Anesthetic effects of alfaxalone-ketamine-midazolam and alfaxalone-ketamine-dexmedetomidine administered intramuscularly in black-tailed prairie dogs (Cynomys ludovicianus)

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OBJECTIVE
To evaluate and compare the anesthetic effects of alfaxalone-ketamine-midazolam (AKM) and alfaxalone-ketamine-dexmedetomidine (AKD) in black-tailed prairie dogs (Cynomys ludovicianus).

ANIMALS
9 male black-tailed prairie dogs.

PROCEDURES
Prairie dogs were anesthetized with AKM (6 mg/kg alfaxalone, 30 mg/kg ketamine, and 1.5 mg/kg midazolam) and AKD (6 mg/kg alfaxalone, 30 mg/kg ketamine, and 0.15 mg/kg dexmedetomidine) in a prospective, complete cross-over study. Atipamezole (1.5 mg/kg) after AKD or flumazenil (0.1 mg/kg) after AKM was administered 45 minutes after induction of anesthesia. Onset of general anesthesia, physiologic parameters, depth of anesthesia, and time to recovery after reversal administration were evaluated for each treatment.

RESULTS
Both AKM and AKD produced a deep plane of anesthesia in black-tailed prairie dogs that varied in duration. The median induction times for AKM and AKD were 82 and 60 seconds, respectively. The median recovery times for AKM and AKD were 27 and 21 minutes, respectively. There were no significant differences between protocols for induction (P = .37) and recovery (P = .51) times. All measured reflexes were absent in all animals at 5 minutes post-induction, with hindlimb reflexes returning prior to forelimb reflexes. Heart rate was lower but respiratory rate was higher in the AKD treatment. Body temperature decreased significantly for both protocols (P < .001) and was significantly lower with AKM than AKD (P < .001).

CLINICAL RELEVANCE
Both AKM and AKD produced a deep plane of anesthesia in black-tailed prairie dogs. For both protocols, heat support and oxygen support are indicated.

Black-tailed prairie dogs (Cynomys ludovicianus) are rodents of the family Sciuridae native to grasslands and prairies of North America and are commonly kept in zoological collections or as companion animals. Handling of black-tailed prairie dogs can be difficult due to their fractious nature, so general anesthesia is often required to perform a thorough examination or any other required procedures. Inhalant anesthesia has been previously evaluated in black-tailed prairie dogs and can produce a stable plane of general anesthesia. While gas anesthesia can provide a stable plane of anesthesia after induction, the use of chamber induction creates risk of waste gas exposure to veterinary personnel, has been associated with a greater odds ratio of anesthetic death in sick dogs and cats, and has been shown to cause greater cardiopulmonary depression than intravenous induction agents in dogs.

The safety and efficacy of injectable anesthetic protocols have only been studied in a limited capacity in black-tailed prairie dogs. A xylazine-ketamine combination was previously evaluated, but resulted in a 3.2% mortality rate, and dexmedetomidine-ketamine-midazolam was previously evaluated and resulted in an unreliable plane of anesthesia. An ideal injectable protocol to produce reliable, deep anesthesia in black-tailed prairie dogs has not been identified to date, and dosing information for alternative protocols must be extrapolated from other rodent species.
Alfaxalone (3α-hydroxy-5α-pregnane-11, 20-dione) is an induction anesthetic labeled for intravenous use in dogs and cats. It has been increasingly investigated as an injectable anesthetic option in a variety of rodent species, particularly via subcutaneous, intramuscular, or intraperitoneal routes.12–22 Alfaxalone is a neuroactive steroid that produces nonreversible, nonanalgesic anesthesia and muscle relaxation through interaction with the gamma-aminobutyric acid (GABA) receptors within the CNS.22 When used in guinea pigs (Cavia porcellus), rats (Rattus norvegicus), and mice (Mus musculus), alfaxalone as a single agent intraperitoneally, subcutaneously, or intramuscularly, it produces inconsistent planes of anesthesia, generally not reaching a surgical plane of anesthesia regardless of dosage.16–20 The combination of alfaxalone with a dissociative agent or alpha-2 agonist has been documented to provide a deeper, more consistent plane of anesthesia in the rodent species evaluated thus far.12–16,20,21 Injectable anesthetic protocols that include alfaxalone have not been evaluated in black-tailed prairie dogs previously.

