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Intramuscular Alfaxalone–Butorphanol–Midazolam Compared with Ketamine–Butorphanol–Midazolam in New Zealand White Rabbits

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Rabbits are a common companion animal and research subject and frequently require sedation to facilitate procedures. The objective of this study was to compare the effects of intramuscular butorphanol and midazolam combined with either alfaxalone or ketamine in rabbits. In a complete crossover study, healthy New Zealand white rabbits (n = 9; age, 6 mo) randomly received midazolam (1 mg/kg IM) and butorphanol (1 mg/kg IM) combined with either alfaxalone (2 mg/kg IM; ABM) or ketamine (5 mg/kg IM; KBM). Time to first effects, recumbency, and standing (recovery) were recorded. Every 5 min during recumbency, an investigator who was blind to treatment group collected serial physiologic parameters and sedation scores. At 5 min after rabbits became recumbent, manipulations were performed to mimic 2-view radiography and a cephalic intravenous catheter was placed. At 30 min after drug injection, flumazenil (0.05 mg/kg IM) was administered for reversal. Food consumption and fecal output were measured for 3 d after each study day. Time to standing and duration of recumbency differed significantly between groups. The median (range) of the total sedation score for ABM was 10 (8 to 10) and for KBM was 10 (6 to 10). Sham radiographs were successful in all rabbits in both groups. Physiologic parameters were not significantly different between groups over time. At 24 h after drug treatment, KBM-treated rabbits showed reduced food intake and both groups showed reduced fecal output. Total sedation scores decreased significantly over time in KBM rabbits ($P < 0.001$) but not in ABM rabbits ($P = 1$). The duration of recumbency was significantly longer in ABM rabbits than in KBM rabbits. Both protocols produced sufficient sedation for radiograph acquisition without clinically significant adverse effects.

Abbreviations: ABM, alfaxalone–butorphanol–midazolam; KBM, ketamine–butorphanol–midazolam

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Introduction

Rabbits are commonly kept as pets and are frequently used in research. They can often experience high levels of stress during veterinary examination and other manipulations.[8] Even routine handling, including lifting and carrying, can elicit fear responses in rabbits.[6] In one study, over half of the rabbits struggled against the handler when being lifted, suggesting fear during handling.[6] In addition to psychologic and welfare concerns secondary to stress, iatrogenic and self-trauma may also occur. The skeleton of rabbits represents approximately 7% to 8% of their total body weight, significantly less than in other mammals of similar size.[8] Their delicate skeleton in combination with the strong musculature in their hind limbs predisposes them to fractures.[13] Stress can also negatively affect gastrointestinal tract motility and potentially lead to rabbit gastrointestinal syndrome.[26] In light of these concerns, sedation is an important tool to facilitate diagnostics and treatment, reduce stress, and minimize the likelihood of iatrogenic trauma during handling in rabbits.

Many sedation protocols for rabbits have been previously described in the literature; these include both single agent and combination protocols. Ketamine, a dissociative anesthetic and antagonist at the n-methyl-d-aspartate receptor, is a mainstay drug in sedation protocols for rabbits.[2,5,9,16,23] Rabbits rapidly eliminate ketamine by both hepatic metabolism and extensive extrahepatic clearance.[4,16,23] In veterinary species, ketamine is frequently combined with other anesthetic drugs for synergistic effects and to provide muscle relaxation.[2,14] Midazolam, a benzodiazepine, and butorphanol, a mixed agonist-antagonist opioid, are often used alone or in combination with other anesthetic agents, such as ketamine, to produce sedation.[2,5,9,14] One study demonstrated that a combination of ketamine 15 mg/kg and midazolam 3 mg/kg administered IM induced loss of righting response and was sufficient for intubation in the majority of the tested rabbits.[14] Another study that evaluated the effects of ketamine 30 mg/kg and midazolam 1 mg/kg IM in rabbits reported good sedation and minimal cardiorespiratory depression with this combination.[9]

