RR = Respiratory rate. SBP = Indirect systolic blood pressure.

IM treatment was hypersalivation in 1 of the 8 rabbits in group A and 1 of the 8 rabbits in group BA.

Mean sedation score did not differ between groups (P = .92). Mean (SD) sedation scores for groups A, BA, and HA were 11.88 (5.06), 11.00 (3.82), and 11.13 (4.91), respectively. Moderate to heavy sedation was achieved within 10 minutes for 6 of the 8 rabbits in group A and 5 of 8 rabbits in groups BA and HA. Some rabbits achieved lateral recumbency prior to the 10-minute mark, but the mean time to achieve recumbency was not different between treatment groups (P = .16). Mean (SD) time in minutes was 6.67 (1.51), 7.72 (2.87), and 10.05 (5.87) for groups A, BA, and HA, respectively. One rabbit from A and another from BA never reached spontaneous lateral recumbency and would return to sternal with the head down when manually placed in lateral.

No difference was found between groups and ease of IV catheter placement (P = .19). Catheter was successfully placed in all rabbits following IM treatment. Mean (SD) scores were 3.19 (1.13) for A, 2.38 (0.81) for BA, and 2.63 (0.60) for HA. Median time from IM drug treatment to first IV catheter attempt was 25.5 minutes (range, 18 to 30 minutes) for A, 21 minutes (range, 15 to 35 minutes) for BA, and 25 minutes (range, 20 to 42 minutes) for HA (P = .24). Partial limb withdrawal was observed in 4 rabbits in groups A and BA and 3 in HA.

Placement of a supraglottic airway device following IM treatment was possible in 1 of 8 rabbits in A and BA and 2 of 8 in HA. Placement of a supraglottic airway device in all remaining rabbits was only possible after induction of anesthesia with ketamine (2.5 mg/kg, IV) and midazolam (0.13 mg/kg, IV).

No differences in surgery duration (P = .7), isoflurane concentration (P = .7), and mean recovery scores (P = .25) were observed between treatment groups. Intraoperative bradycardia was observed in only 1 out of 8 rabbits in group A and successfully treated with glycopyrrolate (0.01 mg/kg). Mean postoperative pain scores were significantly lower in group HA in comparison to groups A (P = .006) and BA (P = .014). Mean ± SD pain scores for A, BA, and HA were 2.25 ± 1.26 (range, 0 to 4.3), 2.06 ± 0.98 (range, 1 to 4), and 0.58 ± 0.43 (range, 0 to 1), respectively. Three rabbits in A and 2 in BA received rescue analgesia postoperatively. The median time to recovery from anesthesia was 30 minutes (range, 15 to 70 minutes), and all rabbits recovered uneventfully and were discharged on the same day. The rescue organization was contacted 1 week after study completion, and no adverse events or fatalities were reported.

Discussion

In the present study, IM administration of alfaxalone (4 mg/kg) alone or in combination with buprenorphine (0.03 mg/kg) or hydromorphone (0.1 mg/kg) provided reliable sedation in healthy rabbits. The combination of alfaxalone with hydromorphone did not provide superior sedation when compared to the other treatment groups but appeared to provide superior analgesia. No severe adverse events were observed in the rabbits included in this study, and no death or morbidity was reported 1 week following study completion.

In groups A and HA, no significant changes in HR were observed. This finding was corroborated by previous studies[8,9] of rabbits administered alfaxalone IM at 4 and 6 mg/kg. Increases in HR were noted after IM treatment with alfaxalone and buprenorphine but remained within normal physiologic values. Additionally, BP was not affected and remained within normal limits. In dogs, a slight increase in HR after IV alfaxalone has been reported, but hypotension also occurred,[24] which was not seen in the present study. Dose-dependent increase in HR has been reported in rats following administration of 0.03 and 0.15 mg/kg of buprenorphine IV.[25] The increase in HR in group BA may have been a result of a synergistic effect of combining the 2 agents. However, the overall cardiac effects noted following all 3 IM treatments were minimal and unlikely to be clinically relevant, which agreed with previous studies.[8,9,26]

Apnea did not occur in rabbits in any of the treatment groups. Respiratory rates in groups BA and HA were significantly lower than baseline but still within acceptable physiologic ranges.[27] This result was corroborated by previous studies[9,11,28] in which significant decreases in RR following alfaxalone administered IM combined with different sedatives and analgesics were observed. No significant changes in RR were noted in rabbits in group A (P = .06). This contrasted with results from a previous study[8] of 10 rabbits in which a significant decrease in RR was noted after the same IM dose of alfaxalone. It is possible the smaller cohort of the present study did not allow for significance to be found. Additionally, the discrepancy between studies may be due to differences in breed-drug sensitivity, environmental factors (eg, stress levels), and possible undiagnosed respiratory disease affecting baseline RR and response to treatment.

Sedation score and time to lateral recumbency did not differ between treatment groups. Most of the rabbits achieved lateral recumbency within 10 minutes. This result was in accordance with previous studies evaluating the sedative effects of IM alfaxalone alone or combined with other agents.[8–11] In the present study, hydromorphone administered IM combined with alfaxalone did not provide higher sedation scores when compared to the other treatment groups. Opioids such as hydromorphone have been shown to work synergistically with sedatives and tranquilizers to enhance sedative effects, quality of sedation, and duration of action in dogs.[13,29–31] In our study, differences between treatment groups may not have been observed because all 3 IM protocols were able to provide smooth and reliable sedation with no obvious adverse effects. The sedation achieved with all 3 treatments could allow for procedures such as radiography, CT, and blood collection to be safely performed in rabbits. Sedation duration was not evaluated and therefore could not be assessed.