The current study evaluated protocols that combined alfaxalone with ketamine and either dexmedetomidine or midazolam. Ketamine is a centrally acting N-methyl-D-aspartate receptor antagonist that is often used for short-term anesthesia in veterinary medicine, and its perioperative analgesic and antidepressive effects have been increasingly recognized in human medicine.23,24 Dexmedetomidine is a reversible alpha-2 agonist often used in veterinary medicine to provide reversible sedation and analgesia in various species, including dogs, cats, and rats, with known cardiovascular effects including bradycardia and initial hypertension and then hypotension.25–28 Administration of atipamezole has been shown to reverse the cardiovascular, sedative, and analgesic effects of dexmedetomidine.26,27 Midazolam is a benzodiazepine commonly used in veterinary medicine for its sedative, anticonvulsant, and muscle-relaxant effects.29 In contrast to dexmedetomidine, midazolam has not been documented to provide analgesic effects and does not produce the same undesirable cardiovascular effects as dexmedetomidine.28–30 The sedative effects of midazolam can be reversed using flumazenil.31

The objective of the current study was to determine the anesthetic effects of two alfaxalone-based, partially reversible injectable anesthesia protocols (alfaxalone-ketamine-midazolam [AKM] and alfaxalone-ketamine-dexmedetomidine [AKD]) in black-tailed prairie dogs. We hypothesized that both protocols would produce a moderate to deep plane of anesthesia for the duration of the anesthetic period and that all prairie dogs would fully recover from anesthesia after administration of reversal agents. The age of all study animals was approximately 6 months, estimated based on timing of emergence from burrows. Mean ± SD body weight on the first day of testing was 622.6 ± 63.6 g and on the second day of testing was 645.8 ± 57.6 g. The prairie dogs were housed in a 15 X 10-ft enclosure, where hay substrate, PVC pipes, and plastic crates were provided. Diet consisted of a commercial rodent pelleted diet, as well as fresh lettuce and other vegetables. One week prior to the first day of testing for the study, each prairie dog was immobilized with varying combinations of intramuscular alfaxalone, ketamine, midazolam, or dexmedetomidine and supplemental isoflurane as needed to facilitate physical examination. Physical examination of each animal did not reveal any abnormalities aside from fractured incisors in several of the study animals. Subsequent examination of each animal showed appropriate regrowth of incisors. Each animal received an identification microchip implanted subcutaneously during the initial examination. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Kansas State University (No. 4594) and the participating zoo.

Experimental design and protocol
All study animals were anesthetized twice in a randomized crossover design with a 16-day washout period between anesthetic events. An automated randomizer (randomization.com) was used to assign anesthetic protocols to each animal. The first anesthetic protocol, AKM, included intramuscular administration of 6 mg/kg alfaxalone (Alfaxan; Jurox), 30 mg/kg ketamine (Ketamine; VetOne), and 1.5 mg/kg midazolam (Versed; Westward). The second anesthetic protocol included intramuscular administration of 6 mg/kg alfaxalone, 30 mg/kg ketamine, and 0.15 mg/kg dexmedetomidine (Dexmedetomidine Hydrochloride; Akorn Inc). Just prior to administration of anesthetic drugs, each subject was weighed on a gram scale to ensure accurate dosing. Each anesthetic drug was injected separately into the epaxial musculature using an insulin syringe with a 27-g needle. Induction time, physiologic parameters, and recovery time were monitored for each study animal after anesthetic injection. The monitoring protocol used in this study was adapted from a prior study evaluating isoflurane and a dexmedetomidine-ketamine-midazolam combination in black-tailed prairie dogs.17 The investigator performing the monitoring of the animals was blind to the administered treatment.

