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# Anesthetic Effects of Alfaxalone–Ketamine, Alfaxalone–Ketamine–Dexmedetomidine, and Alfaxalone–Butorphanol–Midazolam Administered Intramuscularly in Five-striped Palm Squirrels (*Funambulus pennantii*)

David Eshar<sup>1,\*</sup> and Hugues Beaufrère<sup>2</sup>

Injectable anesthesia protocols for five-striped palm squirrels (*Funambulus pennantii*) are poorly described in the literature. In this study, male intact squirrels received intramuscular injections of either alfaxalone (6 mg/kg) and ketamine (40 mg/kg; AK group,  $n = 8$ ); alfaxalone (6 mg/kg), ketamine (20 mg/kg), and dexmedetomidine (0.1 mg/kg; AKD group,  $n = 8$ ); or alfaxalone (8 mg/kg), butorphanol (1 mg/kg), and midazolam (1 mg/kg; ABM group,  $n = 8$ ). Atipamezole (0.15 mg/kg IM) and flumazenil (0.1 mg/kg IM) were administered 40 min after anesthesia induction (defined as loss of the righting reflex) with AKD and ABM, respectively. Heart rate, respiratory rate, rectal temperature, and reflexes were recorded every 5 min during anesthesia. Anesthetic induction was rapid in all groups (AK: median, 49 s; range, 33 to 60 s; AKD, 60 s; 54 to 70 s; and ABM, 15 s; 5 to 58 s). The anesthetic duration (from induction to full recovery) for the AK group was  $62 \pm 3$  min (mean  $\pm$  1 SD). There was no statistically significant difference between the ABM and AKD groups regarding recovery time after partial antagonist administration and was  $51 \pm 5$  and  $48 \pm 5$  min, respectively. All AK animals showed twitching and abnormal vocalization during recovery. The righting reflex was absent in all squirrels for 20 min in the AK treatment group and throughout the 40-min anesthetic period in the AKD and ABM groups. The frontlimb withdrawal response was absent in all squirrels for the 40-min anesthetic period in the AKD and ABM groups, with variable responses for the AK treatment. All tested protocols in this study provided safe and effective immobilization in five-striped palm squirrels, but oxygen and thermal support were indicated. Anesthetic depth must be determined before surgical procedures are performed in palm squirrels anesthetized by using these regimens.

**Abbreviations:** A, alfaxalone; B, butorphanol; D, dexmedetomidine; K, ketamine; M, midazolam

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Five-striped palm squirrels (*Funambulus pennantii*), also known as northern palm squirrels, are rodents in the family *Sciuridae*. This squirrel species is native to Southeast Asia, including India, Pakistan, Nepal, Bangladesh, and Iran. Palm squirrels are an adaptable species that inhabits tropical and subtropical dry deciduous forests, mountain forests, scrublands, plantations, grasslands, arable lands, rural gardens, and urban areas.<sup>6,34</sup> Adult palm squirrels commonly weigh between 135 and 200 g and have a body length of 20 to 30 cm, half of which comprises the tail.<sup>6</sup> This species has been widely studied in a multitude of endocrinologic research studies.<sup>1,2,14,39,40,44,47</sup>

In general, squirrel species can be fractious in nature, and chemical immobilization or anesthesia is often required to enable examinations or clinical procedures.<sup>5,7,9,16,23,25,30,31,36,37,42,51</sup>

With its relatively rapid induction and recovery properties, inhalation anesthesia is commonly used for immobilization of rodent species.<sup>9,20,22,31,37</sup> Because of the small body size of palm squirrels, endotracheal intubation is technically challenging and therefore not routinely performed, and inhalation anesthesia is commonly delivered by using a chamber or face mask. However, the use of a face mask for anesthesia increases the risk of waste gas exposure to personnel.<sup>43,49,53</sup> In addition, in other small rodents, delivering inhalant anesthesia in 100% oxygen via face masks may predispose to fire ignition during surgery.<sup>11,45</sup> Therefore, alternative protocols that exclusively use injectable anesthetic drugs for the induction and maintenance of anesthesia and that reduce waste-gas exposure are desired.<sup>7,9,22,23</sup> Furthermore, intravascular access is challenging to obtain in palm squirrels; consequently, parenteral, nonvascular protocols may provide the most accommodating route for anesthesia induction and maintenance in this species.

