Comparison of intramuscular administration of alfaxalone-ketamine-dexmedetomidine and alfaxalone-butorphanol-midazolam in naked mole-rats (*Heterocephalus glaber*)

David Eshar  DVM  
Gail L. Huckins  DVM  
Trenton C. Shrader  DVM  
Hugues Beaufrère  DVM, PhD

Received April 1, 2019.  
Accepted May 30, 2019.

From the Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 (Eshar, Huckins); Lincoln Children’s Zoo, 122 S 27th St, Lincoln, NE 68502 (Shrader); and the Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada (Beaufrère).

Address correspondence to Dr. Eshar (deshar@vet.k-state.edu).

OBJECTIVE  
To compare anesthetic effects of alfaxalone-ketamine-dexmedetomidine (AKD) and alfaxalone-butorphanol-midazolam (ABM) in naked mole-rats (*Heterocephalus glaber*).

ANIMALS  
20 naked mole-rats.

PROCEDURES  
Naked mole-rats received AKD (alfaxalone, 2 mg/kg; ketamine, 20 mg/kg; and dexmedetomidine, 0.02 mg/kg; n = 10) or ABM (alfaxalone, 2 mg/kg; butorphanol, 2 mg/kg; and midazolam, 1 mg/kg; 9) IM; 1 animal was removed from the study. Atipamezole (1 mg/kg) and flumazenil (0.1 mg/kg) were administered 40 minutes after anesthetic induction (defined as loss of the righting reflex) with AKD and ABM, respectively. Heart rate, respiratory rate, oxygen saturation, and reflexes were recorded every 5 minutes.

RESULTS  
The ABM group had significantly longer median times for induction and recovery than the AKD group. Administration of ABM resulted in significantly lower respiratory rates than administration of AKD from time of anesthetic induction to 10 minutes after induction. Respiratory rate significantly decreased in the AKD group from 10 minutes after induction through the end of the anesthetic period but did not change over time in the ABM group. Males had higher respiratory rates in both groups. Loss of the righting reflex was still evident 40 minutes after induction in both groups. In the AKD group, all tested reflexes were absent from 10 to 40 minutes after induction; the ABM group had variable reflexes that recovered within individual animals over time.

CONCLUSIONS AND CLINICAL RELEVANCE  
Both AKD and ABM provided effective immobilization in naked mole-rats, but AKD appeared to provide more consistent and deeper anesthesia, compared with administration of ABM. (Am J Vet Res 2019;80:1089–1098)
Given their low basal metabolic rate, compared with that for other mammals of similar size, extrapolation of anesthetic doses reported for other rodent species may not be safe and is not recommended. Therefore, additional studies regarding the safety and efficacy of injectable anesthetic regimens in naked mole-rats are required.

Suitable combinations of injectable agents should ideally involve short-acting anesthetics with a wide safety margin that preferably are also reversible. Regimens that involve SC or IM injection of combinations of ketamine and α2-adrenoceptor agonists are routinely used as a practical method for anesthetizing rodents, especially when inhalation anesthesia equipment is not available (eg, field settings) or an animal cannot readily be intubated because of a narrow oral cavity and overall small body size (eg, mole-rats). 

Alfaxalone (3α-hydroxy-5α-pregnan-11, 20-dione) is an increasingly popular option for sedation and anesthesia of rodents. Although the drug is intended primarily for IV administration, it can also be administered via the IM and SC routes. Alfaxalone is a neuroactive steroid molecule that potentiates γ-aminobutyric acid A receptors, which results in centrally mediated muscle relaxation and anesthesia that is not reversible. The anesthetic profile of alfaxalone resembles that of propofol, and the effects of alfaxalone may be potentiated when the drug is combined with sedatives and analgesics. Because alfaxalone does not provide analgesia, premedication with α2-adrenoceptor agonists and opioids can improve the quality of anesthesia, extend the duration of anesthesia, and reduce the required dose and volume of alfaxalone. For example, alfaxalone administered alone to guinea pigs provided only light sedation, but administration of a combination of alfaxalone-dexmedetomidine-buprenorphine increased the duration of sedation and immobility but did not result in general anesthesia. A combination of alfaxalone-medetomidine-butorphanol administered IP and SC to laboratory mice and a combination of alfaxalone-dexmedetomidine-butorphanol administered IM to rabbits resulted in a surgical plane of anesthesia. Addition of low doses of ketamine, a dissociative, centrally acting antagonist of the N-methyl-D-aspartate receptor, can provide a deeper level of sedation and some analgesia, which can facilitate the performance of more invasive procedures or result in a longer duration of immobilization.