Induction time was defined as the time elapsed from the last anesthetic injection to loss of righting reflex. At 45 minutes (T45) postanesthetic induction, 1.5 mg/kg atipamezole (Antisedan; Orion Corp), which resulted in a volume equal to the dexmedetomidine volume, was given intramuscularly into the lumbar epaxial muscles of the animals receiving AKD, and 0.1 mg/kg flumazenil (Flumazenil; Hikma Pharmaceutic) was given intramuscularly into the lumbar epaxial muscles of the animals receiving AKM. If animals were not starting to hold their head up at

Materials and Methods

Animals
Nine juvenile intact male zoo-kept (Sunset Zoo, Manhattan, KS) black-tailed prairie dogs were included in the crossover design of this study. The

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20 minutes postreversal injection, a second reversal injection was given into the lumbar epaxial muscles. All animals received a 35-ml subcutaneous fluid bolus cranially between the shoulder blades using lactated Ringer’s solution immediately before reversal administration. Study animals were placed in a plastic pet carrier once they were able to hold their head up and were monitored until sitting sternal. Heat support was provided after animals were placed in plastic pet carriers with a forced-air warming system (Bair Hugger; Augustine Medical, Eden Prairie, MN). Recovery time was measured for each animal and was defined as the time elapsed from first reversal injection to time when the animal regained the righting reflex and was able to sit or stand sternal.

Throughout the 45-minute anesthetic event, palpebral, forelimb withdrawal, and hindlimb withdrawal reflexes as well as jaw tone were monitored every 5 minutes. Reflexes were scored as 0 (reflex present), 1 (reflex reduced), or 2 (reflex absent). Withdrawal was assessed by using Brown-Adson forceps to pinch a digit and observe for a withdrawal of the limb. When loss of all monitored reflexes was achieved, animals were considered to be under a surgical plane of anesthesia. Physiologic parameters measured every 5 minutes included heart rate (HR), respiratory rate (RR), oxygen saturation (SpO₂), and rectal temperature. The HR was monitored via Doppler probe (Model 811-B Doppler flow detector; Parks Medical Electronics, Las Vegas, NV) placed beneath the sternum of each animal. The RR was measured via direct visualization of chest movement during breaths. Three commercial monitoring systems (UTECH UT100VC VET Vital Signs monitor, Utech Co, Ltd; Nonin PalmSat 2500, Nonin Medical; Masimo Rad-5v Handheld Pulse Oximeter, Masimo) were used in rotation to measure pulse oximetry placed on the footpads of the forelimbs or hindlimbs. All three monitors intermittently stopped producing readings while placed on the animals, so the specific monitor and the placement location varied between time points depending on whether a reading could be obtained. A reading was recorded if the pulse oximeter produced a SpO₂ value with a concurrent heart rate that was similar to the heart rate measured via Doppler probe. Rectal temperature was monitored with a digital thermometer.

**Statistical analysis**

The different outcome variables (HR, RR, temperature, SpO₂) were assessed over time using linear mixed models with time, treatment, and interaction as fixed effects and individual prairie dogs as the random effect. Time was treated as a factor to compare individual time points. An ANOVA was performed on the fixed effects. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were also performed on the residuals for normality assessment. Post hoc analysis was performed with a Turkey adjustment. The ordinal categorical variables (all reflexes) were only reported descriptively.

Differences between the two protocols for induction and recovery time were assessed using Wilcoxon signed-rank tests. The R foundation for statistical computing (Vienna, Austria; http://www.R-project.org/) was used for statistical analysis with an alpha of 0.05 for statistical significance.