Alfaxalone, a neuroactive steroid and γ-aminobutyric acid agonist anesthetic agent, is being used with increasing frequency in exotic pet practice, including in rabbit sedation protocols.[24,34] Alfaxalone produces dose-dependent sedation and cardiorespiratory depression in domestic species including cats and dogs;[25] similar dose dependency has been documented in rabbits.[17] Alfaxalone can be administered by several routes, including by IM injection, making it ideal for use in species in which IV access may be difficult.[34] Additional potential benefits of alfaxalone include its short time to onset of effects and rapid clearance.[24,34] Multiple published studies have assessed the use of alfaxalone in rabbits with largely positive results.[7,17,21,24] The pharmacokinetics of alfaxalone in rabbits have also been described after a dose of 5 mg/kg administered by IM or IV.[24]

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In that study, IM injection resulted in good bioavailability, with a half-life similar to that of IV injection, and clearance was rapid at 1.55 ± 0.27 L/kg/h.[24] Thus, the IM route appeared to be a reasonable alternative to the IV route in rabbits. Adverse effects of alfaxalone have also been reported in rabbits. One study documented cyanosis, nystagmus, and tremors after alfaxalone administration at 5 mg/kg IM and IV; however, these effects were transient and not considered to be life threatening.[23] In another study, alfaxalone at 8 mg/kg IM resulted in apnea and death in a single rabbit.[17] However, lower alfaxalone doses (4 to 6 mg/kg) evaluated in that study did not produce apnea, supporting the dose dependent nature of its adverse effects.[17]

Despite the wide use of both ketamine and alfaxalone in rabbit sedation, these drugs have not been directly compared as part of a drug combination. The purpose of the current study was to determine the clinical efficacy of IM butorphanol (1 mg/kg) and midazolam (1 mg/kg) combined with either alfaxalone (2 mg/kg) or ketamine (5 mg/kg) in New Zealand white rabbits using a complete crossover design. A secondary objective was to determine the short-term effect of each combination on food intake and fecal output. We hypothesized that both protocols would achieve clinically relevant sedation sufficient for a minor, noninvasive procedure and would have minimal adverse effects. We also hypothesized that food intake and fecal output would be reduced during the 24 h period after administration of either combination.

Materials and Methods

New Zealand white rabbits (*Oryctolagus cuniculus*; n = 9, 5 females and 4 males; age, 6 mo) were obtained from a commercial breeder (Charles River Laboratories, Wilmington, MA). Rabbits were reported by the vendor to be free of *Pasteurella multocida*, *Salmonella* spp., *Clostridium piliforme*, *Treponema cuniculi*, *Encephalitozoon cuniculi*, and rabbit hemorrhagic disease virus. Before the study, the rabbits were given a 4-wk acclimation period. At the beginning of the study, the rabbits weighed 3.16 ± 0.12 kg (mean ± 1 SD). Rabbits were individually housed in stainless steel cages (70 cm × 70 cm × 45 cm; Allentown Caging, Allentown, NJ) with perforated plastic flooring, a plastic hide box, enrichment items on rotation, and ad libitum access to water via a water bottle. Urine and feces were collected under each cage on a paper pad that was changed daily. Cages were kept in a climate-controlled facility (range, 20 to 23.9 °C [68 to 75 °F]) with a 12:12-h light:dark cycle using commercial fluorescent lighting. Rabbits were fed 2/3 cup of pelleted rabbit diet (LabDiet, Prolab Laboratory Animal Diet, St Louis, MO) and a large handful of autoclaved grass hay daily. Rabbits were determined to be healthy based on serial physical examinations. The rabbits used in this study were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals*.[20] This study was approved by the IACUC of North Carolina State University (protocol no. 20-276), an AAALAC-accredited facility.