To the authors’ knowledge, data on clinically appropriate injectable anesthetic regimens with a combination of AKD and ABM for use in naked mole-rats have not been published. The objective of the study reported here was to determine the physiologic effects of 2 alfaxalone-based combinations (AKD and ABM) in naked mole-rats. Our primary hypothesis was that both regimens would provide effective immobilization in naked mole-rats. Another hypothesis was that sex of the mole-rats would affect the measured anesthetic variables.

### Materials and Methods

#### Animals

Twenty sexually intact male and female naked mole-rats were included in the study. Mole-rats ranged from 4 to 27 years of age, and mean ± SD body weight was 53.6 ± 12.3 g. The mole-rats were group housed in a climate-controlled room (room temperature, approx 28°C; relative humidity, 50% to 60%) with a light-dark cycle of 14.5 hours of light and 9.5 hours of darkness. The enclosure contained plastic hiding boxes padded with cottonwood shavings and acrylic tubes for environmental enrichment. The mole-rats included in the study were evaluated as part of their annual health examination. In addition, they were observed daily by the caretakers to monitor general appearance and behavior. Mole-rats were fed a rice-based cereal in water, romaine lettuce, apples, carrots, yams, and corn; food was not withheld from the mole-rats before they were anesthetized during the study. The study protocol was reviewed and approved by the Lincoln Children’s Zoo Ethics Committee and the Institutional Animal Care and Use Committee at Kansas State University (No. 4128).

#### Experimental procedures

Mole-rats were placed in individual clear plastic animal containers and moved from the group housing location to a designated procedure room (room temperature, approx 25°C). Each naked mole-rat was anesthetized once. Animals were assigned by use of an online randomizer tool to receive AKD or ABM. The alfaxalone dose used in the study was chosen on the basis of results for preliminary trials in which doses of up to 4 mg/kg caused a profound anesthetic effect and extremely prolonged recovery time in naked mole-rats. The other drug doses were determined on the basis of the minimal practical volume that could be accurately administered to the animals.

In the study reported here, a drug combination was considered effective when all naked mole-rats were in a stable plane of immobilization in a deep sedative state (most reflexes were absent) or a full surgical anesthesia state (complete loss of all reflexes, including deep pain). Naked mole-rats were anesthetized by use of a combination of alfaxalone (2 mg/kg [0.01 mL]), ketamine hydrochloride (10 mg/kg [0.01 mL]), and dexmedetomidine (0.02 mg/kg [0.01 mL]) or a combination of alfaxalone (2 mg/kg [0.01 mL]), butorphanol tartrate (2 mg/kg [0.01 mL]), and midazolam (1 mg/kg [0.01 mL]), which were administered into the thigh musculature as 3 separate injections. Drugs were administered with a 31-gauge, 5/16-inch needle attached to a 0.5-mL insulin syringe. After each mole-rat was injected, it was placed back into a clear plastic container and closely monitored during anesthetic induction.

Anesthetic induction time was defined as the interval from injection of the last of the 3 drugs to loss of the righting reflex. Immediately after loss of
the righting reflex was observed (anesthetic induction; baseline), the mole-rats were positioned in sternal recumbency, and eye lubricant was topically applied bilaterally. The naked mole-rats were allowed to spontaneously breathe room air. Rectal temperature was monitored.