**Results**

Both investigated drug protocols resulted in a deep plane of anesthesia that varied in duration, which was reversed successfully, resulting in no mortality. The median anesthetic induction times for AKM and AKD were 82 seconds (IQR, 34 seconds; range, 233 seconds) and 60 seconds (IQR, 42 seconds; range, 180 seconds), respectively (Figure 1). The median recovery times for AKM and AKD were 27 minutes (IQR, 5 minutes; range, 23 minutes) and 21 minutes (IQR, 8 minutes; range, 47 minutes), respectively. There were no significant differences between protocols for induction time ($P = .37$) and recovery time ($P = .51$). Five of the 9 animals receiving AKM and 3 of the 9 animals receiving AKD required a second reversal injection. All study animals recovered well from anesthesia and were placed back into their group enclosure within 1 to 3 hours after injection of the reversal agents. Within 24 hours of each anesthetic
Heart rate differed significantly with weight \((P = .001)\), increasing by mean ± SEM of 3.4 ± 1.0 beats/min/10 g. On post hoc analysis, AKM resulted in significantly higher heart rate than AKD at all time points (all \(P < .001\)). In animals anesthetized with AKD, heart rate was found to be significantly lower than at T5 only at T45 \((P = .034)\). No difference over time compared to baseline was seen in animals anesthetized with AKM.

Respiratory rate was significantly different between protocols over time (significant treatment X time interaction effect, \(P = .018\)) (Figure 2). The respiratory rate decreased with weight by mean ± SEM of 2.0 ± 0.8 breaths/min/10 g. On post hoc analysis, AKM resulted in significantly higher respiratory rate than AKD at T1 (mean difference of 21 breaths/min, \(P = .029\)) and significantly lower respiratory rate than AKD at T40 (mean difference of 24 breath/min, \(P = .014\)) and T45 (mean difference of 26 breaths/min, \(P = .008\)). In animals anesthetized with AKD, the respiratory rate was stable throughout the anesthesia (all \(P > .05\)). In animals anesthetized with AKM, the respiratory rate decreased over time when compared to baseline, which became significant at T40 (mean decrease of 34, \(P = .018\)) and T45 (mean decrease of 39, \(P = .003\)).

The SpO\(_2\) did not significantly change over time and between protocols (all \(P > .05\)) (Figure 3) but decreased slightly with weight by 1.3 ± 0.3% per 10 g of body weight \((P < .001)\). The pulse oximetry readings obtained throughout the monitoring period were highly variable with readings ranging from 7% to 99%, and readings could not be obtained for 9 of the 180 total time points across the study.

Table 1—Number of black-tailed prairie dogs (Cynomys ludovicianus) anesthetized with alfaxalone-ketamine-midazolam (AKM) or alfaxalone-ketamine-dexmedetomidine (AKD) in which evaluated reflexes were absent at measured time points after anesthetic injection.

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<th>Time (min)</th>
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Baseline indicates the time of anesthetic induction, defined by the loss of the righting reflex. Reflexes were evaluated every 5 minutes after anesthetic induction until administration of reversal agents at 45 minutes postinduction.

Figure 3—Mean ± SEM respiratory rate of 9 black-tailed prairie dogs anesthetized with alfaxalone-ketamine-midazolam (AKM; dashed line) and alfaxalone-ketamine-dexmedetomidine (AKD; solid line) over time. Time indicates time elapsed from anesthetic induction (loss of righting reflex). *Values differed significantly between protocols (\(P < .05\)).
In animals anesthetized with AKM, temperature was found to be significantly lower than baseline at T35 ($P = .022$), T40 ($P = .007$), and T45 ($P = .001$).

**Discussion**

In the current study reported, 9 black-tailed prairie dogs were anesthetized with AKM and AKD administered intramuscularly in a complete cross-over design. Both protocols appeared to produce effective anesthesia in the tested animals with no mortality.

