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Research Article

# Comparison of three sedation protocols using midazolam and opioids in domestic ferrets (*Mustela putorius furo)*

Jessie Vandenbruggen [a], Barbara Ambros [a], Hugues Beaufrère [b], Isabelle Desprez [a,b,*]

[a] Small Animal Clinical Sciences Department, Western College of Veterinary Medicine at the University of Saskatchewan, Saskatoon, SK, Canada
[b] Department of Veterinary Medicine and Epidemiology, UC Davis School of Veterinary Medicine, Davis, CA, USA

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ABSTRACT

*Background:* Performing routine diagnostic tests in ferrets can be challenging without sedation, and intramuscular injections may be problematic due to their limited muscle mass. This study aimed to achieve moderate sedation levels using subcutaneously administered combinations of opioids and midazolam in ferrets. The effects, quality, and duration of sedation, as well as the cardiovascular and respiratory effects of midazolam in combination with three different opioids, were also evaluated.

*Methods:* Twelve healthy adult ferrets were used in a randomized, blinded, crossover study. Three sedation protocols were evaluated: MM group with midazolam (0.5 mg/kg SC) and methadone (0.3 mg/kg SC), MB group with midazolam (0.5 mg/kg SC) and butorphanol (0.2 mg/kg SC) and MH group with midazolam (0.5 mg/kg SC) and hydromorphone (0.2 mg/kg SC). Sedation scores and vital parameters were recorded every five minutes.

*Results:* All 12 ferrets lost their righting reflex in the MB group and 11 out of 12 in the MH group. In the MM group, only 1 out of 12 lost his righting reflex, and for only 2 minutes. The median sedation duration (between loss and return of righting reflex) was 33.5 minutes (4–66) in the MH group and 42 minutes (36–72) in the MB group. MB group had a significantly shorter time to sternal recumbency, lateral recumbency and loss of righting reflex compared to MH group. The heart rate was significantly lower with MB when compared to MH, and MB induced significantly lower respiratory rates compared to both MH and MM. Retching and vomiting were observed in 84% of ferrets in the MH group, 42% in the MM group and 17% in the MB group.

*Conclusions and clinical relevance:* Subcutaneous administration of midazolam at 0.5 mg/kg in combination with either hydromorphone at 0.2 mg/kg or butorphanol at 0.2 mg/kg achieved moderate sedation. The combination of midazolam and butorphanol used in this study showed fewer side effects and variability in sedation duration, which may make it a more appropriate choice in a clinical setting.

## Introduction

Domestic ferrets (*Mustela putorius furo)* are one of the most popular exotic pets [1]. While proper handling techniques can facilitate physical examination or routine diagnostic tests such as imaging (ultrasound, radiographs), blood and urine sampling, this may expose the staff to injury (e.g., bite wounds) or ionizing radiation, for example. Handling may also cause undue stress to ferrets, and sedation may help mitigating these issues. Inhalant anesthetics such as isoflurane and sevoflurane are commonly used to induce rapid anesthesia in ferrets and facilitate routine diagnostic procedures [2]. However, these agents are associated with adverse effects, including dose-dependent vasodilatory effects, negative inotropic effects, and respiratory depression [1,3–5]. Isoflurane administration has been associated with red blood cell sequestration, leading to a decrease in hematocrit from 43% to 38% in ferrets, which can be clinically misleading [5]. Moreover, the odor of inhalant anesthetics can induce stress in ferrets, potentially causing emesis and sialorrhea [1]. Risks of environmental contamination and staff exposure are also to be considered with mask or chamber induction. Injectable sedative drugs are a commonly used alternative to inhalant anesthesia in ferrets and have the advantage of being easily and quickly administered. They also avoid staff exposure or environmental contamination, and the stress associated with an injection is shorter compared with the duration of inhalant induction or the duration of manual restraint for procedures. Various drugs have been reported to be used for ferret sedation, including midazolam and opioids [1,6].

Midazolam, a water-soluble benzodiazepine and a gamma-aminobutyric acid receptor subtype A agonist, offers good quality, dose-dependent, sedation in ferrets, and it has the advantage of being reversible with flumazenil. Possible side effects reported in other species include minimal respiratory depression and a mild reduction of the heart rate. Midazolam can cause paradoxical behavior such as excitement in other

* Corresponding author.
*E-mail address:* isabelle.desprez@usask.ca (I. Desprez).

