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Original Research

Comparison of Subcutaneous and Intramuscular Ketamine–Medetomidine With and Without Reversal by Atipamezole in Dutch Belted Rabbits (*Oryctolagus cuniculus*)

Angelina M Williams,* and Jeff D Wyatt

Forty male Dutch belted rabbits (Oryctolagus cuniculus) enrolled in a minimally invasive pharmacokinetics study were used to compare the efficacy of an anesthetic combination delivered through 2 injection routes. Rabbits were randomly assigned to 4 groups (n = 10/group) to determine the sedative and physiologic effects of ketamine (25 mg/kg)–medetomidine (0.5 mg/kg) given either intramuscularly (IM) or subcutaneously (SC). Palpebral, pedal, ear pinch, and righting reflexes, as well as cardiopulmonary parameters (heart rate, respiratory rate, and arterial blood oxyhemoglobin saturation), were recorded every 5 min. In addition, the reversal effects of an intravenous dose of atipamezole (1 mg/kg), an α2 adrenoreceptor antagonist, were assessed by comparing the return of the righting reflex in rabbits given the reversal agent with those that recovered spontaneously. Compared with the IM route, SC ketamine–medetomidine effectively induced chemical restraint with less than a 2-min difference in onset of anesthesia and markedly less resistance (for example, flinching, kicking, and so forth) during the injection. In all groups, the anesthetic regimen, regardless of the route of administration, provided an adequate level of anesthesia. Reversal with atipamezole improved arterial hemoglobin oxygen saturation for both the SC and IM groups; however, an enhanced rate of recovery from anesthesia was clinically apparent only for animals given the combination by the IM route.

Abbreviations: A, atipamezole; IM, intramuscular; KM, ketamine–medetomidine; SC, subcutaneous; SpO_2, arterial blood oxyhemoglobin saturation

The Dutch Belted rabbit, a commonly used animal model in biomedical research, can be challenging to anesthetize safely. Rabbits in general exhibit variable responses to anesthetics and unreliable reflexes as indicators of depth of anesthesia.[10] When given intramuscularly (IM) in rabbits, the commonly used combination of ketamine, a dissociative anesthetic, and xylazine, an α2 agonist sedative, may produce myonecrosis, vasculitis, and myositis with sciatic neuronal degeneration.[3] In addition, when these drugs are used in combination, anesthesia can be unpredictable, with a reported failure rate of 20% to 40%.[10] More importantly, the anesthetic combination of ketamine-xylazine recently was found to cause myocardial fibrosis in Dutch Belted rabbits.[12] Given these findings, we chose to evaluate ketamine and medetomidine administered in combination subcutaneously (SC) or IM in the Dutch Belted rabbit.

The ketamine–medetomidine combination (KM) offers several advantages over ketamine and xylazine. KM combinations provide adequate immobilization and the depth of sedation necessary to perform minor procedures in New Zealand White rabbits.[4,9] Medetomidine, a sedative agent that is 10 times more selective for α2 adrenoreceptors than is xylazine, compensates for the poor muscle-relaxing and analgesic effects of ketamine. The cardiac-stimulating properties of ketamine partially compensate for medetomidine-induced bradycardia.[15,19]

In the present study, Dutch Belted rabbits were anesthetized for a single intravitreal injection of a test compound as part of a pharmacokinetics study. The anesthetic dosages chosen for this experiment (ketamine, 25 mg/kg; medetomidine, 0.5 mg/kg) were based on published recommendations that all suggested an IM route of administration.[6,10,18] We hypothesized that the SC route may provide a similar depth of anesthesia while avoiding the potential local tissue irritation and pain associated with the intramuscular route. We also compared the reversal effects of intravenous atipamezole, a highly specific α2 antagonist, in rabbits anesthetized by the IM or SC route.

Materials and Methods

Animals. Forty male Dutch Belted rabbits (*Oryctolagus cuniculus*; weight, 2.0 ± 0.36 kg) were obtained from Covance Research Products (Denver, PA). Vendor health-screening reports indicated that these animals were specific-pathogen free for *Pastuerella multocida*, *Encephalitozoon cuniculi*, *Bordetella bronchiseptica*, *Salmonella spp.*, and cilia-associated respiratory bacillus. The rabbits were housed individually in stainless-steel cages (27 × 27 × 18 in.) with perforated floors and were maintained under climate-controlled conditions at 20 ± 2 °C, 50% ± 20% relative humidity, 12:12-h light:dark cycle, and 10 to 15 air

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Department of Comparative Medicine, University of Rochester Medical Center, Rochester, NY.
Corresponding author. Email: angelina_williams@urmc.rochester.edu

changes per hour. The animals received a commercial pelleted diet (HF5326, Purina, St Louis, MO) and water ad libitum. Rabbits were acclimated for 1 wk prior to the study and were not fasted prior to anesthesia.