The induction times for both studied protocols were rapid and smooth with no obvious adverse events. The smooth induction quality observed in this study is similar to what has been observed in naked mole rats (*Heterocephalus glaber*) and five-striped palm squirrels (*Funambulus pennantii*) receiving various combinations of alfaxalone with ketamine, dexmedetomidine, butorphanol, or midazolam. In contrast, chinchillas (*Chinchilla lanigera*) anesthetized with alfaxalone-butorphanol experienced rolling, twitching, and tremors throughout induction of anesthesia, mice given alfaxalone or alfaxalone-xylazine experienced erratic jumping and limb jerking during induction, and rats frequently twitched during recovery when given alfaxalone intraperitoneally. This could perhaps be attributed to the use of significantly higher alfaxalone doses in rats and mice; however, the chinchillas in the aforementioned study received comparable alfaxalone doses to the dose evaluated in this study. The use of ketamine and either midazolam or dexmedetomidine with alfaxalone appears to ameliorate the undesirable induction qualities previously observed in mice, rats, and chinchillas.

The recovery quality for both studied protocols was smooth with no obvious adverse events, similar to the observed recovery quality of naked mole rats after receiving alfaxalone based anesthetic protocols. Prior studies evaluating alfaxalone in rats and mice or alfaxalone combined with xylazine in mice observed erratic jumping behavior, intense facial scratching, limb jerking, and facial twitching during the recovery period. Five-striped palm squirrels experienced twitching, tail lifting, and vocalization throughout recovery after receiving...
alfaxalone-ketamine, but no adverse responses when receiving alfaxalone-ketamine-dexmedetomidine or alfaxalone-butorphanol-midazolam. Our results further support that recovery quality is variable in rodents receiving alfaxalone, which can be either species or dosage specific.

Although recovery was smooth in the current study, nearly half of the animals in this study required second reversal injections at 20 minutes after the initial injection to expedite their recovery. Despite this, the median recovery times reported in the current study are shorter than the previously reported median recovery times for black-tailed prairie dogs anesthetized with dexmedetomidine-ketamine-midazolam. In the prior study, anesthesia induced hypothermia was cited as a possible contributing factor to the prolonged recoveries. Prolonged anesthetic recovery was also noted in Five-striped palm squirrels when given AKM and AKD, and all became markedly hypothermic throughout the duration of anesthesia.

Furthermore, when alfaxalone-butorphanol was evaluated in chinchillas, all but one chinchilla in the current study. In humans, dexmedetomidine has established normal physiologic values (40 to 60 breaths/minute).

Although it is not generally accepted as an indication of a surgical plane of anesthesia, the use of AKD may need to be limited to brief procedures. In contrast, AKM maintained a surgical plane of anesthesia for 20 minutes, after which 4 of 9 animals gradually regained hindlimb and forelimb withdrawal, jaw tone, and palpebral reflexes. Eight of 9 animals in the AKM group maintained a surgical plane of anesthesia until reversal administration at 45 minutes postinduction. All animals in both groups maintained loss of righting reflex until reversal injections were administered. It appears that AKD reliably produces a brief surgical plane of anesthesia that gradually progresses to a light to moderate plane of anesthesia. Unless additional gas anesthetics are used to maintain anesthetic depth, the use of AKD may need to be limited to brief procedures. In contrast, AKM maintained a surgical plane of anesthesia for the duration of the anesthetic period in almost all animals and may be suitable for longer procedures. However, anesthetic depth should be carefully evaluated as prolonged deep anesthesia was not achieved in 1 of the 9 study animals. Regardless, if planning to perform painful, invasive procedures, the use of an analgesic in addition to AKM or AKD is recommended.

The current study found several differences in physiologic parameters both between protocols and over time for both protocols. AKD anesthesia resulted in significantly lower heart rates than AKM at all time points and a decrease in heart rate over time. This was an expected finding in this study based on the known effects of dexmedetomidine, an α2 adrenoceptor agonist. Bradycardia was also noted to a similar degree in black-tailed prairie dogs anesthetized with dexmedetomidine-ketamine-midazolam, which can likely be attributed to the administration of dexmedetomidine rather than midazolam based on the known effects of dexmedetomidine in other species and the comparative results of the current study. AKD and AKM anesthesia also differed in the effects on respiratory rate. Despite the comparable decrease in respiratory rate in the AKM group, mean respiratory rates for both protocols evaluated in the current study were never lower than previously published normal physiologic values (40 to 60 breaths/minute).