For establishment of baseline values, body weight, pelleted diet intake, and fecal output were measured for 48 h on each rabbit prior to the first study day. Data was collected between 7am and 9am each morning. For the pelleted diet, the total weight of that day's ration and any previously uneaten food was recorded to determine the amount of food consumed each day. Fecal material was collected and weighed. Subjective and objective quantification of hay intake was unreliable for this cohort; therefore, this value was not recorded for any rabbit. On test days, after body weight acquisition but at least 30 min before testing, rabbits were manually restrained for a brief physical examination, including collection of baseline heart rate, respiratory rate, rectal temperature, and sedation score. Food was removed from all cages at the time of injection of the first study animal. On the first test day, rabbits were randomly assigned to one of 2 treatment groups by pulling numbers out of a hat. After a 7-d washout period, treatment groups were reversed for each rabbit on the second test day by using a complete crossover study design. In addition, treatment order was randomized in a similar manner for both study days. Rabbits received either alfaxalone (2 mg/kg IM; Alfaxan Multidose, 10 mg/mL, Jurox, Kansas City, MO), butorphanol (1 mg/kg IM; Butorphanol tartrate, 10 mg/mL, Patterson Veterinary, Greeley, CO), and midazolam (1 mg/kg IM; Preservative-free midazolam, 5 mg/mL, Hospira, Lake Forest, IL; ABM, n = 9) or ketamine (5 mg/kg IM; Ketaset, ketamine hydrochloride, 100 mg/mL, Zoetis, Kalamazoo, MI), butorphanol (1 mg/kg IM), and midazolam (1 mg/kg IM; KBM, n = 9) on each test day. For both groups, drug doses were calculated and butorphanol and midazolam were combined in a single syringe immediately prior to injection. Rabbits were manually restrained for injection. The butorphanol and midazolam mixture was administered in the right caudal epaxial muscles, followed immediately afterward by injection of either alfaxalone or ketamine into the left caudal epaxial muscles. Rabbits were then placed in an individual kennel (66 cm × 51 cm × 51 cm) that was partially covered with a towel to decrease visual stimulation of the rabbit but still allow direct observation of the animal.

The times after injections to first effects (described below) and recumbency were recorded. Respiratory rate was recorded every 5 min after injection until the rabbit was again standing. Five minutes after they became recumbent, rabbits were removed from the kennel and placed in left lateral recumbency on a towel-covered hot-water blanket (Stryker T/Pump, Portage, MI). Serial measurements of heart rate via auscultation, rectal temperature, noninvasive oscillometric blood pressure (no. 2 cuff placed on the right hindlimb; Cardell Veterinary Monitor 9402, Midmark, Tampa, FL), oxygen saturation of hemoglobin (probe placed on tongue or foot; Cardell Veterinary Monitor 9402, Midmark), and sedation score (assigned by an investigator who was blind to treatment group [JB]) were collected every 5 min during recumbency. The sedation score included posture, resistance to placement in dorsal recumbency, jaw tone, and palpebral reflex, as adapted from a previous study in rabbits (Figure 1).[2]

After collection of the first set of parameters, manipulations mimicking those needed for 2-view radiographs (ventrodorsal and then right lateral) were performed, followed by placement

Variable	Score	Response
Posture		
	0	Normal
	1	Sitting, with head up
	2	Lying sternally, with head down
	3	Lying laterally
	4	Lying dorsally, responsive to stimuli
	5	Lying dorsally, unresponsive to stimuli
Resistance to dorsal recumbency		
	0	Strong/normal resistance
	1	Moderate resistance
	2	Slight resistance
	3	No resistance
Jaw tone		
	0	Normal
	1	No resistance to opening mouth
Palpebral reflex		
	0	Normal
	1	Decreased
	2	Absent

Figure 1. Scoring system for assessment of sedation in rabbits. Adapted from reference 2.