Experimental procedure. All described procedures were approved by the Institutional Animal Care and Use Committee in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. The rabbits were assigned randomly to 4 groups consisting of 10 animals per group. All groups received ketamine (25 mg/kg; Ketamine, 200 mg/ml, The Medicine Shoppe, Canandaigua, NY) and medetomidine (0.5 mg/kg; Domitor, 1.0 mg/ml, Pfizer Animal Health, Exton, PA) combined in a single syringe. Each rabbit underwent slit-lamp examination and intravitreal injection after administration of the anesthetic regimen. One group (SC-KMA) received the KM mixture SC with intravenous injection of atipamezole (1.0 mg/kg; Antisedan, 4.0 mg/ml, Pfizer Animal Health) into a marginal ear vein approximately 20 min after the anesthetic injection. A second group (IM-KMA) was given the same dose of KM by the IM route, followed by injection of atipamezole 20 min after administration of the anesthetic. The remaining groups received KM by the SC (SC-KM) or IM (IM-KM) routes but were allowed to recover spontaneously.

The behavioral reaction of each rabbit to the initial injection was graded based on a scale of 0 to 4 (Table 1). The same investigator scored the reaction of all animals. Baseline heart rate and respiratory rate recordings were taken prior to the initial injection. A pulse oximeter (V3402, SurgiVet, Waukesha, WI) was used to record heart rates and arterial blood oxyhemoglobin saturation (SpO_2). After an animal was in lateral recumbency, the pulse oximetry probe was positioned on a digit of either forelimb or hindlimb. Respiratory rates were measured by auscultation or visualization of spontaneous breaths. Depth of anesthesia was assessed by using the following parameters, which were recorded every 5 min: righting reflex, palpebral reflex, pedal reflex, ear pinch reflex, heart rate, respiratory rate, and arterial blood oxyhemoglobin saturation. The righting reflex was considered absent if the animal made no attempt to right itself from a lateral recumbent position. To evaluate the palpebral reflex, the investigator touched the animal near the medial canthus of the eye. Pedal reflex was tested by pinching of 1 digit of the hindlimb and watching for its withdrawal. Ear pinch reflex was evaluated by pinching the pinna and monitoring for vocalization or head shaking.

After intravitreal injections were performed, rabbits in the SC-KMA and IM-KMA groups received intravenous atipamezole. Heart rates, respiratory rates, SpO_2, and reflex responses were monitored every 5 min for all 4 groups, regardless of administration of the reversal agent, until full recovery (identified by the return of the righting reflex) from anesthesia.

Statistical analyses. The time interval between administration of the injection and the subsequent loss and return of reflexes are reported as mean ± standard error. The percentage of rabbits that lost the palpebral reflex is reported also. The data were analyzed by using SAS statistical software (version 9.1, SAS Institute, Cary, NC). For continuous variables, 2-sample *t* tests were used to compare the means from 2 different groups, and 1-way analysis of variance was used to compare means from more than 2 groups. The Fisher exact test was used to analyze proportional data. Variables that changed with time, (for example, heart rate, respiratory rate, and SpO_2) were reported as means and standard errors in a graph, and a mixed effect model was used to compare group differences. Each rabbit was considered to be a random effect; treatment group and time were considered as fixed effects. Empirical sandwich estimators were used to estimate variance. The mixed-effect model was chosen because it takes into account the correlation between measurements obtained from the same rabbit. The threshold for statistical significance was set at $P = 0.05$.