In addition to respiratory rate, the current study attempted to evaluate blood oxygen saturation using pulse oximetry. Statistical analysis of the recorded values showed no difference in SpO2 readings between animals receiving AKM and animals anesthetized with dexmedetomidine-ketamine-midazolam. Naked mole rats also showed a discrepancy between return of hindlimb and forelimb withdrawal after alfaxalone-ketamine anesthesia, but the order in which reflexes returned varied between animals. When assessing anesthetic depth in black-tailed prairie dogs, both the forelimb and hindlimb withdrawal should be evaluated separately.
receiving AKD. However, readings were highly variable throughout the anesthetic period, and it was often difficult to obtain reliable readings for all time points despite using 3 different brands of monitors. Variables such as poor perfusion or variations in venous pulsation due to the probe clamp being too tight can cause prevent a pulse oximeter from producing accurate readings.59 Hemodynamic parameters aside from heart rate were not monitored, so it is entirely possible that poor perfusion could have contributed to the variable pulse oximetry results recorded in this study. Additionally, the probes used in this study were not specially designed for rodent extremities, so it is possible that the pressure of the clamps led to variations in venous pulsations.

In addition to evaluating two different anesthetic protocols in black-tailed prairie dogs, the current study illustrates species specific difference in anesthetic dosing requirements. The alfaxalone dose in the current study was 6 mg/kg, which is much lower than the alfaxalone doses evaluated in rats, mice, and guinea pigs (20 to 80 mg/kg); was similar to the alfaxalone dose evaluated in five-striped palm squirrels (6 mg/kg); and was higher than the alfaxalone dose evaluated in naked mole rats (2 mg/kg).13,35–39 These discrepancies could in part be due to discrepancies in metabolic rates between species or different routes of administration. The current study used intramuscular administration and the prior studies using higher doses of alfaxalone in guinea pigs, rats, and mice used subcutaneous or intraperitoneal administration. These examples highlight the need for species specific dosing recommendations for anesthetic protocols for rodents.

There were several limitations within the current study. The study population consisted of all males, so differences between sex could not be accounted for. Previous studies17,21,22 evaluating alfaxalone in rats and mice have documented significant differences in sedative effects between males and females, with males requiring higher doses. In contrast, a study15 evaluating alfaxalone-ketamine-dexmedetomidine and alfaxalone-butorphanol-midazolam in naked mole rats did not find a significant difference in anesthetic induction, depth, or recovery between males and females. These findings show that it is difficult to predict how anesthetic effects will vary between male and female rodents, so future anesthetic studies in black-tailed prairie dogs should ideally include both female and male animals. The study population was also limited with a total of 9 animals, and although expanding the study population would give a more comprehensive observation of the effects of AKM and AKD on black-tailed prairie dogs, this number of subject animals was also used in other anesthesia studies in a variety of species.14,18,40–42

Additionally, the physiologic parameters measured in the current study could have been expanded for a more comprehensive evaluation of cardiovascular and respiratory effects of AKM and AKD. As noted above, the pulse oximetry readings obtained in the current study were difficult to interpret due to the degree of variability, so blood gas analysis could have provided a valuable measure of ventilation and oxygenation in addition to respiratory rate. Furthermore, the current study did not evaluate blood pressure throughout the anesthetic period. A previous study5 evaluating isoflurane and dexmedetomidine-ketamine-midazolam recorded indirect oscillometric blood pressure measurements but found that the measurements were not highly reliable. Since blood pressure can be an indirect indicator of cardiac output,43 measurement of blood pressure, ideally via direct arterial measurement, could be considered in addition to heart rate monitoring in future studies to gain a better understanding of the cardiovascular effects of anesthetic protocols in black-tailed prairie dogs.

In conclusion, both AKM and AKD produced a deep, surgical plane of anesthesia suitable to facilitate examination and brief clinical procedures provided that anesthetic depth is closely monitored. Heat and oxygen support are strongly recommended when using both AKM and AKD protocols.

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