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species such as cats or dogs [7–11]. Recommended dosages for midazolam in ferrets range from 0.2 to 0.5 mg/kg intravenously (IV), intramuscularly (IM) or subcutaneously (SC) [1,12,13].

Various opioids have been reported in ferrets. While they all can be reversed using naloxone, it has been anecdotally noted that ferrets tend to be more sensitive to opioids in general compared to dogs and cats, exhibiting a greater respiratory depressant effect [14–16].

Hydromorphone, a water-soluble mu-receptor agonist opioid, is metabolized by the liver, is more potent than morphine and is used for moderate to severe pain. Adverse effects reported in dogs and cats [17] include nausea, emesis, respiratory depression, and sedation. Another study, however [18], suggested no detrimental clinical side effects in ferrets. Proposed dosages for ferrets range from 0.1 to 0.2 mg/kg IM [18]. However, recently published studies [19,20] indicate that a dosage of 0.2 mg/kg IV, or SC seems more appropriate.

Methadone, a synthetic mu-opioid receptor agonist has additional analgesic effects due to its antagonistic action on the N-methyl-D-aspartate (NDMA) receptor. In other species such as dogs, cats and humans, adverse effects include nausea and vomiting, although these seem less common than with morphine [21–24]. Respiratory depression and sedation are also reported in dogs, cats and guinea pigs [23,25,26]. However, there are currently no available studies on the use of methadone for sedation or analgesia in ferrets. Dosages found in textbooks for ferrets range from 0.2 to 0.5 mg/kg IV, IM or SC [1]. However, a recent pharmacokinetic study of methadone administered subcutaneously and intravenously showed that a dosage of 0.3 mg/kg SC or IV was insufficient to reach plasmatic concentration associated with analgesia in other species [27].

Butorphanol, a kappa receptor agonist and mu antagonist, has a short half-life and is commonly utilized for sedation in ferrets in combination with other drugs [1]. It also serves as an analgesic for mild to moderate pain. Compared to hydromorphone and methadone, butorphanol induces less respiratory depression in dogs and cats [28]. Dosages studied in ferrets range from 0.2 mg/kg [1,13,29,30] to 0.3 mg/kg IM or SC [1,18].

While IV, IM and SC routes are reported for midazolam and opioids, IV injection is often considered impractical in ferrets due to the difficulty of restraining them for IV injection, defeating the intent of lowering their stress levels. IM administration is the most used route for sedation in ferrets. However, because they have a proportionally smaller muscle mass compared with dogs and cats, the required volume for IM injections is proportionally larger. This may lead to having to administer multiple IM injections in various sites, which may increase stress levels and lower the ferret's tolerance [31]. Subcutaneous injection of preanesthetic drugs has been shown to be less painful than the IM route in dogs, with similar sedation scores reported [32]. Additionally, short-duration sedation has been achieved using SC injection of alfaxalone and butorphanol in hyperthyroid cats [33]. Subcutaneous drug administration has also been described in exotic animals. For instance, in African pygmy hedgehogs, due to the anatomical challenges of IM injection, sedative drugs can be administered SC [34,35]. In ferrets, SC injection of hydromorphone has been described as an alternative route of administration [19,20], while sedation with SC administration of alfaxalone has been shown to achieve moderate sedation, making SC administration of other sedative drugs interesting to investigate [31].

The objective of this study was to evaluate the sedative effect of subcutaneously administered combinations of opioids and midazolam. This study also evaluated the cardiovascular and respiratory effects of these drug combinations. The secondary objective was to describe the quality and duration of sedation and identify any side effects observed during the study.

The hypothesis was that all three sedation protocols would achieve a moderate level of sedation suitable for minimally invasive procedures such as blood collection, imaging, and urine collection. Furthermore, it was expected that the combination of midazolam with methadone and hydromorphone would cause more pronounced cardiorespiratory depression compared to midazolam-butorphanol. Anticipated side effects for all groups included emesis, retching, and lip licking, as these are commonly reported in other species [17,21,36,37].

## Materials and methods

This research project received approval from the University of Saskatchewan Animal Research Ethics Board (no. 20230007) and adhered to the Canadian Council guidelines for humane and animal use.