Atipamezole<sup>h</sup> (1 mg/kg) and flumazenil<sup>i</sup> (0.1 mg/kg) were administered IM 40 minutes after induction of anesthesia with AKD and ABM, respectively. During recovery from anesthesia, the naked mole-rats were placed in a heated clear plastic container, and vital signs were monitored. Once each mole-rat was fully responsive, it was transported back to the group holding facility.

Measurements of vital signs were obtained at baseline and at 5-minute intervals in the following order: heart rate, respiratory rate, rectal temperature, and Sp<sub>O2</sub>. Heart rate was determined by use of Doppler ultrasonography<sup>j</sup> with the probe placed over the thorax. Respiratory rate was measured by direct visual observation of chest movements. The Sp<sub>O2</sub> was measured by use of a handheld pulse oximeter<sup>k</sup> that was placed on the paw of a hind limb.

After vital signs were recorded at each time point during the anesthetic period, reflexes were evaluated in the following order: loss of the righting reflex, loss of the palpebral reflex, forelimb withdrawal reflex, and hind limb withdrawal reflex. Reflexes were scored on a scale of 0 to 2 (0 = response was present, 1 = response was reduced, and 2 = response was absent).<sup>16</sup> Loss of the righting reflex was assessed by gently rolling each naked mole-rat into lateral recumbency and evaluating attempts to right itself into sternal recumbency. Loss of the palpebral reflex was evaluated by gently touching the rostral canthus of the eye 2 times with a cotton-tipped applicator. Both of the withdrawal reflexes were assessed by use of a toe pinch (a plastic forceps was used to pinch the metacarpal and metatarsal digits with increasing amounts of applied pressure [each pressure was applied 2 times at each location] until a response was determined). A surgical plane of anesthesia was defined as the loss of all monitored reflexes. Recovery was defined as the return of all reflexes.

**Statistical analysis**

Outcome variables were assessed over time by use of linear mixed models, with time, group, sex, and interaction terms as fixed effects and mole-rats nested within treatment as a random effect. Time was treated as a factor for comparison with baseline values. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots of the residuals were also used to assess normality. Post hoc analysis was performed with a Tukey adjustment. Ordinal categorical variables (all reflexes) were only reported as descriptive statistics. Differences between the 2 drug combinations for induction and recovery times were assessed by use of Mann-Whitney U tests. All variables analyzed by use of linear mixed models were reported as mean and SD or SEM, and all variables analyzed by use of the Mann-Whitney U tests were reported as median and IQR. All analyses were performed with a statistical program.<sup>l</sup> Values were considered significant at \( P < 0.05 \).

**Results**

The AKD group consisted of 10 naked mole-rats (4 females and 6 males), and the ABM group consisted of 9 naked mole-rats (5 females and 4 males). One mole-rat was removed from the ABN group because an underlying disease was identified. Mean ± SD body weight was significantly \( (P = 0.011) \) higher for the males (60 ± 9.6 g) than the females (46.4 ± 11.2 g).

---

**Figure 1**—Box-and-whisker plots of anesthetic induction time for 10 naked mole-rats (*Heterocephalus glaber*) anesthetized with AKD and 9 naked mole-rats anesthetized with ABM. Each drug was injected IM separately; anesthetic induction time was defined as the interval from injection of the last drug to loss of the righting reflex. Each box represents the IQR, the horizontal line in each box represents the median, whiskers represent the highest and lowest values, and circles represent outliers. *Median value differs significantly \( (P = 0.019) \) from the median value for the ABM regimen.

**Figure 2**—Box-and-whisker plots of recovery time for 10 naked mole-rats anesthetized with AKD and 9 naked mole-rats anesthetized with ABM. Mole-rats were administered reversal agents 40 minutes after anesthetic induction. *Median value differs significantly \( (P = 0.030) \) from the median value for the ABM regimen. See Figure 1 for remainder of key.
Anesthetic induction was rapid for both groups, but anesthetic induction time was significantly ($P = 0.019$) greater for the ABM group (median, 240 seconds; IQR, 150 seconds) than the AKD group (median, 140 seconds; IQR, 52.5 seconds; Figure 1).