Alfaxalone (3 $\alpha$ -hydroxy-5 $\alpha$ -pregnane-11, 20-dione) is a neuroactive steroid molecule that potentiates  $\gamma$ -aminobutyric acid A receptors, resulting in centrally mediated muscle relaxation

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and anesthesia that is not chemically reversible in an anesthetic profile that resembles that of propofol.<sup>4,23</sup> Alfaxalone is an increasingly popular option for the sedation and anesthesia of rodents, and although intended primarily for intravenous administration, the drug can also be administered via the practical intramuscular and subcutaneous routes.<sup>4,12,15,24,38</sup> The addition of  $\alpha_2$ -adrenoceptor agonists and opioids can improve the quality of anesthesia, provide analgesia, and reduce the required dose and volume of alfaxalone.<sup>8,12,13,24,26,28,38,41,46</sup> For example in guinea pigs, alfaxalone administered as a sole agent provided only light sedation, but a alfaxalone–dexmedetomidine–buprenorphine combination increased the duration of sedation and immobility.<sup>12,13</sup> Combinations of alfaxalone–butorphanol–medetomidine (ABM) administered intraperitoneally and subcutaneously to laboratory mice<sup>24</sup> and of alfaxalone–dexmedetomidine (D)–butorphanol administered intramuscularly to rabbits<sup>8</sup> resulted in a surgical plane of anesthesia. The addition of ketamine (K)—a dissociative, centrally acting antagonist of the *N*-methyl-D-aspartate receptor—can extend the duration of immobilization, achieve a deeper level of sedation, and provide some analgesia, thus facilitating the performance of invasive procedures.<sup>33,48</sup>

Most reports in the literature that involve injectable anesthesia of palm squirrels poorly describe the anesthetic effects of the used protocols.<sup>16</sup> In a terminal hematology study, five-striped squirrels were immobilized by using isoflurane gas induction followed by intramuscular administration of high-dose ketamine (100 to 150 mg/kg).<sup>6</sup> In a recent prospective study, squirrels were safely and effectively anesthetized via a combination of dexmedetomidine (0.1 mg/kg), ketamine hydrochloride (30 mg/kg), and midazolam (0.75 mg/kg) administered intramuscularly.<sup>16</sup> The objective of our current study was to determine the anesthetic effects of 3 alfaxalone-based combinations (AK, AKD, and ABM) in five-striped palm squirrels. Our primary hypothesis was that all regimens would provide effective immobilization in palm squirrels for a length of time suitable for most testing and clinical procedures.

## Materials and Methods

This study protocol was reviewed and approved by the zoo ethics committee and the IACUC at Kansas State University (no. 4205). Twenty-four male intact, five-striped palm squirrels of unknown age (weight, 78 to 145 g) were included in the study. The squirrels were confiscated from local illegally owned private collections and were kept temporarily at a local zoo, where they were group-housed in a climate-controlled room (approximately 24 °C) with a 14:10-h light:dark cycle. Their indoor wire enclosures contained wooden hiding boxes, with wood shavings, branches, and canvas ropes for environmental enrichment. Squirrels had free access to water and a diet consisting of seeds, nuts, and fruit. The squirrels were not fasted before they were anesthetized during this study.

Squirrels were placed in individual clear plastic animal containers (31 × 19 × 20 cm) and moved from the housing location to the designated examination room (approximately 25 °C). Each squirrel was anesthetized once via the protocol that was assigned by using an online randomization tool (<http://www.randomization.com>). The anesthesia drug doses used in this study were chosen on the basis of results from preliminary experiments using other palm squirrels, in which lower dosages than those used here final study provided just partial or brief immobilization.

Drug combinations used in the study reported here were considered effective when squirrels showed loss of the righting reflex (light anesthetic state) or a full surgical anesthetic state

(loss of all responses, including deep pain) for at least 20 min. For each protocol, 8 different squirrels were anesthetized by using either alfaxalone (6 mg/kg; Alfaxan, Jurox, Kansas City, MO) and ketamine (40 mg/kg; Ketaset, Hospira, Lake Forest, IL; AK regimen); alfaxalone (6 mg/kg), ketamine (20 mg/kg), and dexmedetomidine (0.1 mg/kg; Dexdomitor, Orion, Espoo, Finland; AKD protocol); or alfaxalone (8 mg/kg), butorphanol (1 mg/kg; Torbugesic-SA, Zoetis, Florham Park, NJ), and midazolam (1 mg/kg; Hospira; ABM combination). Squirrels were manually restrained by a handler wearing a thin leather glove, and the investigator administered each drug individually into the thigh musculature through a 31-gauge, 5/16-in needle attached to a 0.3-mL insulin syringe (BD Ultra-Fine II, Becton Dickinson, Franklin Lakes, NJ).

After drug administration, the squirrels were replaced into the clear plastic containers and closely monitored during the induction period. Anesthetic induction time was defined as the interval from drug injection to loss of the righting reflex. Once the righting reflex was lost (time 0), squirrels were positioned in sternal recumbency, and eye lubricant was applied bilaterally.

Atipamezole (0.15 mg/kg; Orion, Espoo, Finland) and flumazenil (0.1 mg/kg; Flumazenil, Hikma Farmaceutica, Terrugem, Portugal) were administered intramuscularly at 40 min after anesthesia induction in the AKD and ABM groups, respectively. While under anesthesia, the squirrels were allowed to spontaneously breathe room air without supplemental oxygen or active thermal support. During their recovery from anesthesia (after recovery of the righting reflex or antagonist administration), squirrels were placed in a heated cage for continuous monitoring of vital signs. Once squirrels were observed to be fully responsive, they were returned to the holding facility.