### *Animals and husbandry*

Twelve healthy ferrets (7 spayed females and 5 neutered males) were used for this study. Their ages ranged from 39 to 51 months, with weight spanning from 0.61 to 1.45 kilograms. Ferrets were acquired from a commercial breeder (Triple F farms, Gillet, PE, USA) and housed as two groups in one room separated into 2 pens. Each group was free roam in its side of the room and had access to a 5 feet-tall cat tree, hammocks, blankets and litterboxes. Various toys (e.g., tunnels, balls, foraging toys, digging bins) were provided and rotated every week. The temperature in the room was thermostat-controlled and maintained between 19 and 21° C, with a 12:12-h light cycle. The ferrets received a kibble diet (Mazuri Ferret Diet, Mazuri) ad libitum with fresh tap water provided ad libitum in ball-tipped water bottles and water dishes. Prior to the study, ferrets were determined to be healthy based on physical examination. Two hours prior to sedation, ferrets were placed into smaller stainless-steel holding cages (4 feet x 2.5 feet x 1.5 feet) for fasting purposes. For each ferret, body weight, heart rate, respiratory rate, and temperature were acquired immediately prior to sedation administration.

### *Sedation assessment and scoring*

Sedation was defined by the loss of the righting reflex and sedation duration was the time between the loss and return of the righting reflex. Sedation variables were recorded by a single, blinded observer (JV). A previously published sedation score was used (Table 1) [31], including body position, front limb withdrawal, jaw resistance and toe pinch. Time of drug administration, sternal recumbency, loss of righting reflex, first spontaneous movement, return of righting reflex, and standing/walking were recorded.

After drug administration, body posture and righting reflex were evaluated every 5 minutes. When righting reflex was lost, sedation score was assessed every 5 minutes until the return of righting reflex. Jaw resistance was assessed using one finger to gently open the mouth. Front limb withdrawal was performed by using gentle traction applied to one front leg. Finally, the toe pinch was assessed using a Doyen intestinal forceps, applied at one alternating digit per time point.

Quality of induction and recovery was evaluated, in addition to any side effects noticed during the observation period.

### *Study design and data collection*

This was a randomized, blinded, crossover study evaluating three SC sedation protocols: midazolam (0.5 mg/kg, Midazolam 5 mg/mL, Fresenius Kabi Canada Ltd, Toronto, ON, Canada) and methadone (0.3 mg/kg, Comfortan 10 mg/mL, Dechra, Eurovet Animal Heath B.V, Bladel, Netherlands) (MM group), midazolam 0.5 mg/kg with butorphanol (0.2 mg/kg, Torbugesic 10 mg/mL, Zoetis Canada Inc, Quebec, Canada) (MB group), midazolam 0.5 mg/kg with hydromorphone (0.2 mg/kg, Hydromorphone Hydrochloride Injection USP 50 mg/5 mL, Sandoz Canada Inc, Quebec, Canada) (MH group). Randomization of treatments was achieved through available software (http://www.randomizer.org/), and a minimum of 3-day washout period was maintained between each sedation protocol. Prior to the start of the study, all ferrets' body weight, heart rate, respiratory rate, and temperature were obtained. Each ferret received the sedative drugs mixed in a single syringe and using a 26-G needle. Drugs were administered SC in the

**Table 1**
Sedation Scoring System Used to Evaluate the Level of Sedation Following Subcutaneous Administration of Benzodiazepine in Combination of Opioid Drugs in Twelve Healthy Adult Ferrets [31]

| Criteria | Score Description |
|---|---|
| Body posture | 0: Ambulate normally |
| | 1: Mild to moderate ataxia, can stand and walk more than 5 feet |
| | 2: Recumbent (lateral and sternal). Can stand when manipulated. Walks less than 5 feet. Marked ataxia |
| | 3: Recumbent. Unable to stand |
| Righting reflex | 0: Impossible to place in dorsal recumbency |
| | 1: Can place in dorsal recumbency, but animal immediately rights itself |
| | 2: Can place in dorsal recumbency, but the animal continues to lift head (not body) |
| | 3: No resistance to being placed in dorsal recumbency and remains stationary |
| Jaw resistance test (ability to open mouth; tested using one finger to open lower jaw) | 0: Notable resistance, cannot open the mouth with one finger |
| | 1: Mild resistance, can open mouth with one finger |
| | 2: No resistance, can easily open the mouth with one finger |
| Reaction to front limb withdrawal (gentle traction applied to one front leg at a time) | 0: Immediate withdrawal |
| | 1: Mild delay in withdrawal |
| | 2: Marked delay in withdrawal |
| | 3: No reaction to withdrawal |
| Reaction to toe pinch (using hemostats attempting one digit per time point) | 0: Immediate withdrawal from application of hemostats |
| | 1: Mild delay in withdrawal, can apply hemostats for about 1–2 s |
| | 2: Marked delay in withdrawal, can apply hemostats for > 2 s |