Recovery time was significantly greater ($P = 0.030$) for the ABM group (median, 26 minutes; IQR, 16 minutes) than the AKD group (median, 11.5 minutes; IQR, 5.5 minutes; Figure 2). There was no significant difference between sexes in induction or recovery times for both drug combinations. None of the naked mole-rats twitched or vocalized through-out the procedures (induction, anesthesia, and recovery). All mole-rats recovered well from anesthesia. They began eating and were transported back to the group holding facility within 2 to 3 hours after administration of the reversal agents.

Reflexes were measured during the anesthetic period (Table 1). In the AKD group, all 4 reflexes were absent from 10 to 40 minutes after anesthetic induction, whereas there was a more variable individual response for the ABM group that diminished with time.

Vital signs were measured. Heart rate did not differ significantly ($P = 0.71$) between treatment groups (Figure 3). However, heart rate decreased over time in both groups and was significantly ($P < 0.001$) lower than at baseline from 10 minutes after anesthetic induction through the end of the study. There was a significant ($P < 0.001$) time-by-group interaction effect for respiratory rate (Figure 4). The ABM combination resulted in a significantly ($P = 0.009$) lower respiratory rate than the AKD combination from time of induction through 10 minutes after anesthetic induction; however, respiratory rates did not differ significantly ($P = 0.18$) between the 2 drug combinations from 15 minutes after anesthetic induction until the end of the study. For the ABM group, the respiratory rate did not change significantly ($P = 0.069$) over time, compared with the respiratory rate at time of induction. In contrast, respiratory rate for the AKD group was significantly ($P < 0.001$) lower, compared with the respiratory rate at time of induction, from 10 minutes after induction through the end of the study. Sex also had a significant effect on the respiratory rate; males had significantly ($P = 0.031$) higher respiratory rates (mean ± SD, 8 ± 3 breaths/min higher) than did females for both drug combinations. The $SpO_2$ did not differ significantly ($P = 0.99$) between drug combinations (Figure 5). However, $SpO_2$ increased over time for both drug combinations and was significantly ($P =$...
provide safe and effective immobilization but with differences in the anesthetic effects attributable to the drug regimen and sex of the mole-rats.

To the authors’ knowledge, the study described here was the first one conducted to evaluate the anesthetic effects of clinically appropriate injectable anesthetic regimens with AKD and ABM in naked mole-rats. In 1 study,10 naked mole-rats were anesthetized with tribromoethanol (30 mg/100 g), and buprenorphine (0.05 mg/kg) was administered as postoperative analgesia. In other studies,5,7 naked mole-rats anesthetized by IP administration of ketamine (80 mg/kg) and xylazine (16 mg/kg) or ketamine (100 mg/kg) and xylazine (2 mg/kg) recovered without complications. Naked mole-rats anesthetized with ketamine (15 to 20 mg/kg) and xylazine (0.6 to 1.0 mg/kg) in another study6 recovered from the experimental procedures, which suggested that lower doses of these drugs can provide anesthesia in this species. A similar dose of ketamine was used in the present study.

The drug regimens evaluated in the present study were designed to provide balanced and partially reversible anesthesia that can be used for prolonged or pain-inducing procedures that might require more than brief or deep sedation. The authors are not aware of any similar regimens that have been reported for use in naked mole-rats, but Damaraland mole-rats (Fukomys damarensis) have been anesthetized with a combination of ketamine (4 to 6 mg/kg), medetomidine (0.06 to 0.15 mg/kg), and buprenorphine (0.05 to 0.1 mg/kg), and atipamezole (0.3 to 0.7 mg/kg) was used to reverse the effects of medetomidine.11