of an IV catheter. For sham radiographic positioning, rabbits were placed in dorsal or right lateral recumbency, with all 4 legs manually outstretched. Positioning was considered successful when the rabbit did not struggle excessively during positioning and subsequently remained in each position for 10 s. After sham radiographic positioning, placement of an IV catheter was attempted in the right cephalic vein of each rabbit. For placement, rabbits were manually restrained with the right forelimb held in an extended position, a small square of fur was clipped over the right antebrachium, and the site was prepared by using dilute chlorhexidine scrub (Hibiclens Antimicrobial Skin Liquid Soap, chlorhexidine gluconate 4.0%, Mölnlycke Health Care, Norcross, GA) and alcohol (70% isopropyl alcohol, Medline, Northfield, IL). A small stab incision was made in the skin adjacent to the vessel by using a 25-gauge needle (Monoject, Cardinal Health, Dublin, OH), and a 24-gauge IV catheter (Becton Dickinson Infusion Therapy Systems, Sandy, UT) was placed in the cephalic vein. A maximum of 5 min was allotted for catheter placement. Placement was deemed successful if blood appeared in the hub of the catheter; when noted, the catheter was advanced and taped in place, flushed with 1 mL of 0.9% sodium chloride (Monoject, Cardinal Health), and maintained for the remainder of the monitoring period. If IV catheterization was unsuccessful, a pressure bandage was placed and maintained for the same period of time. During radiograph acquisition and IV catheterization attempts, the degree of struggling against restraint was scored (0, 2 or more attempts to withdraw or struggle; 1, 1 or 2 attempts to withdraw or struggle; 2, no attempt to withdraw or struggle).

At 30 min after anesthetic injection, response to noxious stimulus was assessed by using a single clamp of a hemostat on the fourth digit of the right hindlimb. The IV catheter or pressure bandage was then removed, and flumazenil (0.05 mg/kg IM; Hikma Farmaceutica, Terrugem, Portugal) was administered in the left caudal epaxial muscles. A pressure bandage was placed when an IV catheter was removed. The rabbit was then returned to its individual kennel, which was resting on a hot-water blanket and partially covered with a towel, as described above. Time to standing, which provided our benchmark for recovery from sedation, was recorded and a rectal temperature was collected once the rabbit was standing. When the rectal temperature was 100 °F (37.8 °C) or greater, the pressure bandage was removed, if present, and the rabbit was returned to its permanent cage with immediate access to food and water. If the rectal temperature was less than 100 °F, the rabbit was maintained on heat support, and the rectal temperature was rechecked every 15 min until it exceeded 100 °F. If a rabbit was not standing by 20 min after flumazenil administration, serial rectal temperatures were measured at that time point and every 15 min thereafter. In addition, any adverse clinical signs were recorded throughout the study. After return to their permanent cages, the body weight, pelleted diet intake, and fecal output of each rabbit were measured once daily for 72 h, as described above.

Data were analyzed by using the R statistical software (version 3.6.2 with lme4 and lmerTest packages).[29] Linear mixed models were fit with main effects for treatment order, time period, and treatment. A random intercept was included for each subject. A time effect and an interaction with treatment were included for variables that were recorded throughout a sedation episode. Normality was assessed by using the Shapiro–Wilk test. Normal data were reported as mean ± 1 SD, and nonnormal data were reported as median and range. Values were considered significant when the P value was less than 0.05; P values were adjusted for multiple comparisons by using Bonferroni correction.

Results

Times from injection to first effects, recumbency, and standing, and the duration of recumbency are summarized in Table 1. The first effects included mild ataxia, closing of the eyes, and nystagmus. All rabbits in both groups became recumbent. Times to first effects and recumbency did not differ significantly between groups. Physiologic measurements are summarized in Table 2. Mean oxygen saturation of hemoglobin, blood pressure (systolic, mean, and diastolic), heart rate, and rectal temperature did not differ significantly over time between the 2 groups ($P = 1$), nor did mean respiratory rate ($P = 0.28$). No rabbit in either group became hypothermic (rectal temperature less than 100 °F [37.8 °C]) at any time point. All heart rates, respiratory rates, and rectal temperatures remained within normal clinical limits throughout the study, and none of the rabbits showed apnea.[10]