interscapular area. After the injection, the ferret was placed in a stainless-steel cage (4 feet x 2.5 feet x 1.5 foot) and observed closely. Respiratory rate was recorded and dorsal recumbency attempted every 5 minutes until the ferret was successfully placed in dorsal recumbency. The ferret was then placed in dorsal recumbency on a stainless-steel surgical table on top of a warming blanket (HotDog Patient Warming System with medium sized blanket). Time of drug administration, sternal recumbency, and loss of righting reflex were recorded.

Once the ferret lost righting reflex, vital parameters were monitored every 5 minutes. Heart rate was evaluated using a stethoscope (Littmann Infant stethoscope) and respiratory rate by visual assessment. Rectal temperature was measured with a digital thermometer (Physio Logic 016-638 Accuflex Pro Digital Oral Thermometer). Noninvasive blood pressure (NIBP) (VET HDO High Definition Oscillometry) was monitored using an appropriate size cuff, (Midmark #SV2 4–8 cm cuff) placed at the base of the tail. Systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressure were recorded. Oxygen saturation ($SpO_2$) was determined using a pulse oximeter (Nonin Palmsat 2500 Series Handheld Pulse Oximeter) placed on the palmar aspect of the ferret forelimbs. If $SpO_2$ dropped below 90% for more than 1 minute, 1 L/min oxygen supplementation was initiated via a tight-fitting face mask (Surgivet anesthesia mask). If the ferret was not responsive to oxygen supplementation, intubation and manual ventilation were implemented. Time to first spontaneous movement, return to righting reflex and standing/walking were recorded.

*Reversal and recovery*

If the righting reflex had not returned 60 minutes after drugs administration, midazolam was reversed using flumazenil (Flumazenil 0.5 mg/5 mL, Sandoz Canada Inc, Québec, Canada) 100 µg/kg administered IM into the epaxial muscle. In case of an emergency, such as prolonged hypoxia that was unresponsive to oxygen administration, cardiopulmonary arrest, marked bradycardia, marked hypo- or hyperthermia, naloxone (Naloxone 4 mg/10 mL, Sandoz Canada Inc, Québec, Canada) was available. Once the righting reflex returned, measurements of rectal temperature, $SpO_2$ and blood pressure were discontinued. Ferrets were monitored until complete recovery (able to stand and walk normally) and returned to their room.

*Statistical analysis*

Univariate statistics on paired data (time-to-effect variables) were performed using paired t-test for normally distributed data with homogeneous variances or Wilcoxon-signed rank tests for nonparametric data. The 95% confidence interval of the mean difference or 95% confidence interval of the median difference was reported (larger dose−lower dose), respectively.

Normality was assessed using Shapiro-Wilk tests and quantile plots and homogeneity of variances were assessed using Levene's tests.

Repeated measure data (anesthesia monitoring variables) were analyzed using linear mixed models with the anesthetic variables as outcome variables (in separate models); treatment, time, and their interaction as fixed variables; and individual ferrets as the random variable. A compound symmetry covariance structure was used. Assumptions of linearity, normality, homoscedasticity of residuals and lack of outliers were assessed graphically on standardized residual plots. The presence of autocorrelation over time was also assessed in the models. When more than 2 treatments were included, an ANOVA was performed on the fixed effect, and post hoc testing was performed with a Tukey adjustment.

Categorical scoring data were evaluated similarly but using mixed ordinal logistic regression.

Model fit was assessed graphically. Proportional odds ratios (OR) were obtained by exponentiation of parameter estimates. Missing data were handled by listwise deletion.

R (version 4.2.0, 2022, R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. Plots were generated with ggplot2. An alpha of 0.05 was used for statistical significance.

## Results

No local adverse effects were noticed at the injection site in any of the ferrets. All twelve ferrets in the MB group achieved a moderate level of sedation, characterized by the loss of righting reflex for at least 10 minutes. In the MH group, eleven out of twelve ferrets (92%) achieved a moderate level of sedation. Only one out of twelve ferrets (8%) in the MM group lost their righting reflex, and for only 2 minutes. Consequently, the MM group was excluded from all statistical comparisons except for respiratory rate, for which there was enough data.