Dexmedetomidine is an α2-adrenoceptor agonist that is reversible and provides analgesia, sedation, and muscle relaxation.13,29 Midazolam, a benzodiazepine, is also reversible and commonly used for sedation in rodents, with minimal adverse effects.13 Butorphanol is a κ-opioid receptor agonist that provides both analgesia and sedation and can be administered IM and SC.13,18,19,22,23 The addition of butorphanol, midazolam, dexmedetomidine, and butorphanol-dexmedetomidine to alfaxalone appears to potentiate sedation and anesthesia in rabbits, compared with the effects for alfaxalone administered alone.21 Administration of ABM to Beagles provides

**Discussion**

In the study reported here, naked mole-rats were anesthetized with a combination of AKD or ABM administered IM. Both drug combinations appeared to
excellent quality of induction of anesthesia with minimal cardiopulmonary effects; for this reason, ABM was evaluated in the present study. Both drug combinations used in the study reported here appeared to provide improvements in anesthesia, compared with results of preliminary experiments conducted with higher doses of alfaxalone alone.

Achieving and maintaining a consistent plane of immobilization can be challenging when drugs are not administered IV. The IM route of administration was chosen for the present study because it generally allows for uniform and rapid drug absorption. The doses used in the present study were determined on the basis of the lowest effective and practical drug volume used in preliminary experiments. Selection of the doses of the various anesthetic drugs was challenging, given the low body weights of the naked mole-rats and the relatively minute volumes for injection. To ensure accurate drug delivery, each of the 3 drugs in each combination was administered IM, rather than combining all 3 drugs in 1 syringe and administration at a single site, which could have led to greater tissue trauma from the larger volume and pain in these small animals, as has been reported for chinchillas. Dilution of the drugs was considered but was not performed because of the concern about increased soft tissue trauma from a larger injectate volume. Because IP administration of alfaxalone has resulted in inconsistent anesthetic responses and potentially dangerous responses in other rodent species, this route of administration was not used in the study reported here. Use of the SC route of administration may have helped address these concerns and can be considered in future studies. However, the SC route was not as effective, compared with effects after IM administration of alfaxalone and other drugs in chinchillas; thus, SC administration was not used in the present study. Furthermore, to reduce the volume injected into skeletal muscles, it is recommended that the dose of alfaxalone administered IM should not exceed 5 mg/kg (0.05 mL) at each injection site. The drug formulation used in the study reported here (10 mg/mL) resulted in a dose and volume (2 mg/kg and 0.01 mL, respectively) that were below the recommended cutoff values.

The alfaxalone dose in the study reported here was relatively low, compared with doses used in studies of other rodent species. In laboratory mice, IP administration of alfaxalone (80 mg/kg) alone or with xylazine (10 mg/kg) resulted in similar mean induction times of 2.4 and 2.3 minutes, respectively. Also, administration of alfaxalone (100 mg/kg, IP or SC) alone resulted in extremely low anesthetic scores, whereas SC administration of a combination of alfaxalone (60 mg/kg), medetomidine (0.3 mg/kg), and butorphanol (5 mg/kg) was required to achieve a surgical plane of anesthesia. Mean ± SD durations of anesthesia for rats receiving alfaxalone at 2 and 5 mg/kg, IV, were only 10.4 ± 6.6 minutes and 12.6 ± 3.3 minutes, respectively. In guinea pigs, alfaxalone (5 mg/kg, SC) provided only mild sedation, whereas higher doses of alfaxalone (15 to 20 mg/kg) alone or in combination with dexmedetomidine and butorphanol resulted in a longer induction time (7 to 8 minutes), compared with that for naked mole-rats in the present study. The IM administration of a combination of alfaxalone (5 mg/kg) and butorphanol (0.5 mg/kg) to chinchillas resulted in rapid induction (median, 2 minutes; range, 1 to 4 minutes) of short-term anesthesia that was inconsistent in depth and duration. Synergistic effects between alfaxalone and midazolam have been reported for dogs, cats, and rabbits. It appears that the addition of butorphanol and dexmedetomidine in the study reported here resulted in more balanced anesthesia of longer duration, which is similar to results described for Beagles, cats, and rabbits. It also was possible that the effectiveness of the low dose of alfaxalone administered in combination with other drugs to the naked mole-rats of the present study could be attributed to the physiologic adaptations of these subterranean rodents, including a lower metabolic rate. This emphasizes the need for establishing species-specific anesthesia regimens.