Table 1. Grading scale for assessment of rabbits' reactions to the anesthetic injection

Score	Reaction to injection
0	None
1	Shows minor signs of discomfort (for example, flinching)
2	Resists or attempts to move away from handler
3	Kicking or foot thumping without vocalization
4	Kicking or foot thumping with vocalization

Results

Behavioral response to injection. Overall, rabbits that received SC administration of KM had fewer ($P = 0.01$) behavioral reactions than did those injected by the IM route ($P = 0.01$) The SC route elicited little or no reaction from the rabbits, with an average response score of 0.25 ± 0.23; 2 animals responded with a score of 1, and another scored a 3 after SC injection of the anesthetic. In contrast, rabbits dosed via the IM route yielded an average score of 1.5 ± 0.43. Among the 20 rabbits in this group, 14 (70%) displayed signs of pain or discomfort (for example, kicking, vocalization). In addition, 1 rabbit was injected twice because of its reaction to the initial injection, and the site of the initial injection bled slightly.

Depth of anesthesia. The onset of anesthesia, as indicated by loss of the righting, ear pinch, and pedal reflexes, was characterized by muscle relaxation in all groups and occurred within 10 min of injection, but the actual time of onset differed significantly ($P < 0.05$) between groups (Table 2). Loss of the palpebral and pedal reflex responses was more rapid in the IM-KMA and IM-KM groups than in the SC-KMA and SC-KM groups. The palpebral reflex was not lost consistently in any of the groups, but this reflex often is considered an unreliable indication of anesthetic depth in rabbits.[6,7,18] Specifically, 1 of the 20 rabbits (5%) in the SC groups and 6 of 20 (30%) of those in the IM groups never lost the palpebral reflex, whereas the remaining rabbits became unable to blink within 15 min of the initial injection. However according to Fisher's exact test, these groups did not differ significantly.

Cardiopulmonary effects. All animals experienced moderate bradycardia after induction of anesthesia. The decrease in heart rate was greater ($P = 0.0014$) in the SC-KMA group than in the IM-KMA group (Figure 1). Heart rates in both of these groups returned to near baseline measurements shortly after reversal with atipamezole. Heart rates did not differ significantly between the SC-KM and IM-KM groups.

Respiratory rates were reduced after induction of anesthesia but did not differ significantly between the SC-KMA and IM-KMA groups (Figure 2). Respiratory rates in both of these groups increased after administration of atipamezole. The respiratory rates of the SC-KM and IM-KM groups did not differ significantly ($P = 0.38$) and seemed to stabilize shortly after the initial depression. In addition, all 4 groups displayed hypoxemia to varying degrees (Figure 3). The average SpO_2 in group IM-KMA were significantly ($P = 0.04$) lower than in Group SC-KMA; oxyhemoglobin saturations improved and returned to normal in these groups after injection of atipamezole. SpO_2 levels did not differ significantly ($P = 0.80$) between the SC-KM

Table 2. Time (min; mean ± standard error) to onset of loss of reflex responses after administration of ketamine–medetomidine in Dutch belted rabbits

Route of administration	Righting reflex ($P = 0.0497$)	Ear pinch reflex ($P = 0.0089$)	Palpebral reflex[a] ($P = 0.09$)	Pedal reflex ($P = 0.03$)
SC	3.9 ± 0.1	4.5 ± 0.3	10 ± 1.0	8.9 ± 0.9
IM	3.5 ± 0.1	3.5 ± 0.1	8.8 ± 1.0	6.5 ± 0.7

[a]Palpebral reflex was lost in 1 of 20 rabbits (5%) from the SC-induced groups and 6 of 20 rabbits (30%) from the IM-induced groups.

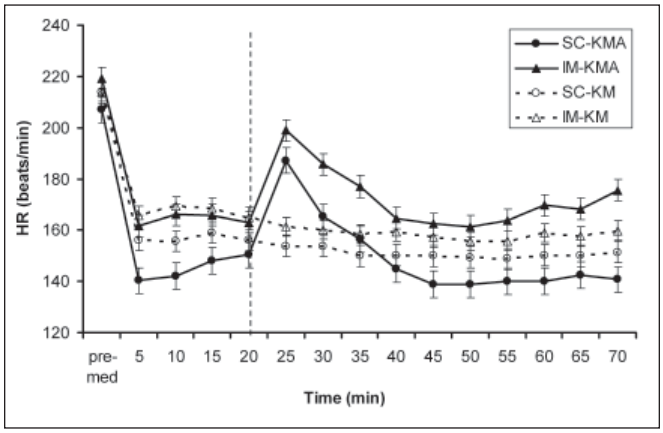

Figure 1. Heart rate (HR) after administration of ketamine–medetomidine by SC or IM route. Anesthesia in groups SC-KMA and IM-KMA was reversed with atipamezole (1 mg/kg) at 20 min after injection of anesthetic.