Sedation and restraint scores for both radiographic positioning and IV catheter placement are shown in Table 3. Rabbits that received KBM had an initial total sedation score that was significantly ($P = 0.013$) higher than that of ABM rabbits. Total sedation scores decreased significantly over time in the KBM group ($P < 0.001$) but not in ABM rabbits ($P = 1$), with the ABM group having higher sedation scores, on average, than KBM rabbits throughout the sedation period. Positioning for sham radiographs was successful in all rabbits in both groups, and IV catheter placement was successful in 3 (33.3%) KBM rabbits and 2 (22.2%) ABM animals. We considered poor compliance as the cause of unsuccessful IV catheterization in 2 ABM and 3 KBM rabbits, with the majority of unsuccessful attempts attributed to technique failure. All rabbits in both groups responded to noxious stimuli. Transient nystagmus was observed at various time points throughout the sedation period in all 9 ABM rabbits and in 5 (56%) KBM rabbits. All rabbits recovered uneventfully, and no additional adverse effects were observed in any rabbit.

Baseline pelleted food intake and fecal output were not significantly different between the 2 d before the first study day when including all rabbits ($P = 1$). During the first 24 h after sedation, KBM rabbits had a significant decrease in pellet intake ($P = 0.01$) compared with baseline; a difference was not detected in ABM rabbits ($P = 0.06$). Fecal output during the first 24 h was significantly lower in both ABM ($P = 0.01$) and KBM ($P = 0.005$) rabbits compared with baseline but was not different between groups ($P = 1$). At the 72-h time point following each study day, food intake and fecal output were not significantly different from baseline for either ABM ($P = 0.28$ and $P = 0.82$) or KBM ($P = 0.06$ and $P = 0.43$).

Discussion

At the doses used in the current study, both KBM and ABM produced a level of sedation in New Zealand white rabbits that

Table 1. Time (min; mean ± 1 SD, $n = 9$) from injection to first effects, recumbency, and standing, and duration (min) of recumbency for New Zealand white rabbits (*Oryctolagus cuniculus*) after the administration of butorphanol (1 mg/kg IM) and midazolam (1 mg/kg IM) combined with either alfaxalone (2mg/kg IM; ABM) or ketamine (5 mg/kg IM; KBM)

	ABM	KBM	*P*
Time to first effects	1.6 ± 0.5	1.8 ± 0.4	0.78
Time to recumbency	2.7 ± 0.6	3.4 ± 1.0	0.11
Time to standing	48.9 ± 4.5	32.7 ± 1.3	< 0.001
Duration of recumbency	46.2 ± 4.6	29.3 ± 1.2	< 0.001

Differences are considered significant at $P < 0.05$.

Table 2. Median and range of heart rate, respiratory rate, rectal temperature, oxygen saturation of hemoglobin, and noninvasive oscillometric blood pressure (systolic, diastolic, and mean) measurements for New Zealand white rabbits (*Oryctolagus cuniculus*; *n* = 9) after the administration of butorphanol (1 mg/kg IM) and midazolam (1 mg/kg IM) combined with either alfaxalone (2mg/kg IM; ABM) or ketamine (5 mg/kg IM; KBM)

	ABM		KBM		*P*
Heart rate (median and range of beats per minute)	198	(152–244)	180	(124–230)	1
Respiratory rate (median and range of breaths per minute)	20	(8–96)	28	(16–164)	0.28
Rectal temperature (°F) (mean and SD)	101.3	± 0.4	101.5	± 0.5	0.73
Oxygen saturation (%) (median and range)	85.5	(68–97)	90.5	(81–100)	0.71
Systolic blood pressure (mm Hg) (median and range)	85	(64–114)	87	(70–121)	1
Mean blood pressure (mm Hg) (median and range)	49.5	(37–96)	57	(37–93)	1
Diastolic blood pressure (mm Hg) (median and range)	34	(25–63)	32	(24–71)	1

P values were determined for the treatment effect based on the linear mixed models. Differences are considered statistically significant when $P < 0.05$.