The induction time for both drug combinations in the present study was less than the mean induction time for Ansell mole-rats (4 minutes) and giant mole-rats (9 minutes) anesthetized with ketamine-xylazine. However, the dose of ketamine (20 mg/kg) used in the present study was higher than the dose (6 mg/kg) used for the other 2 mole-rat species. Differences in the induction times among mole-rats may be related to dose, drug, and species, and direct comparisons without use of the same anesthetic procedures for all mole-rat species may not be valid. Shorter induction times were detected for rabbits after the addition of midazolam, dexmedetomidine, and butorphanol-dexmedetomidine to alfaxalone, which is similar to results for the naked mole-rats of the present study.

Compared with results for the ABM group, mole-rats in the AKD group had a significantly shorter and more consistent recovery after administration of the reversal agent. Recovery times for the ABM and AKD groups were similar to those for chinchillas anesthetized with a combination of alfaxalone-butorphanol or dexmedetomidine-ketamine, respectively. Because a shorter recovery time is generally desired, especially in field settings, the AKD combination appeared to be advantageous, compared with the ABM combination. Lateral recumbency (or loss of the righting reflex) is considered the delineation between sedation and anesthesia in laboratory rodents because it suggests loss of consciousness. In the study reported here, all naked mole-rats in the AKD and ABM groups had loss of the righting reflex between the time of induction and 45 and 35 minutes after induction, respectively. This suggested that both combinations can provide anesthetic immobolization for this range of time in

1094

AJVR • Vol 80 • No. 12 • December 2019
this species. Because reversal agents were administered 40 minutes after induction, it was impossible to determine the exact duration of anesthesia that could potentially be provided by both drug combinations. Some of the mole-rats in the ABM group started to regain some reflexes before the administration of flumazenil, which suggested that this drug combination may not provide anesthesia for a prolonged period. Future studies that involve administration of these anesthesia regimens to naked mole-rats could be designed to allow the animals to spontaneously recover from anesthesia and thus reveal the full anesthetic duration of these drug combinations.

The AKD combination provided a consistent surgical plane of anesthesia, as indicated by the loss of all monitored reflexes between 10 and 40 minutes after induction in all the naked mole-rats in this group. However, although all mole-rats receiving ABM were anesthetized from the time of induction to 35 minutes after induction, only 3 of 9 mole-rats in this group had a surgical plane of anesthesia from 5 to 25 minutes after induction. Apart from the intragroup variability, it is important to mention that there were differences in the observed withdrawal responses between the forelimbs and hind limbs of the ABM group, which suggested that both the forelimbs and hind limbs should be used to determine the plane of anesthesia before application of invasive or pain-inducing procedures.

In the present study, heart rate decreased over time for both drug combinations, which is a generally expected deep anesthetic effect that has been observed in other species. Because alfaxalone was administered in combination with other drugs in the study reported here, it was challenging to determine which drug had the greatest effect on heart rate. For example, dexmedetomidine (an α2-adrenoceptor agonist) causes peripheral vasoconstriction because of its action on adrenergic receptors, which leads to increased peripheral vascular resistance and eventually a decrease in heart rate and cardiac output.

Administration of ABM resulted in a significantly lower respiratory rate than did administration of AKD in the naked mole-rats of the present study. Rabbits anesthetized with alfaxalone-butorphanol and alfaxalone-midazolam combinations also had a relative decrease in respiratory rate, compared with the respiratory rate for rabbits anesthetized with alfaxalone or dexmedetomidine alone, thus indicating the respiratory suppressive effect of these 2 drugs. Hyperthyroid cats sedated with alfaxalone-butorphanol (3 and 0.2 mg/kg, respectively, SC) also became bradypneic. In contrast, chinchillas anesthetized with alfaxalone-butorphanol had higher respiratory rates, compared with respiratory rates for chinchillas anesthetized with dexmedetomidine-ketamine. Because alfaxalone-related bradypnea is a dose- or species-dependent effect, it is likely that lower doses of butorphanol or midazolam in the ABM combination will result in less respiratory suppression but may also reduce the quality and duration of anesthesia for the ABM combination, which should be considered when administering ABM to naked mole-rats.