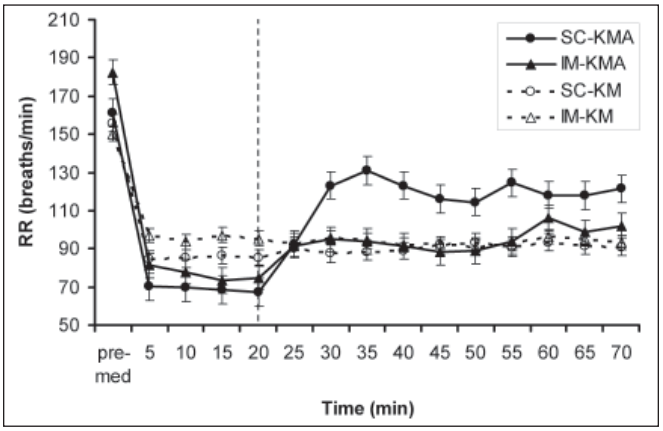

Figure 2. Respiratory rate (RR) after administration of ketamine–medetomidine by SC or IM route. Anesthesia in groups SC-KMA and IM-KMA was reversed with atipamezole (1 mg/kg) at 20 min after injection of anesthetic.

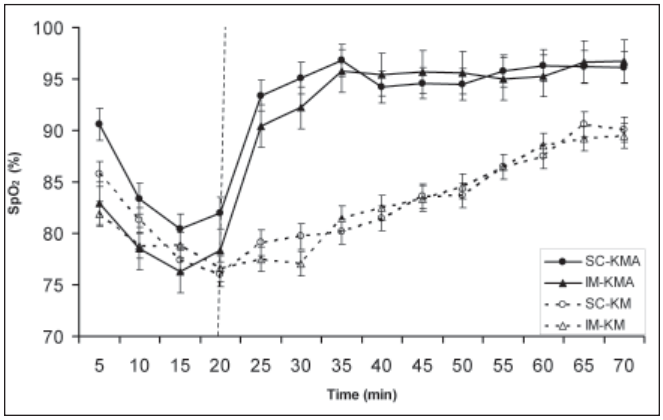

Figure 3. Arterial blood oxyhemoglobin saturation (SpO_2) after administration of ketamine–medetomidine by SC or IM route. Anesthesia in groups SC-KMA and IM-KMA was reversed with atipamezole (1 mg/kg) at 20 min after injection of anesthetic.

and IM-KM groups and remained lower than those in the groups receiving atipamezole.

Recovery from anesthesia. Full recovery was defined as the return of all reflexes but primarily of the righting reflex. Using linear contrasts in a 1-way analysis of variance, we compared the time for return of the righting reflex between the SC-KMA and SC-KM groups and between the IM-KMA and IM-KM groups. Although the time to return of the righting reflex differed significantly ($P = 0.0004$) between the IM-KMA (68.5 ± 5.6 min) and IM-KM (95.5 ± 3.5 min) groups, it did not differ ($P = 0.0681$) between the SC-induced groups (Table 3). Therefore, time to full recovery after SC-KM was not shortened by atimapezole administration.

Adverse events. Side effects during recovery included nystagmus and periods of apnea followed by rapid respiratory patterns in all groups. No other complications or anesthetic-related morbidity or mortality occurred in these rabbits.

Discussion

In an attempt to refine the administration of a suitable anesthetic regimen for intravitreal injection and other minor procedures in Dutch belted rabbits, we compared the anesthetic effects of a combination of ketamine and medetomidine given IM versus SC. Our findings indicated that both routes of administration achieved comparable planes of anesthesia. However, 85% of the animals tolerated the SC injections without displaying behaviors such as foot stomping, thumping, and so forth). Therefore, SC administration may produce less distress to animals than does IM dosing. Because local tissue inflammation with fibrosis and histiocytic infiltration was noted in rats after a single injection of KM,[17] tissue damage might occur in rabbits undergoing multiple IM injections of ketamine anesthetic combinations. Furthermore, SC administration is relatively simple to perform.