No rabbits required additional sedation for IV catheter placement, and no difference in ease of

catheter placement was found between treatment groups. Some rabbits, albeit in lateral recumbency, responded to catheter insertion with partial limb withdrawal. This could have been due to an inadequate level of analgesia or sedative effects wearing off. Lack of withdrawal response associated with toe pinch has been reported after IM administration of alfaxalone (6 mg/kg), butorphanol (0.3 mg/kg), and dexmedetomidine (0.2 mg/kg).[9] Analgesia and muscle relaxation provided by the protocol could explain the lack of response to the noxious stimulus. In rabbits, the duration of action of alfaxalone administered IM is approximately 52 minutes following 6 mg/kg and 37 minutes following 4 mg/kg.[8] Therefore, administration of higher doses of alfaxalone IM, higher doses of analgesics, or both may be able to mitigate the response to IV catheter placement observed in our study.

The IM treatments did not provide sufficient anesthesia to allow for supraglottic airway device placement in most rabbits. Device insertion was possible in 1 rabbit from group A and 2 from BA and HA. Reabel et al[11] reported inability to perform endoscopic-guided intubation in rabbits following IM administration of alfaxalone at 2 mg/kg combined with hydromorphone (0.1 mg/kg) and dexmedetomidine (0.005 mg/kg). Intubation was achieved in most rabbits when higher doses of alfaxalone IM, 5 and 7 mg/kg, were administered.[11] However, larger volumes of drugs, exceeding the recommended 0.25 mL/kg per muscle bed,[32] may be required, and respiratory and cardiovascular depression may be more profound when higher doses of alfaxalone are administered.[8,9] Greater and longer respiratory depression was reported in rabbits receiving 6 and 8 mg of alfaxalone/kg IM when compared to rabbits receiving 4 mg/kg.[8] Apnea and subsequent arrest occurred in 1 rabbit receiving 8 mg of alfaxalone/kg IM.[8] If the placement of a supraglottic airway device is desired following IM treatment with alfaxalone alone or combined with buprenorphine (0.03 mg/kg) or hydromorphone (0.1 mg/kg), higher doses of alfaxalone (5 to 7 mg/kg) could be considered. In the present study, combining the assigned sedatives into 1 syringe (a form of compounding) for administration was done so that each rabbit received only 1 IM injection. Veterinarians should adhere to compounding regulations and be aware that pharmacokinetic properties may differ among compounded products.

Postoperative pain scores were lower in rabbits treated IM with hydromorphone and alfaxalone in comparison to rabbits that received alfaxalone IM alone or combined with buprenorphine. Full μ-opioid receptor agonists such as hydromorphone provide excellent analgesia, whereas buprenorphine provides mild to moderate analgesia in veterinary species.[31] In chinchillas, 0.2 mg of buprenorphine/kg SC, but not 0.05 to 0.1 mg/kg, significantly increased limb withdrawal latencies in response to a thermal noxious stimulus.[33] Although studies evaluating antinociceptive effects of opioids in rabbits are lacking, on the basis of results of this study, IM administration of hydromorphone may be a better choice for rabbits undergoing painful procedures. Studies evaluating higher doses of IM administration of buprenorphine and antinociception are required.

In this clinical study, rabbits received meloxicam SC and ketamine IV prior to skin incision. The addition of these drugs was deemed necessary to provide adequate analgesia. This represented a study limitation and affected postoperative pain scores. However, all rabbits in all treatment groups received the same added drugs and doses after sedation and IV catheter placement data were collected. Another limitation was the use of the RbtGS. Although this grimace scale was specifically designed for rabbits, facial indicators such as whisker position may be difficult to interpret and could result in erroneous assessments. Additionally, the different rabbit breeds included in this study could have also affected the accuracy of the RbtGS scores as previous study models evaluated the accuracy of this pain scale on only 2 breeds: New Zealand White or Dutch Belted rabbits.[22,23] All animals were scored using the same scale by the same blinded evaluators to minimize any bias or errors.

In conclusion, alfaxalone (4 mg/kg, IM) alone or with either buprenorphine or hydromorphone provided reliable and uneventful sedation in rabbits and allowed for successful IV catheter placement. Intramuscular hydromorphone-based protocol may provide superior analgesia in comparison to buprenorphine in rabbits undergoing elective orchiectomy. Additional research is warranted.

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Appendix

Semiquantitative rating scale to assess sedation levels for 24 rescue-owned healthy male rabbits that received IM administration of either alfaxalone (4 mg/kg) alone (n = 8), alfaxalone (4 mg/kg) combined with buprenorphine (0.03 mg/kg; 8), or alfaxalone (4 mg/kg) combined with hydromorphone (0.1 mg/kg; 8) before receiving ketamine (2.5 mg/kg, IV), midazolam (0.13 mg/kg, IV), and meloxicam (0.5 mg/kg, SC) for elective orchiectomy during a randomized controlled clinical trial between August 21, 2021, and November 6, 2021. Scores for each category were summed to determine the total score for each treatment group (maximum possible score of 15).

General appearance
0 Awake and normal
1 Tranquil
2 Stuporous

Spontaneous posture
0 Normal
1 Sitting with head up
2 Lying sternal, head up
3 Lying sternal, head down
4 Lying laterally, head up
5 Lying laterally, head down

Response to placement in lateral recumbency
0 Cannot be placed in lateral recumbency
1 Can be placed in lateral, returned to sternal recumbency < 5 seconds
2 Remained in lateral recumbency > 5 seconds, without muscle tone

Jaw tone
0 Normal muscle tone
1 Decreased
2 No resistance to mouth being opened

Response to toe pick
0 Removed limb quickly
1 Removed limb slowly
2 No movement

Palpebral reflex
0 Normal
1 Slow
2 Not present