Evaluators were blinded to the treatment administered to the anesthetized squirrels. Measurements obtained immediately after induction (time 0) were used for subsequent comparisons. Vital signs measurements were obtained at time 0 and at 5-min intervals in the following order: heart rate, respiratory rate, and rectal temperature. Heart rate was determined by using Doppler ultrasonography (model 811-B Doppler flow detector, Parks Medical Electronics, Las Vegas, NV), with the probe placed over the thorax. SpO<sub>2</sub> reading were obtained by using 2 pulse oximeters (BCI 3301, Smiths Medical PM, Waukesha, WI; PalmSat 2500, Nonin Medical, Plymouth, MN) placed over a distal limb. Respiratory rate was assessed by direct visual observation of chest movements. In addition, heart and respiratory rates were monitored between the 5-min time points by using a stethoscope. Rectal temperature was measured by using a handheld digital thermometer.

Once the vital signs were recorded, the following responses were also tested at every 5-min time point, during the anesthetic period in the following order: righting reflex, palpebral reflex, forelimb withdrawal, and hindlimb withdrawal. The responses were scored on a scale of 0 to 2, with 0 indicating an intact response, 1 a decreased response, and 2 the absence of a response. The righting reflex was assessed as the response to a gentle lateral roll of the animal and its attempt to right itself sternally. The palpebral reflex was evaluated by gently touching a fine cotton-tip applicator twice to the rostral canthus of the eye. Plastic forceps were used to assess toe-pinch response (i.e., withdrawal reflexes) of the metacarpal and metatarsal digits. A deep (surgical) plane of anesthesia was defined as the loss of all monitored responses. Full recovery was defined when animals showed all responses and purposeful movement, either spontaneously (AK) or after the administration of reversal agent (AKD and ABM).

**Statistical analysis.** Outcome variables were assessed over time by using linear mixed models, with time, group, weight, and interactions as fixed effects and animal as the random effect. Time was treated as a factor to compare with baseline. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were performed on residuals for normality assessment. Posthoc analysis was performed through Tukey adjustment. Differences between the 3 protocols in regard to induction and recovery times were assessed by using Kruskal–Wallis ANOVA. Posthoc analysis was performed by using Wilcoxon rank-sum tests with Bonferroni correction. Assumptions were validated. All analyses were performed by using a statistical program (R package, version 3.1-121, R Foundation for Statistical Computing, Vienna, Austria). Values were considered significant at an  $\alpha$  value of 0.05 or less.

## Results

All squirrels showed stable physiologic and no major adverse responses throughout the perianesthetic period.

Anesthetic induction was rapid and without complications in all squirrels; median (range) times to induction were 49 s (33 to 60 s) in the AK group, 60 s (54 to 70 s) in AKD animals, and 15 s (5 to 58 s) after ABM (Figure 1). Induction time was significantly different between treatments (Kruskal–Wallis,  $P = 0.005$ ). Specifically, the AKD group had a significantly ( $P = 0.016$ ) longer induction time than the ABM group. Other differences were not detected on posthoc analysis.

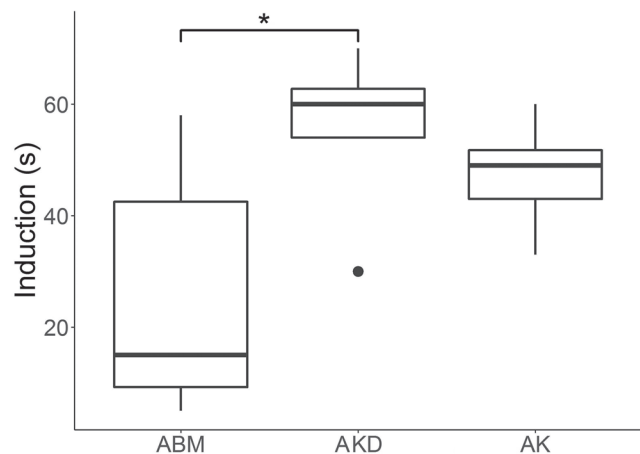
Anesthetic duration (from induction to full recovery) for the AK group was  $62 \pm 3$  min (mean  $\pm$  1 SD). There was no statistically significant difference between the ABM and AKD groups regarding recovery time after partial antagonist administration, which was  $51 \pm 5$  and  $48 \pm 5$  min, respectively (Figure 2). All animals in the AK treatment group showed twitching, tail lifting, and abnormal vocalization during their recovery.

Heart rate showed a significant ( $P < 0.001$ ) time  $\times$  treatment effect (Figure 3). For the ABM group, heart rate was significantly lower than baseline starting at time 15 ( $P = 0.02$ ) and remaining throughout the rest of the anesthesia (all  $P < 0.001$ ). For the AKD group, heart rate was significantly lower than baseline starting at time 15 ( $P = 0.008$ ) and throughout the rest of the anesthesia (all  $P < 0.001$ ). For the AK group, the heart rate did not significantly change over time (all  $P > 0.16$ ).