Rabbits display wide interindividual variability and strain-specific differences in response to anesthetic drugs.[1,2] Moreover, the level of sedation achieved by using KM appears to be dose-dependent because previously published studies have demonstrated inconsistent success with various doses of this combination.[9,8,14] New Zealand White rabbits given ketamine (15 mg/kg) and medetomidine (0.25 mg/kg) by the IM or SQ routes failed to reach a surgical plane of anesthesia, even with the addition of butorphanol.[8] In contrast, another study reported that all New Zealand White rabbits given slightly different doses of ketamine (5 mg/kg) and medetomidine (0.35 mg/kg) were immobilized and showed good muscle relaxation with no side effects.[11] In the present study, Dutch Belted rabbits were anesthetized successfully with ketamine (25 mg/kg) and medetomidine (0.5 mg/kg), with no anesthetic complications. On the basis of the pedal and ear pinch reflexes, the rabbits in the SC-KM and IM-KM groups were in a surgical plane of anesthesia for 28 and 20 min, respectively.

Several factors play key roles in the effect and absorption of anesthetic drugs. Stress and injection route influence the

Table 3. Time (min; mean ± standard error) to return of ear pinch, palpebral, pedal and righting reflexes

Group	Righting reflex	Ear pinch reflex	Palpebral reflex[a]	Pedal reflex
SC-KMA	105.5 ± 6.0	31 ± 4.9	23.5 ± 3.8	19 ± 1.2
IM-KMA	68.5 ± 5.6	37 ± 9.4	37.5 ± 11.1	27.5 ± 2.0
SC-KM	118.5 ± 4.0	76 ± 5.0	34 ± 9.9	37 ± 4.2
IM-KM	95.5 ± 3.5	64 ± 4.3	50 ± 11.7	27 ± 4.0

[a]Palpebral reflex was lost in 1 of 20 rabbits (5%) in the SC-induced groups and 6 of 20 rabbits (30%) from the IM-induced groups.

distribution of medetomidine.[5,16] In our study, an acceptable level of sedation was achieved in all animals, regardless of administration route or possible stress associated with the anesthetic injections. The IM route led to more rapid drug effects whereas the duration of sedation was longer in the SC groups, even with the aid of a reversal agent. Prolonged recovery, seen mainly in rabbits anesthetized by the SC route, may reflect the greater bioavailability of medetomidine compared with that in the IM groups, where medetomidine absorption and distribution were more rapid.

Although intravenous atipamezole at doses of 1 mg/kg or lower has been reported to immediately reverse KM anesthesia in rabbits,[13] our study did not replicate those findings. In the IM groups, the animals that received atipamezole recovered about 27 min sooner than those allowed to recover spontaneously. Conversely, the SC groups (including both rabbits that were and were not given atipamezole) recovered within 10 to 15 min of one another. In the SC groups, atipamezole did not speed recovery from anesthesia. However, the immediate increase in SpO_2 that occurred after intravenous administration of atipamezole suggests that the hypoxemia was related to the pharmacologic effects of anesthesia and that reversal may have reduced ventilation–perfusion mismatch or improved alveolar ventilation.

The heart rates and respiratory rates were stable during anesthesia, but oxyhemoglobin saturations dropped below baseline values, which were taken 5 min after the injection of KM. Anesthetic combinations of KM produce moderate to marked hypoxia, which is thought to be a direct consequence of peripheral vasoconstriction associated with medetomidine.[14] Therefore, oxygen supplementation should be provided when inducing anesthesia by using these drugs in combination. Further, investigators should consider the potential effect of profound and extended hypoxemia (as demonstrated by animals in this study) on research results. For instance, postoperative tissue damage due to hypoxemic hypoxia may occur, particularly in rabbits used in cardiovascular procedures.

Potential weaknesses in the study design include a lack of mean arterial blood pressure monitoring for evaluation of tissue perfusion, but this monitoring could not be performed due to the restrictions of the pharmacokinetics study in which the rabbits were enrolled. In addition, an intravenous vehicle-only injection was not administered as a stimulus control for the atipamezole-dosed groups, in light of the well-documented effect described for this drug in rabbits. Another improvement of the study design would have been histopathologic evaluation of injection sites for possible tissue reaction. However, the pharmacokinetic study did not permit such evaluations due to the various endpoints assigned to these animals.

In conclusion, ketamine (25 mg/kg) and medetomidine (0.5 mg/kg) can be combined into a safe and effective anesthetic regimen that can be given either IM or SC for short-term procedures in Dutch Belted rabbits. The SC route was technically easy to administer by a single animal handler without additional mechanical restraint of the rabbits, due to minimal resistance of the animal during injection. Intravenous administration of atipamezole (1 mg/kg) markedly improved SpO_2 values in both SC- and IM-treated groups but did not rapidly reverse anesthesia in animals induced by the SC route. Further research may be necessary to optimize the dosage of the reversal agent for use in Dutch Belted rabbits.