Median sedation duration was 42 minutes for the MB group and 33.5 minutes for the MH group. Sedation duration ranged from 36 to 72 minutes for the MB group and 4 to 66 minutes for the MH group (Table 2). The MB group resulted in significantly shorter time to sternal recumbency (Wilcoxon, $P = 0.018$, median difference: 5 minutes), time to lateral recumbency (Wilcoxon, $P = 0.018$, median difference: 5 minutes) and time to loss of righting reflex (Wilcoxon, $P = 0.009$,

**Table 2**
Time (Minutes, Median, and Range) from Injection to Sternal Recumbency, to Loss of Righting Reflex, Duration of Loss of Righting Reflex for Healthy Adult Ferrets ($n$ = 12) After the Subcutaneous Administration of Midazolam (0.5 mg/kg) - Butorphanol (0.3 mg/kg) (MB), Midazolam (0.5 mg/kg) - Hydromorphone (0.2 mg/kg) (MH) and Midazolam (0.5 mg/kg) - Methadone (0.2 mg/kg) (MM)

| Group | Midazolam-Butorphanol (MB) | | Midazolam-Hydromorphone (MH) | | Midazolam-Methadone (MM) | |
|---|---|---|---|---|---|---|
| Time to sternal recumbency | 6.45 | 4–10 | 10.25 | 6–14 | 26 | 16–26 |
| Time to loss of righting reflex | 8.09 | 5–11 | 13.08 | 8–23 | | N/A |
| Duration of loss of righting reflex | 42 | 36–72 | 33.5 | 4–66 | | N/A |

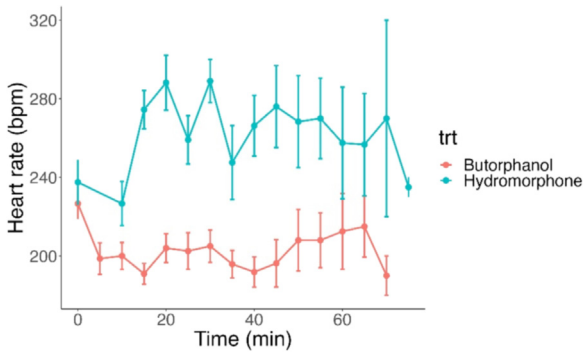


**Fig. 1.** Line plot of mean heart rate ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg. There was a significant difference between treatments for respiratory rate ($P < 0.001$) and time ($P < 0.001$).

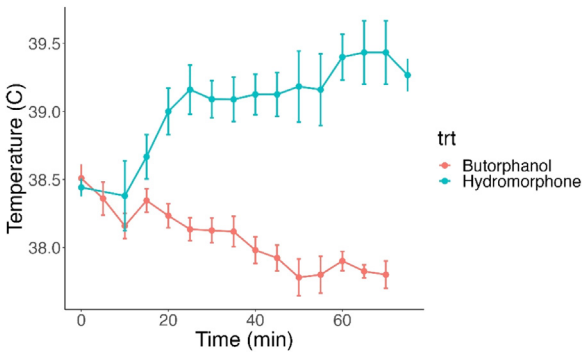


**Fig. 3.** Line plot of mean temperature ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg.

median difference: 6.5 minutes) than MH. The heart rate was significantly lower with MB when compared to MH (by 59 ± 4 bpm/min) (Fig. 1). For treatments, MB induced significantly lower respiratory rates than both MH (by 11 ± 3 bpm, $P < 0.001$) and MM (by 8 ± 3 bpm, $P = 0.02$) (Fig. 2). For times, the respiratory rate only significantly decreased 5 minutes after injections (all $P < 0.001$) but remained stable thereafter (all $P > 0.05$).

Body temperatures were generally higher with MH ($P < 0.001$). MH was associated with significantly increasing temperature overtime while MB was associated with significantly decreasing temperature over time ($P < 0.001$) (Fig. 3).