Respiratory rate did not change over time for the ABM group, whereas it significantly decreased for the AKD group, compared with the baseline value. However, this had no effect on the SpO2 because the SpO2 increased over time for both groups throughout the remainder of the study. Several injectable anesthetic regimens that include α2-adrenoceptor agonists reportedly cause respiratory depression, hypoxemia, and hypercapnia in small animals and rodent species. In the present study, oxygen saturation was measured by the use of pulse oximetry, which can result in inaccurate measurements with decreased tissue perfusion, especially in cases of vasoconstriction. Also, hemoglobin of naked mole-rats has a higher affinity for oxygen, compared with that of humans. Because pulse oximeters are designed on the basis of the oxygenation curve of humans, naked mole-rats comparatively can have a left-ward shift in the oxygenation curve, which would suggest that the pulse oximetry measurements of naked mole-rats could possibly overestimate blood oxygenation. Therefore, interpretation of SpO2 without corresponding arterial Po2 in naked mole-rats is difficult, and results of the study reported here should be considered as patterns rather than absolute measurements. Further studies involving blood gas measurements will be required to determine whether the observed decrease in respiratory rate is associated with hypoxemia and to better correlate SpO2 and blood oxygenation.

In the present study, males had significantly higher respiratory rates than did females. Laboratory mice (C57BL/6) have significant sex-associated differences in anesthetic sensitivity, with males requiring higher doses of alfaxalone (80 to 120 mg/kg) than females (40 to 80 mg/kg) when administered in combination with xylazine (10 mg/kg) to achieve a surgical plane of anesthesia. Because female naked mole-rats in the study reported here had lower body weights, it was possible that the small volumes of the administered injectable drugs could have led to subtle differences in accuracy of doses and greater anesthetic effects on the females. However, no sex-related differences were evident for the induction and recovery times or any other anesthetic variables. Rats anesthetized with isoflurane followed by IV administration of alfaxalone had sex-related differences in the pharmacokinetics and pharmacodynamics of the alfaxalone.

In that study, female rats had a lower arterial blood pressure, lower blood pH, and higher PaCO2, which suggests that consideration should be given regarding the dose of alfaxalone administered to male and female rats. A study that involved the administration of older alfaxalone formulations to rats revealed that males required 4 times the dose required by females.
to reach a surgical plane of anesthesia, which suggests that differences in sex hormones (mainly estrogen) may have an effect on alfaxalone, a neuroactive steroid that resembles progesterone. Analysis of results of a pharmacokinetics study of alfaxalone in rats suggested that differences were attributable to the various formulations of alfaxalone and assay methods rather than to actual anesthetic-response differences between male and female rats. However, a more recent study of rats given alfaxalone (25, 35, and 45 mg/kg) alone revealed that anesthesia time was 2- to 3-fold longer in females than in males, and males required a dose that was 3 times as high to achieve a similar duration of anesthesia. It is also possible that the small number of naked mole-rats of each sex could have affected the results reported for the present study. However, studies conducted to compare cardiovascular effects of various anesthetic drugs also used similar numbers of animals per treatment group.