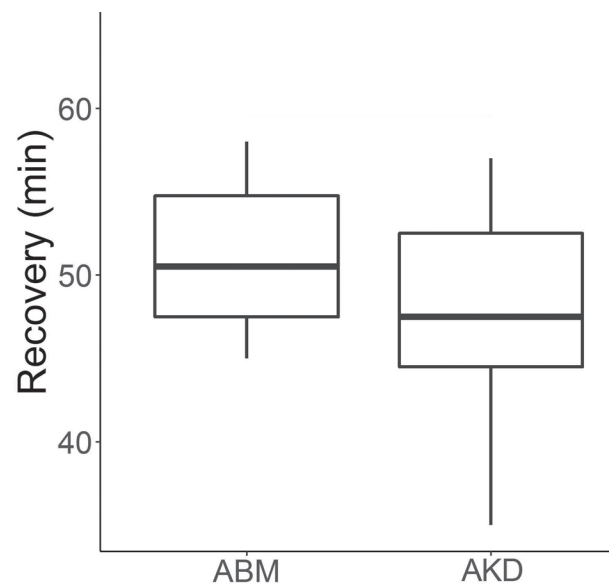
Respiratory rate demonstrated ( $P < 0.001$ ) a significant time  $\times$  treatment effect (Figure 4). Animals in the ABM group had significantly lower respiratory rates than the AKD group from time 0 to 20 (all  $P < 0.008$ ) and from the AK group throughout anesthesia (all  $P < 0.001$ ). The AKD group had significantly lower respiratory rates than the AK group at time 0 ( $P = 0.008$ ) and from time 30 to 40 (all  $P < 0.02$ ). For the ABM group, the respiratory rate did not significantly change over time (all  $P > 0.42$ ). For the AKD group, the respiratory rate was significantly lower than baseline starting at time 20 ( $P = 0.018$ ) and throughout the rest of anesthesia (all  $P < 0.001$ ). For the AK group, the respiratory rate was significantly lower than baseline starting at time 10 ( $P = 0.042$ ) and throughout the rest of anesthesia (all  $P < 0.001$ ).

Rectal temperature showed a significant ( $P = 0.009$ ) time  $\times$  treatment effect (Figure 5). When compared with baseline, all groups had decreased temperatures starting at time 5 (all  $P < 0.04$ ) and onward (all  $P < 0.001$ ). The ABM group had significantly lower temperatures than the AKD group at time 5 ( $P = 0.005$ ) and time 10 ( $P = 0.037$ ), but temperatures did not differ significantly for the remainder of anesthesia.

Responses were tested during the anesthetic period (Table 1). In the AK group, the righting reflex was the only response to



**Figure 1.** Anesthesia induction time of palm squirrels anesthetized with either alfaxalone–ketamine (AK;  $n = 8$ ), alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ) or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ ). \*, significant difference ( $P < 0.05$ ).



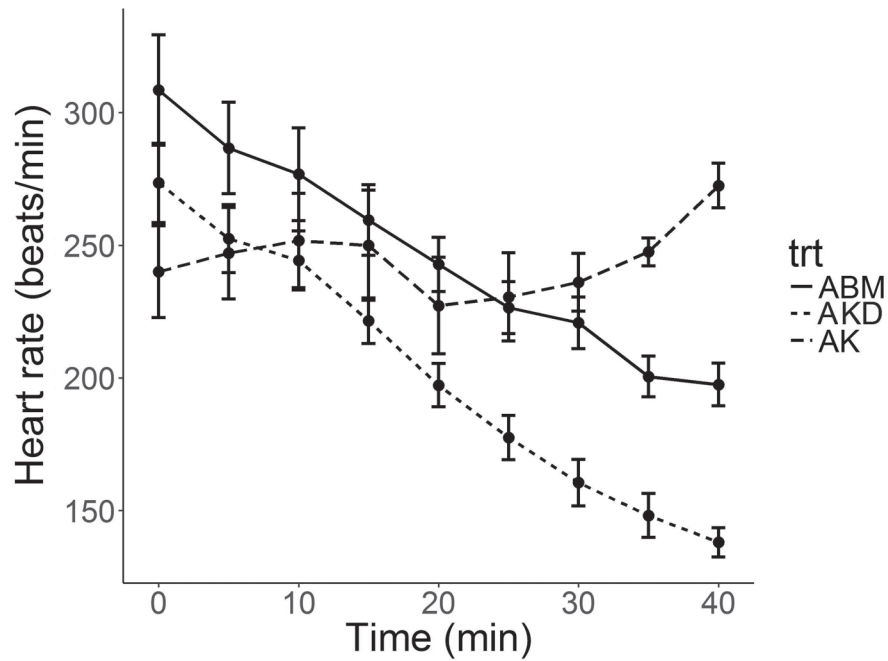
**Figure 2.** Recovery time of palm squirrels anesthetized with alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ) or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ ).

differ throughout anesthesia, being absent in all animals from time 0 to 20 and recovered in all animals by time 30. In the AKD group, all 4 tested responses were absent from 15 to 40 min after anesthetic induction, whereas the ABM group showed complete loss of 3 tested responses (i.e., righting reflex, palpebral reflex, and forelimb withdrawal) and a variable individual response for hindlimb withdrawal during that period.