For NIBP, there was a significant time*treatment effect ($P < 0.05$ for SAP, MAP, and DAP). NIBP initially decreased in both treatments and was generally higher with MH and tended to increase over time after the initial decrease ($P = 0.003$ for SAP, $P = 0.001$ for MAP, and $P < 0.001$

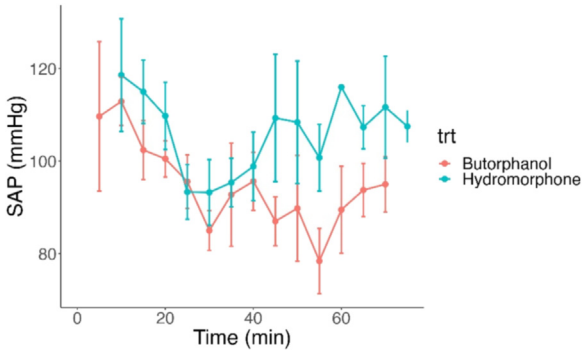


**Fig. 4.** Line plot of mean systolic arterial pressure ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg.

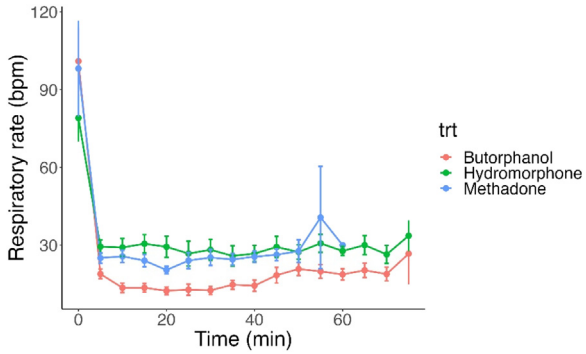


**Fig. 2.** Line plot of mean respiratory rate ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg, methadone 0.3 mg/kg or hydromorphone 0.2 mg/kg.

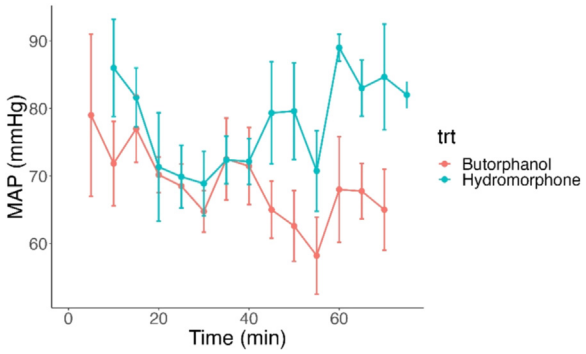


**Fig. 5.** Line plot of mean arterial pressure ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg.

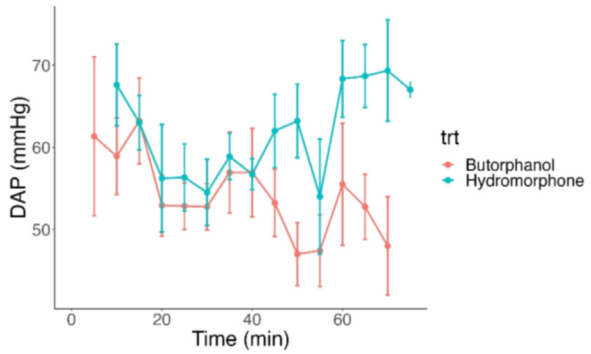


**Fig. 6.** Line plot of mean diastolic arterial pressure ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg.

for DAP) whereas it stayed at a lower level in the MB treatment (Figs. 4, 5, and 6).

Oxygen saturation significantly increased overtime in all groups ($P < 0.001$) (Fig. 7). Oxygen saturation dropped below 90% in nine out of twelve ferrets from the MB and MM groups and ten out of twelve ferrets from the MH group. Oxygen supplementation at a flow rate of 1 L/min via a tight-fitting mask increased the oxygen saturation above 90%. No ferrets required intubation or manual ventilation.

The MH treatment was associated with decreased odds of an increase in any sedation scores controlling for time and sex compared to the MB treatment: posture score (prop OR: 0.4/unit increase in score, 95% CI: 0.2–0.8, $P = 0.011$) and dorsal recumbency score (prop OR: 0.04/unit increase in score, 95% CI: 0.01–0.14, $P < 0.001$). MH resulted in increasing odds of a higher toe pinch score (prop OR: 35, 95% CI: 14–88, $P < 0.001$) and higher limb withdrawal scores (prop OR: 2.8, 95% CI: 1.4–5.5, $P = 0.002$).