Table 3. Total sedation scores (median [range]) and restraint scores (mean ± 1 SD) for New Zealand white rabbits (*Oryctolagus cuniculus*; *n* = 9) after the administration of butorphanol (1 mg/kg IM) and midazolam (1 mg/kg IM) combined with either alfaxalone (2mg/kg IM; ABM) or ketamine (5 mg/kg IM; KBM)

	ABM		KBM		*P*
Total sedation score	10	(8–10)	10	(6–10)	0.008
Radiographic positioning restraint score (ventrodorsal view)	2		1.78	± 0.44	0.51
Radiographic positioning restraint score (right lateral view)	2		1.9	± 0.3	1
Intravenous catheter restraint score	1.4	± 0.7	1	± 0.9	0.83

was sufficient for minor, noninvasive procedures. Times to first effects and recumbency were rapid in both groups, with recumbency occurring in less than 5 min in all rabbits. Compared with ABM, KBM produced higher initial total sedation scores, lower scores on average throughout the remainder of the sedation episode, and faster recovery times. These findings are consistent with previous pharmacokinetic studies of ketamine and alfaxalone in rabbits.[23,24] Ketamine is metabolized and cleared more rapidly in rabbits as compared with other species, and so rabbits also recover faster from ketamine sedation.[23] Both of the protocols we tested provided sedation that was sufficient for acquiring 2 views of radiographs, a common clinical diagnostic modality used in rabbits. In addition, IV catheter placement was possible without excessive struggling, with the high failure rate predominantly attributed to the time limits we set and to human error rather than to patient compliance. We selected the cephalic vein for catheter placement over the lateral auricular or another vein to better approximate techniques performed in clinical patients, given that the cephalic vein is typically used for medication and fluid administration in pet rabbits.[8] All rabbits responded to a noxious stimulus at 30 min after anesthetic injection, suggesting that the tested protocols may not be sufficient for more invasive or painful procedures. Although a potentially more stimulating route, we used IM rather than IV administration of flumazenil to better emulate what is performed in the clinical setting and to maintain consistency for rabbits in which IV catheterization was unsuccessful. Transient nystagmus was observed in all rabbits in the ABM group and in over half of those given KBM, consistent with observations from other studies of these drugs, particularly alfaxalone; however, transient nystagmus has minimal clinical significance.[9,21,24] We did not observe other side effects, including those reported with alfaxalone use in other studies (for example, clinically significant respiratory depression, muscle tremors).[21,24]

The sedation scoring system used in our study was adapted from a previously published sedation study in rabbits.[2] A more recent rabbit sedation scale provides more consistent and reliable scoring than does a previously published scale.[29] Our study was conducted before the publication of the updated sedation scale, and we recommended that future rabbit sedation studies use the newer approach.[30]

Hypotension, defined as a mean arterial blood pressure less than 60 mm Hg or systolic arterial blood pressure less than 80 mm Hg, occurred in the majority of rabbits in the current study.[15] All but one rabbit in each group had at least one mean arterial blood pressure measurement that was considered hypotensive. Previous studies comparing direct and indirect (noninvasive) blood pressure measurement suggest that oscillometric blood pressure measurement has poor agreement with direct arterial blood pressure measurement, both in rabbits and other species.[1] A study in dogs reported that the sensitivity of oscillometric blood pressure measurement to correctly detect hypotension was only 40%.[32] Thus, the measurements in the current study may have underestimated the arterial blood pressure, yet hypotension cannot be excluded. Hypotension was not reported in previous rabbit studies using ketamine and alfaxalone, which were often used at higher doses than we tested.[9,21] In one study, average direct mean arterial blood pressure values in rabbits given ketamine at 30 mg/kg IM and midazolam at 3 mg/kg IM remained over 60 mm Hg at all time points.[9] Another group determined that rabbits given alfaxalone alone at doses up to 5 mg/kg IM remained normotensive by oscillometric blood pressure measurement.[21] Although not included as part of the initial methodology, we assessed capillary refill time and femoral pulse on a subset of rabbits with documented hypotension, and both were within clinically acceptable limits (capillary refill time less than 2 s, good to excellent pulse quality). Although these assessments are subjective and were not conducted on all rabbits, they support the notion that rabbits were well-perfused during the sedation period. Alternatively, blood pressure may have been overestimated in the current study, as suggested by another study investigating the agreement between an indirect oscillometric blood pressure monitor and direct blood pressure measurement in rabbits.[3] For future studies, blood pressure measurement using a Doppler device or an arterial catheter (direct) might achieve a more accurate assessment of arterial blood pressure.[15] Invasive blood pressure monitoring is not routinely performed in sedated rabbits in clinical practice and was outside the scope of the current study.