Regardless of the drug combination that was administered, none of the naked mole-rats of the present study had any adverse behavioral responses associated with alfaxalone-induced anesthesia, although hypotension and hypoxemia could not be ruled out. Co-administration of several drugs for the same anesthetic combination can decrease the dose of the main agent (which was alfaxalone in the present study), thus minimizing its potential adverse effects. Mice anesthetized with alfaxalone alone or a combination of alfaxalone-xylazine displayed twitching (popcorn-like jumping), face scratching, hyperresponsiveness to noises or touching, and limb jerking during recovery. Rats anesthetized with alfaxalone had apnea that lasted for 95 to 170 seconds (2 of 5 rats receiving alfaxalone at 2 mg/kg, IV, and 4 of 5 rats receiving alfaxalone at 5 mg/kg, IV), and several of the rats displayed facial twitching during induction and recovery, regardless of the route (IP or IV) or administered dose. Guinea pigs anesthetized with alfaxalone alone or in combination with dexmedetomidine or buprenorphine had dose-dependent respiratory depression and twitching or bruxism when receiving higher doses. Tremors, twitching, and rolling were observed in most of the chinchillas anesthetized with alfaxalone (5 mg/kg) and butorphanol (0.5 mg/kg) but none of the chinchillas anesthetized with dexmedetomidine-ketamine. Dogs anesthetized with alfaxalone alone had adverse responses, such as apnea, tachypnea, hypotension, hypoxia, and excitement. In another study, dogs anesthetized with alfaxalone combined with butorphanol or medetomidine displayed excitement, paddling, twitching, apnea, and cyanosis. However, Beagles anesthetized with ABM had a good quality of anesthesia, which was similar to results for naked mole-rats anesthetized with ABM in the present study. It is likely that many of the adverse responses are dose-dependent effects or related to species-sensitivity phenomena, so direct comparisons among the various species may not be applicable.

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 6 rabbits, 7 cats, 8 and 10 African mole-rats, and 8 chinchillas. Performing a complete crossover study, where each mole-rat would have received both 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 naked mole-rat be anesthetized twice, thus placing them at greater anesthetic risk. To determine the full anesthetic effects of drug combinations used in this study, the naked mole-rats were allowed to breathe room air spontaneously without supplemental oxygen and active thermal support in the heated examination room. Although all the naked mole-rats made a full recovery, perhaps because of their ability to survive relative hypoxemia and hypercapnia, supplemental oxygen and thermal support are strongly recommended for all animals during general anesthesia. Hematologic variables, including venous blood gas concentrations, were not measured, and measurement of these concentrations may be worthwhile in future studies to enable investigators to identify acid-base disorders and hypoventilation. Additional studies to compare the safety and efficacy of inhalation anesthetics (eg, isoflurane or sevoflurane) and the injectable combinations of AKD and ABM could also prove useful.

Both injectable drug combinations evaluated in the present study appeared to provide safe and effective anesthesia of naked mole-rats. They induced a stable plane of surgical anesthesia and satisfactory immobilization for AKD and ABM of 40 and 35 minutes after induction, respectively. Anesthetic differences between the 2 drug combinations and between males and females should be taken into consideration before administration of these injectable agents to naked mole-rats.

**Acknowledgments**

Supported by an internal research grant (MCAT) from the College of Veterinary Medicine at Kansas State University.

The authors declare that there were no conflicts of interest.

The authors thank Elizabeth Loos, Carolyn Mark, Kallie Woodruff, and Sarah Ostrom for technical assistance.

**Footnotes**


b. Alfaxan, Jurox, Kansas City, Mo.

c. Ketaset, Hospira, Lake Forest, Ill.

d. Dexdomitor, Orion Corp, Espoo, Finland.

e. Torbugesic-SA, Zoetis, Florham Park, NJ.

f. Midazolam, Hospira, Lake Forest, Ill.

g. BD Ultra-Fine II, Becton, Dickinson and Co, Franklin Lakes, NJ.

Chinchilla lanigera
Cynomys ludovicianus

Heterocephalus glaber

Cavia porcellus

Mus musculus


AJVR • Vol 80 • No. 12 • December 2019 1097


39. White KL, Paine S, Harris J. A clinical evaluation of the pharmacokinetics and pharmacodynamics of intravenous alfalfalone in cyclopedrinx in male and female rats following a