No significant differences were detected between treatments for time to first movement (t-test, $P = 0.61$), return of righting reflex (t-test, $P = 0.37$), and time to stand/walk (t-test, $P = 0.59$). There was no effect of time ($P = 0.87$) or sex ($P = 0.70$) on heart rate. Respiratory rate between treatment groups MH and MM did not significantly differ ($P = 0.61$). For times, the respiratory rate only significantly decreased 5 minutes after injections (all $P < 0.001$) but remained stable thereafter (all $P > 0.05$). No significant time*treatment effect was observed for body temperature ($P > 0.05$). There was no significant difference between groups for $SpO_2$ ($P = 0.27$) and it significantly increased over time ($P < 0.001$). There was no effect of sex for any variable (all $P > 0.05$). There was no difference between treatments on jaw resistance score ($P = 0.99$).

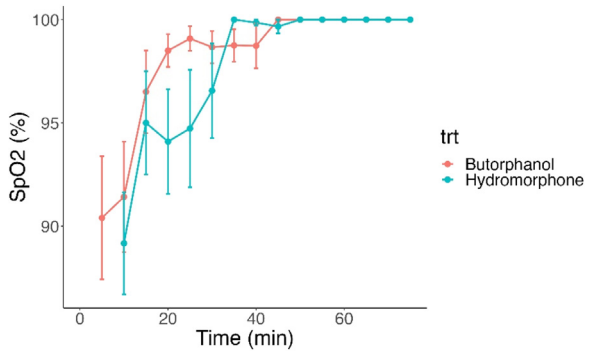


**Fig. 7.** Line plot of mean peripheral oxygen saturation (SpO2) ± SEM changes overtime in twelve healthy adult ferrets after administration of a single subcutaneous dose of a combination of midazolam 0.5 mg/kg with either butorphanol 0.2 mg/kg or hydromorphone 0.2 mg/kg.

Side effects noticed during this study included lip licking, retching, vomiting and shivering. Twelve out of twelve ferrets (100%) from the MH group experienced retching and lip licking. Four out of twelve ferrets (33%) from the same group vomited. Lip licking and retching were also observed in six out of twelve ferrets (50%) in the MM group, while one ferret (8%) vomited. Only one ferret experienced retching and lip licking in the MB group, and none vomited. Shivering was noticed in nine out of twelve (75%), seven out of twelve (58%) and four out of twelve (33%) ferrets shivered in the MM, MH and MB groups, respectively.

## Discussion

The combination of SC midazolam (0.5 mg/kg) and butorphanol (0.2 mg/kg) provided moderate sedation in 100% of the ferrets with a duration of sedation ranging from 36 to 72 minutes. After MB administration, fewer side effects (retching, vomiting, shivering) were observed than after administration of MM and MH. Midazolam and butorphanol is widely utilized for sedation in ferrets, and SC administration could be used as an alternative to IM injection to reach a moderate level of sedation, allowing for routine procedures such as physical examination, imaging, or blood sampling. However, notable variability in sedation duration was observed, ranging from 36 to 72 minutes. Prolonged sedation may not be necessary to perform the diagnostic procedure, and reversal of midazolam with flumazenil may be required in some ferrets if sedation lasts too long.

The combination of SC midazolam at 0.5 mg/kg with hydromorphone at 0.2 mg/kg resulted in a moderate level of sedation, but notable variability in sedation duration was observed. Indeed, the range of sedation duration spanned from 4 to 66 minutes. Performing noninvasive diagnostic tests such as blood sampling or imaging within this timeframe could be challenging for the shorter end of the range.

In the present study, SC injection of midazolam at 0.5 mg/kg combined with methadone at 0.3 mg/kg failed to achieve sedation. A recently published pharmacokinetic study compared SC and IV administration of 0.3 mg/kg methadone in ferrets [27]. It demonstrated a rapid absorption and elimination of methadone following SC administration. The study suggested that the elimination of methadone occurred faster than its absorption and that a dose of 0.3 mg/kg did not reach the minimum effective concentration (MEC), indicating that a higher dosage might be necessary for ferrets. Additionally, the results obtained in the present study might also be explained by the variability in plasma concentrations, that has been observed after SC administration of methadone in dogs [38].

Lower heart and respiratory rates were observed in the MB group compared to the MH group. In other species such as humans and dogs, hydromorphone causes a more profound cardiorespiratory depressant effect than butorphanol [39,40]. In this study, the superior level of sedation after MB administration compared to MH in ferrets could have contributed to the cardiorespiratory depressant effect. Although heart and respiratory rates were lower after MB administration, they remained within normal limits for this species.