The oxygen saturation of hemoglobin in our study was lower than expected. Subjectively, the pulse oximeter readings

fluctuated significantly throughout the monitoring period and were highly variable between 5-min assessment periods. Thus, we suspect that these readings were an unreliable measure of the true oxygen saturation in our study. Similar inconsistencies in oxygen saturation measurements have been encountered in other rabbit sedation studies.[7,21] Although the accuracy of the oxygen saturation measurements is questionable, hypoxemia cannot be entirely excluded. Alfaxalone has been documented to cause respiratory depression in rabbits.[17,21] In one study, rabbits had low oxygen saturation after receiving alfaxalone at 2.5 or 5 mg/kg IM.[21] In the current study, rabbits showed a clinically normal respiratory rate while sedated, but a reduction in tidal volume might have contributed to hypoxemia. Maintenance in lateral recumbency and lack of supplemental oxygen may have contributed also. Arterial blood gas analysis was outside the scope of this study but could be considered for future studies. Oxygen supplementation of sedated rabbits should be considered in future studies and in clinical patients.

Although both ABM and KBM provided sufficient sedation for minor procedures, ABM resulted in deeper sedation as indicated by higher average sedation scores, particularly at later time points. Alfaxalone has been evaluated in rabbits at doses of 2 to 8 mg/kg and does not always result in a predictable level of sedation due to its nonlinear pharmacokinetic properties, as demonstrated in both rabbits and cats.[7,14,17,24,33] However, although higher doses of alfaxalone can provide both deeper and prolonged sedation, the adverse effects of alfaxalone in mammals, notably cardiorespiratory depression, are also dose-dependent.[17,25] Thus, increasing the dose of alfaxalone beyond 2 mg/kg may provide deeper sedation but may also increase the likelihood of adverse side effects. Like alfaxalone, ketamine provides dose-dependent sedation and has anesthetic properties at higher doses. Ketamine (and midazolam) have been used in rabbits at much higher doses than those used in the current study. In one study, ketamine at 15 mg/kg IM and midazolam at 3 mg/kg IM had no clinically apparent complications.[14] Another study used ketamine at 30 mg/kg IM in combination with midazolam at 1 mg/kg IM; side effects were minimal and included urination, defecation, and penis exposure.[9] Ketamine doses as high as 50 mg/kg are listed for rabbits in some formularies.[10] The ketamine dose we used in the current study was selected based on previously reported dosages and our prior experience with successful clinical sedation in rabbits. Overall, the use of lower dosages may avoid some of the adverse effects of these drugs, which is particularly important in rabbits because they are susceptible to gastrointestinal stasis syndrome. Given the wide range of drug dosages reported in the literature, future studies could investigate higher doses of these drugs in combination for use in situations that require a deeper level of sedation or a longer duration of effect.