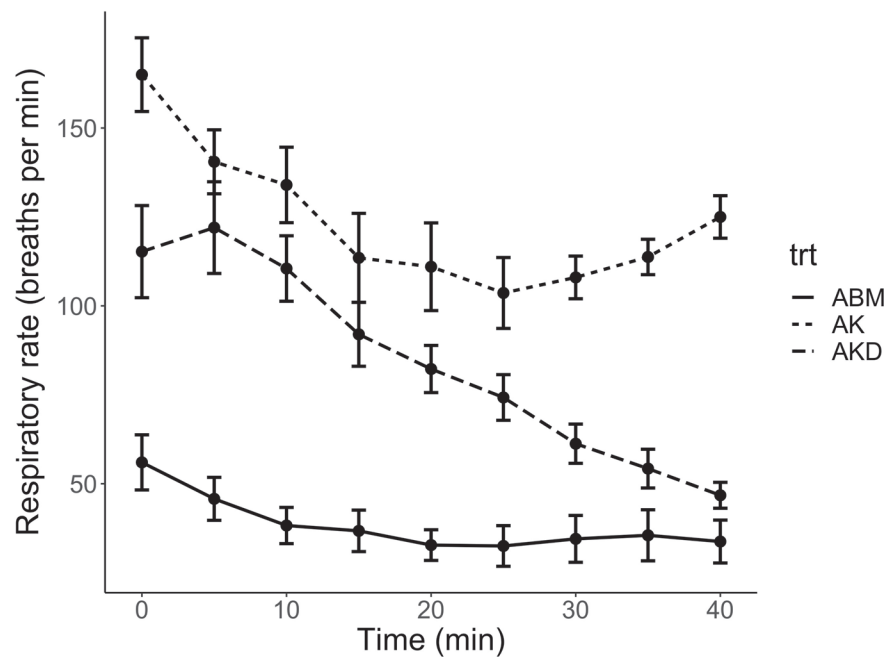
None of the squirrels that we tested showed any signs of injection-site reactions on follow-up examinations.

## Discussion

In the study reported here, five-striped palm squirrels were anesthetized by using 3 alfaxalone-based combinations administered intramuscularly, and all regimens appeared to provide safe and effective anesthesia in this species. A systematic review of the literature showed that no similar regimens have been used previously in palm squirrels, and the combination of agents tested in the present study was designed to provide



**Figure 3.** Heart rate (mean  $\pm$  SEM) of palm squirrels anesthetized with either alfaxalone–ketamine (AK;  $n = 8$ ), alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ) or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ ).

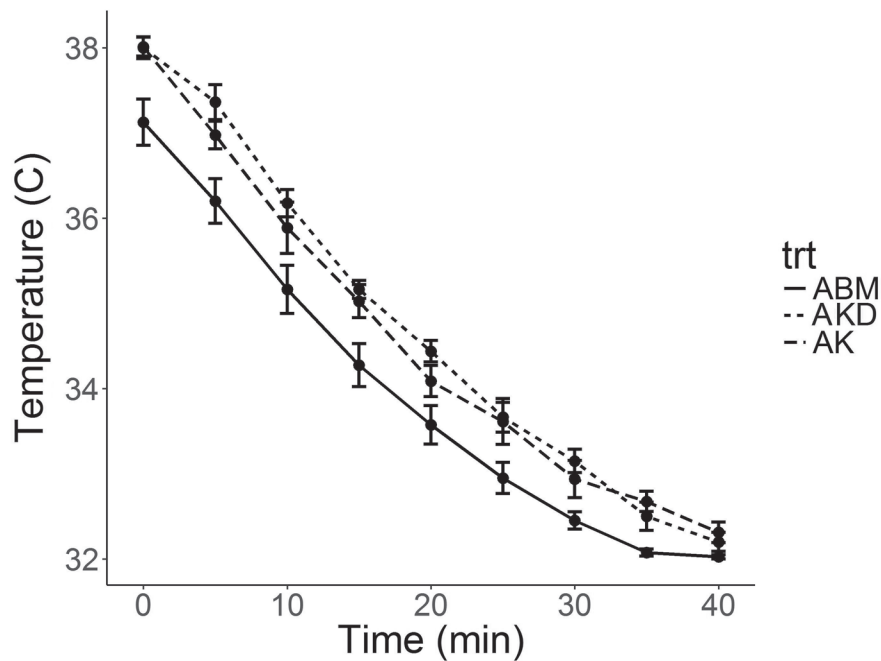


**Figure 4.** Respiratory rate (mean  $\pm$  SEM) of palm squirrels anesthetized with either alfaxalone–ketamine (AK;  $n = 8$ ), alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ) or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ ).

balanced and—depending on the protocol—partially reversible anesthesia. On the basis of our current results, combinations of AK, AKD, and ABM are viable options for the induction and maintenance of anesthesia in palm squirrels.