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References

1. **Aeschbacher G.** 2001. Rabbit anaesthesia. Exot Anim Med **17:**1003–1010.
2. **Avsaroglu H, Versluis A, Hellebrekes L, Haberman Z, van Zutphen.** 2003. Strain differences in response to propofol, ketamine and medetomidine in rabbits. Vet Rec **152:**300.
3. **Beyers TM, Richardson JA, Prince MD.** 1991. Axonal degeneration and self-mutilation as a complication of the intramuscular use of ketamine and xylazine in rabbits. Lab Anim Sci **41:**519–520.
4. **Difilippo S, Norber P, Suson U, Savino A, Reim D.** 2004. A comparison of xylazine and medetomidine in an anesthetic combination in New Zealand white rabbits. Contemp Top Lab Anim Sci **43:**32–37.
5. **England GCW, Clarke KW.** 1989. The effect of route of administration upon the efficacy of medetomidine. Journal of the Association of Veterinary Anaesthetists **16:**32–34.
6. **Flecknell PA.** 1996. Laboratory animal anaesthesia, 2nd ed. San Diego: Academic Press.
7. **Gil AG, Silvan G, Illera JC**. 2005. Effects of barbituate administration on hepatic and renal biochemical parameters in New Zealand white rabbits. Contemp Top Lab Anim Sci **44:**43–45.
8. **Hedenqvist P, Orr HE, Roughan JV, Antunes LM, Flecknell PA.** 2002. Anaesthesia with ketamine/medetomidine in the rabbit: influence of route of administration and the effect of combination with butorphanol. Vet Anaesth Analg **29:**14–19.
9. **Henke J, Astner S, Brill T, Eissner B, Busch R, Erhardt W.** 2005. Comparative study of three intramuscular anaesthetic combinations (medetomidine/ketamine, medetomidine/fentanyl/midazolam and xylazine/ketamine) in rabbits. Vet Anaesth Analg **32:**261–270.
10. **Hrapkiewicz K, Medina L, Holmes D.** 1998. Clinical laboratory animal medicine: an introduction, 2nd ed. Ames (IA): Iowa State University Press. p 146–147.
11. **Kim MS, Jeoing SM, Park JH, Nam TC, Seo KM.** 2004. Reversal of medetomidine-ketamine combination anesthesia in rabbits by atipamezole. Exp Anim **53:**423–428.
12. **Marini RP, Li X, Harpster NK, Dangler C.** 1999. Cardiovascular pathology possibly associated with ketamine/xylazine anesthesia in Dutch belted rabbits. Lab Anim Sci **49:**153–160.
13. **Nevalainen T, Pyhala L, Voipio HM, Viranten R.** 1989. Evaluation of anaesthetic potency of medetomidine-ketamine combination in rats, guinea-pigs and rabbits. Acta Vet Scand Suppl **85:**139–143.
14. **Orr H, Roughan J, Flecknell P.** 2005. Assessment of ketamine and medetomidine anaesthesia in the domestic rabbit. Vet Anaesth Analg **32:**271–279.
15. **Plumb DC.** 2005. Plumb's veterinary drug handbook, 5th ed. Stockholm (WI): PharmaVet Publishing.

16. **Raekallo M, Ansah OB, Kuusela E, Vanio O.** 2002. Some factors influencing the level of clinical sedation induced by medetomidine in rabbits. J Vet Pharmacol Therap **25:**39–42.
17. **Sun FJ, Wright DE, Pinson DM.** 2003. Comparison of ketamine versus combination of ketamine and medetomidine in injectable anaesthetic protocols: Chemical immobilization in macaques and tissue reaction in rats. Contemp Top Lab Anim Sci **42:**32–37.
18. **Swindle MM, Vogler GA, Fulton LK, Marini RP, Popilskis S.** 2002. Preanesthesia, anesthesia, analgesia, and euthanasia: rabbits. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. San Diego (CA): Academic Press. p 966–971.
19. **Vergesten J, Fargetton X, Donnay I, Ectors F.** 1991. An evaluation of medetomidine/ketamine and other drug combinations for anesthesia in cats. Vet Rec **128:**32–35.