Administration of anesthetic drugs can affect gastrointestinal tract function, but the magnitude of effect and outcomes vary among species. Ketamine, for example, reduces gastrointestinal motility in pigs but has no effect on gastrointestinal transit time in horses or dogs.[11,12,31] In comparison, midazolam increases gastrointestinal transit time in mice.[19] In rabbits, gastrointestinal motility is of particular importance because a healthy, functional gastrointestinal tract is critical to hindgut fermentation. Like other species, rabbits are susceptible to perianesthetic gastrointestinal side effects, which have been investigated in at least two other studies.[22,26] One study evaluated the effects of an anesthetic cocktail comprised of butorphanol at 0.5 mg/kg IM, midazolam at 0.5 mg/kg IM, and ketamine at 15 mg/kg IM and determined that gastrointestinal motility as assessed via ultrasound was not decreased as compared with baseline.[28] In a second study involving isoflurane-anesthetized rabbits induced with ketamine (30 mg/kg IM) plus either midazolam (3 mg/kg IM) or medetomidine (0.25 mg/kg IM), gastrointestinal transit time was significantly longer in the ketamine–medetomidine group compared with the ketamine–midazolam and control groups.[5] In the current study, pelleted food intake and fecal output were assessed after sedation. Significant decreases in fecal output were noted in both ABM and KBM groups during the first 24-h period after testing. This finding could indicate reduced gastrointestinal tract motility. Such a reduction could be a direct effect of the administered drugs on gastrointestinal transit time, but other causes, including stress associated with sedation or handling,[27] cannot be excluded. In addition, KBM rabbits had a significant reduction in pelleted food intake during the 24-h period after sedation, with no statistically significant change noted in ABM rabbits. This decrease in food consumption may have contributed to the reduction in fecal output. Although food preferences after sedation have not been determined in rabbits, a relative increase in hay consumption may have occurred during this period to compensate for the reduction in pelleted food intake. Because we were could not quantify hay consumption—a limitation of this study—this hypothesis is speculative. Reduced activity and residual sedation may be other explanations for a reduction in pelleted food intake after each study day, but because sedation scores were not performed on rabbits after administration of the anesthetic reversal agent, the ultimate contribution of this factor remains unknown. Assessment of sedation scores after recovery could be considered for future studies to account for residual drug effects that may have contributed to a reduction in food intake. Although the decreased consumption of pelleted food could have various causes, including the stress of handling, it may in and of itself indicate reduced gastrointestinal motility. Regardless of the etiology, decreases in food consumption and fecal output are components of rabbit gastrointestinal stasis syndrome and a potential adverse effect to consider when selecting a sedation protocol.[18,22,27] Other methods for assessment of gastrointestinal tract motility, such as ultrasonographic evaluation as described in a previous study,[28] could be performed in future research but were outside the scope of our study. By 72 h after each study day, pelleted food intake and fecal production in rabbits in the current study was no longer significantly different from baseline, demonstrating that the effects were transient. The reduced pelleted food intake and fecal output resolved without intervention, making underlying disease an unlikely cause. In addition, our study was performed using young, clinically healthy rabbits, and these negative effects may be more apparent in systemically ill rabbits. For future work, the use of a control group to measure pelleted food consumption and fecal output for comparison with sedated rabbits would be beneficial.

In summary, combinations of alfaxalone, butorphanol, and midazolam or ketamine, butorphanol, and midazolam given IM to New Zealand white rabbits resulted in rapid recumbency and sufficient sedation for minor, noninvasive procedures (sham radiographs, IV catheter placement), with the maintenance of heart rate, respiratory rate, and rectal temperature within clinically acceptable limits. Duration of recumbency was clinically relevant, with ABM rabbits having a longer time to recovery than did KBM rabbits. The KBM group had higher initial total sedation scores and faster recovery times, but the ABM protocol produced a deeper level of sedation throughout the sedation period with no major adverse effects observed. After sedation, KBM rabbits had a reduction in pelleted food intake, and both

ABM and KBM rabbits had a reduction in fecal output; however, these effects were transient and resolved without intervention. This study supports the use of IM KBM or ABM to sedate New Zealand white rabbits for minor, noninvasive procedures.

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