Dexmedetomidine is an  $\alpha_2$ -adrenoceptor agonist that is reversible and provides analgesia, sedation, and muscle relaxation.<sup>10,20,29</sup> Midazolam, a benzodiazepine, is reversible and commonly used for sedation in rodents, with minimal adverse effects.<sup>20</sup> Butorphanol is a  $\kappa$ -opioid receptor agonist that provides both analgesia and sedation and can be administered intramuscularly or subcutaneously.<sup>20</sup> The

addition of butorphanol, midazolam, dexmedetomidine, and butorphanol–dexmedetomidine to alfaxalone appeared to potentiate sedation and anesthesia in rabbits, compared with the effects for alfaxalone administered as a sole agent.<sup>8</sup> Administration of an ABM protocol to beagles provided excellent quality of induction of anesthesia with minimal cardiopulmonary effects;<sup>46</sup> for this reason, we evaluated ABM in the present study. Both the AKD and the ABM drug combinations that we used here appeared to improve most anesthesia parameters when compared with results of the AK protocol.



**Figure 5.** Rectal temperature over 40 min of palm squirrels anesthetized with alfaxalone–ketamine (AK;  $n = 8$ ), alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ) or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ ).

Achieving and maintaining a consistent plane of immobilization can be challenging when drugs are not administered intravenously. We used the intramuscular route of administration in this study because it generally allows for uniform and rapid drug absorption.<sup>50</sup> Because intraperitoneal administration of alfaxalone has resulted in inconsistent anesthesia and potentially dangerous responses in other rodent species,<sup>4,24,27</sup> we avoided this route of administration in our study. The use of subcutaneous administration may help to address potential concerns regarding multiple intramuscular injections and can be considered in future studies. In chinchillas, the subcutaneous route was not as effective as intramuscular administration of alfaxalone and other drugs;<sup>38</sup> consequently we did not use subcutaneous administration in the current study. None of the squirrels that we tested showed any signs of injection-site reactions on follow-up examinations.

The times until anesthetic induction and full recovery in our study were relatively short, which generally is desirable, especially when anesthetizing small animals.<sup>5,7,23</sup> In another study, five-striped palm squirrels anesthetized by using a combination of dexmedetomidine–ketamine–midazolam showed a similar induction time (67.5 s; interquartile [25th to 75th percentile] range, 5.5 s) to that of our squirrels and a much shorter recovery time ( $147 \pm 79$  s) after the administration of reversal agents.<sup>16</sup> Differences in anesthetic responses to different immobilization protocols within the same species emphasize the need for species-specific anesthetic regimens, as was determined for the palm squirrels in the study reported here.

Lateral recumbency (or loss of the righting reflex) after drug administration to laboratory rodents is considered a marker of anesthesia onset.<sup>21</sup> In our current study, all squirrels in all protocols lacked the righting reflex from times 0 to 40 in the ABM and AKD groups and from times 0 to 20 in the AK group, thus suggesting that these protocols can provide immobilization for this range of time in this species. Because drug antagonists were administered at 40 min after anesthetic induction, we could not determine the potential duration of anesthesia that the AKD and ABM protocols can provide. Future studies in palm squir-

rels could allow the animals to spontaneously recover without the administration of reversal agents and thus reveal the full anesthetic duration of these protocols.

The absence or marked suppression of withdrawal responses has been suggested as an indicator of deep or surgical anesthesia.<sup>20</sup> The AKD and ABM (to a slightly lesser extent) regimens provided consistent, deep anesthesia in the squirrels in the current study. Despite the increased dose of ketamine in this protocol, administration of AK did not provide a consistent surgical plane of anesthesia, as indicated by the variable responses to toe pinch (i.e., limb withdrawal reflex), suggesting that supplemental drugs (anesthesia and analgesia) or increases in the doses of both alfaxalone and ketamine might be required for invasive and pain-inducing procedures. In addition to the intragroup variability, observed responses differed between the forelimb and hindlimb withdrawal reflexes in the ABM and AKD treatments, suggesting that both reflexes should be tested to determine the level of anesthesia and before application of invasive or painful treatments. In rodents, hindlimb withdrawal typically is lost at a lighter plane of anesthesia than forelimb withdrawal.<sup>20</sup> However, in the current study, forelimb withdrawal was lost first, and hindlimb withdrawal was either absent or reduced in the AKD group, likely depending on the level of anesthesia. Regardless, response to a toe pinch might merely be an indicator of the depth of anesthesia,<sup>35</sup> and the potential antinociceptive effect of ABM and AKD in palm squirrels should be evaluated further before a painful treatment is applied.

Loss of the palpebral reflex usually indicates a deep plane of anesthesia but can be difficult to assess or may appear variable in small rodents.<sup>20</sup> In the current study, squirrels in the AK group showed variable palpebral responses, when compared with the deeply anesthetized animals in the AKD and ABM groups, which consistently showed loss of palpebral reflex during anesthesia. This finding suggests that loss of the palpebral reflex can be used in five-striped palm squirrels to assess depth of anesthesia.