In the present study, NIBP stabilized at a lower level in the MB group compared to MH. While all measurements remained normotensive in the MH group, the lower levels observed in the MB group are compatible with hypotension based on reference intervals obtained using the same technology and measurement site [13]. Hypotension was not treated in our study but is a possible complication of sedation with MB. NIBP should be monitored, especially if MB sedation is administered to debilitated ferrets, in which hypotension may become more clinically significant and require treatment. A time effect was also observed in our study, indicating that early reversal may prevent hypotension from occurring.

The majority of ferrets from all groups required oxygen supplementation as their $SpO_2$ levels dropped below 90%. The results of the study

showed that respiratory rate within normal range alone did not necessarily indicate adequate ventilation and highlights the importance of monitoring oxygen saturation in sedated animals. Factors that can contribute to hypoventilation include anesthetic drugs, equipment malfunction, body position, muscle relaxation, or concomitant diseases [41]. In the present study respiratory rates decreased markedly but stayed within normal ranges following drug administration. Tidal volume was not monitored; a reduced tidal volume could have contributed to the desaturation observed in the ferrets [42].

Temperature over time increased in the MH group and decreased in the MB group. Increased body temperature and hyperthermia are common findings when mu-opioids such as hydromorphone are used in cats [43]. The results of our study indicated that temperature could fluctuate over time with these two protocols but would likely remain within normal limits. No hyperthermia was observed in our study.

The MB treatment resulted in faster onset of sedation in ferrets, with a shorter time to sternal recumbency and loss of righting reflex compared to the MH treatment. There was no significant difference between the MH and MB groups in terms of sedation recovery. As hypothesized, a decreased reaction to mechanical noxious stimuli (toe pinch and withdrawal score) was observed in the MH group, most likely due to the higher analgesic properties of hydromorphone. There was no difference in jaw resistance score between the MH and MB groups. Only two ferrets from the MB group allowed to open the mouth using one finger, and only for 5 minutes. All the ferrets from the MH group exhibited notable jaw resistance. As a result, these protocols may not be appropriate to perform a thorough oral examination.

Regarding adverse effects, emesis and clinical signs that could be associated with nausea in animals (such as retching and lip licking) are commonly observed as a side effect of opioid administration in dogs [17,21,37]. In cats, SC administration of hydromorphone, compared to IM and IV administration, has been associated with an increased risk of emesis [36]. For this research, we anticipated similar side effects to those seen in dogs and cats, including retching, lip licking, and emesis. In the MH group, all ferrets experienced retching and lip licking, and 1/3$^{rd}$ of the ferrets vomited. The MB group had the lowest rate, with one ferret experiencing retching and lip licking, and none who vomited. When observed, retching, lip licking or vomiting occurred a few minutes after the administration of the sedative drugs. Once ferrets were sedated, all signs stopped.

Shivering after drug administration was a commonly observed side effect, especially in the MM and MH groups (75% and 58% of ferrets, respectively). The MB group had the lowest number of ferrets that shivered during sedation (33%). In this study, shivering was not associated with low body temperature. Shivering after opioid administration is not a side effect reported in other species but humans [44,45] and may pose challenges performing certain procedures, such as imaging, for which this may cause motion artifacts. A recent study [27] also observed shivering and lip licking during the first 20 minutes after administration of methadone IV or SC. In humans, the use of high doses of remifentanil has been shown to induce shivering, especially in nonpainful conditions [44,45]. In our study, only healthy, pain-free ferrets were included, and the observed shivering side effect may not manifest in nonhealthy, painful ferrets.

Limitations of this study include small sample size, and lack of comparison with other routes of drug administration. In addition, this study only included middle-aged, healthy ferrets. The inclusion of older or sick ferrets would allow for the assessment of the influence of comorbidities on the sedation level achieved for each treatment.

In conclusion, SC administration of midazolam (0.5 mg/kg) and butorphanol (0.2 mg/kg) offers a moderate level of sedation and fewer side effects compared to the other drug combinations tested in this study. Emesis, retching, and lip licking were commonly observed across all groups, highlighting the importance of fasting ferrets before sedation. Additionally, oxygen supplementation is recommended, as oxygen saturation dropped below 90% in most ferrets in all treatment groups. Further studies are needed to determine the drug dosage and effects in ferrets of various ages and health status.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Jessie Vandenbruggen:** Writing – original draft, Methodology, Investigation, Data curation. **Barbara Ambros:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Hugues Beaufrère:** Writing – review & editing, Methodology, Formal analysis. **Isabelle Desprez:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization.

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