In this study, heart rate decreased over time for all drug combinations; this is a generally expected deep anesthetic

**Table 1.** Numbers of palm squirrels with absent responses during 40 min of anesthesia due to alfaxalone–ketamine (AK;  $n = 8$ ), alfaxalone–ketamine–dexmedetomidine (AKD;  $n = 8$ ), or alfaxalone–butorphanol–midazolam (ABM;  $n = 8$ )

Time (min)	Protocol	Loss of righting reflex	Loss of palpebral reflex	Loss of frontlimb withdrawal	Loss of hindlimb withdrawal
0	AK	8	1	3	2
	AKD	8	5	8	6
	ABM	8	6	8	4
5	AK	8	1	4	2
	AKD	8	8	8	7
	ABM	8	8	8	4
10	AK	8	1	1	1
	AKD	8	8	8	7
	ABM	8	8	8	6
15	AK	8	1	1	1
	AKD	8	8	8	8
	ABM	8	8	8	6
20	AK	8	1	1	1
	AKD	8	8	8	8
	ABM	8	8	8	6
25	AK	6	1	1	0
	AKD	8	8	8	8
	ABM	8	8	8	6
30	AK	2	0	0	0
	AKD	8	8	8	8
	ABM	8	8	8	5
35	AK	0	0	0	0
	AKD	8	8	8	8
	ABM	8	8	8	5
40	AK	0	0	0	0
	AKD	8	8	8	8
	ABM	8	8	8	5

Note that drug reversals for the AKD and ABM protocols were administered after the 40-min time point.

effect that has been observed in other species.<sup>4,15,38,46</sup> Because we administered alfaxalone in combination with other drugs, it was challenging to determine which drug had the greatest effect on the heart rate. The AKD group showed significantly lower mean heart rates when compared with the other groups, possibly due to the dexmedetomidine included in this protocol. Dexmedetomidine (an  $\alpha_2$ -adrenoceptor agonist) causes peripheral vasoconstriction because of its action on adrenergic receptors, leading to increased peripheral vascular resistance and eventually decreases in heart rate and cardiac output.<sup>29</sup> In rabbits, mean heart rates were significantly decreased after the administration of alfaxalone–dexmedetomidine and were lower (in order) than those after the administration of alfaxalone–butorphanol–dexmedetomidine, alfaxalone–midazolam, alfaxalone as a sole agent, and alfaxalone–butorphanol.<sup>8</sup>

In the palm squirrels of the present study, the administration of ABM resulted in a significantly lower respiratory rate than did AK and AKD. Because alfaxalone-related bradypnea can be a dose- or species-dependent effect,<sup>12,32,38</sup> the increased

alfaxalone dose in the ABM treatment might be responsible for the lower mean respiratory rates observed with this treatment. Similarly, rabbits anesthetized with alfaxalone–butorphanol and alfaxalone–midazolam combinations showed relative decreases in respiratory rate, compared with those in rabbits anesthetized with alfaxalone alone or dexmedetomidine alone, thus indicating the respiratory suppressive effect of these 2 drugs alone or in combination with alfaxalone.<sup>8</sup> The lower doses of butorphanol or midazolam in the ABM combination might reduce respiratory suppression but might also reduce the anesthetic quality and duration of this combination; these possibilities should be considered when administering ABM to palm squirrels. Despite the use of 2 different pulse oximeters, with placement alternating over all 4 limbs, reliable SpO<sub>2</sub> readings could not be obtained in the squirrels in this study. Regardless, further studies involving blood gas measurements and other pulse oximetry technologies, are required to determine whether the observed decreased respiratory rate is associated with hypoventilation, hypoxemia, and other blood

gas abnormalities in palm squirrels under AK, AKD, or ABM anesthesia.<sup>9,52</sup>

Regardless of the anesthesia protocol, all squirrels in our study became profoundly hypothermic, with a decreased (mean, 5.5 °C) rectal temperature over the procedure time. Regardless of the drugs used, anesthesia in general can lead to hypothermia.<sup>20</sup> Alpine marmots (*Marmota marmota*) anesthetized with ketamine–xylazine or ketamine–medetomidine in field settings had a decrease in body temperature to as low as 30 °C but made full recovery.<sup>7</sup> Anesthetic hypothermia can negatively affect the immune system, delay wound healing, slow blood clotting, and prolong recovery from anesthesia.<sup>7</sup> Although our palm squirrels made a full recovery from anesthesia, active thermal support should be provided when AK, AKD, or ABM is used to anesthetize this species.

Only male squirrels were tested in this study. Male laboratory mice (C57BL/6J) required higher doses of alfaxalone (80 to 120 mg/kg) than females (40 to 80 mg/kg), when combined with xylazine (10 mg/kg) to achieve a surgical plane of anesthesia.<sup>15</sup> A study in rats, using an older alfaxalone formulation, revealed that males required 4 times the dose for females to reach a surgical plane of anesthesia, suggesting that differences in sex hormones (mainly estrogen) may have an effect on alfaxalone, a neuroactive steroid that resembles progesterone.<sup>19</sup> An alfaxalone pharmacokinetics study in rats suggested that these sex-related differences were due to the various formulations of alfaxalone tested and assay methods rather than actual anesthetic-response differences between male and female rats.<sup>27</sup> However, a more recent study in rats given alfaxalone only (25, 35, and 45 mg/kg) revealed that anesthesia time was 2- to 3-fold longer in females than in males and that males required a dose that was 3 times higher to achieve a similar duration of anesthesia.<sup>4</sup> Rats anesthetized with isoflurane followed by intravenous administration of alfaxalone showed sex-related differences in the pharmacokinetics and pharmacodynamics of alfaxalone.<sup>52</sup> In that study, female rats had lower arterial blood pressure and blood pH and higher PaCO<sub>2</sub> than males.<sup>52</sup> The accumulated data in rats suggest that consideration should be given regarding the dose of alfaxalone administered to male and female rodents. Likewise female squirrels should be tested in future studies of alfaxalone-based combinations to determine whether there are differences in anesthetic responses between sexes in this species.

Squirrels in the AK treatment group had all showed vocalization and twitching ('popcorn-like' jumping) during their recovery period, whereas no adverse responses were observed after the AKD and ABM treatments. Mice anesthetized with alfaxalone alone or a combination of alfaxalone–xylazine displayed twitching, face scratching, hyperresponsiveness to noise or touching, and limb jerking during recovery.<sup>48</sup> Rats anesthetized with alfaxalone (intraperitoneally or intravenously) experienced short transitory dose-dependent apnea, and several of these rats also displayed facial twitching during induction and recovery.<sup>27</sup> Guinea pigs anesthetized with alfaxalone alone or in combination with dexmedetomidine or buprenorphine had dose-dependent respiratory depression and twitching or bruxism at increased doses.<sup>12</sup> Tremors, twitching, and rolling were observed in most of the chinchillas anesthetized with alfaxalone–butorphanol but in none anesthetized with dexmedetomidine–ketamine.<sup>38</sup> Dogs anesthetized with alfaxalone as a sole agent showed adverse responses, including apnea, tachypnea, hypotension, hypoxia, and excitement.<sup>18,32,46</sup> Coadministration of several drugs in an anesthetic combination can decrease the dose of the main agent (i.e., alfaxalone in the present study), thus minimizing its potential adverse effects.<sup>54</sup>

However, the squirrels in our study that received AK showed adverse responses despite receiving a lower dose of alfaxalone when compared with the ABM group, which showed none of these responses. Beagles anesthetized with ABM had a good quality of anesthesia,<sup>46</sup> similar to the results for our squirrels anesthetized with ABM in the current study. Alternatively, ketamine reactions occur in 5% to 30% of human patients and may include delirium, confusion, and excitement.<sup>3</sup> Ketamine reactions typically occur in the first hour after administration, subside within 1 to 2 h, and usually disappear on full wakening, perhaps similarly to what we observed in the AK group in our study.<sup>3</sup> A higher incidence of ketamine reactions is associated with a large dose and rapid intravenous administration,<sup>3</sup> perhaps the higher dose (40 mg/kg) in the AK treatment when compared with the lower dose (20 mg/kg) in the AKD treatment is the cause of this abnormal recovery behavior. Regardless of the anesthesia protocol, all squirrels tested in this study made full recovery and were returned to their main holding area within 2 to 3 h after the end of the procedure.

The study reported here had several limitations, including a small sample size, which could have led to a type II error for some variables. However, studies conducted to evaluate anesthesia in other species have used similar numbers of animals, including 5 to 6 mice,<sup>24</sup> 6 rabbits,<sup>8</sup> and 8 chinchillas.<sup>22</sup> Performing a complete crossover study, where each squirrel would have received all 3 drug combinations, could have eliminated potential confounding factors, increased statistical power, and increased the sampling pool; however, such a study was not performed because it would have required that each animal be anesthetized 3 times, thus placing them at greater anesthetic risk. To determine the full anesthetic effects of drug combinations used in this study, the squirrels were allowed to breathe room air spontaneously without supplemental oxygen and active thermal support in the heated examination room. Although all the squirrels made a full recovery, supplemental oxygen and thermal support are strongly recommended for all animals during general anesthesia. Additional studies to compare the safety and efficacy of inhalation anesthetics and the injectable combinations of AK, AKD and ABM could also prove useful.

The AK, AKD, and ABM regimens evaluated in the study reported here appear to be safe and effective methods for immobilizing five-striped palm squirrels. The data in this study suggest that the AK protocol allows for brief (25 to 30 min) immobilization, whereas the AKD and ABM protocols provide deeper, surgical anesthesia for at least 40 min before partial drug reversal. However, anesthetic depth must be determined individually before surgical procedures are performed in palm squirrels that are anesthetized by using one of these drug combinations.

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