DSPACE

https://dspace.org/

Comparison of three sedation combinations in isofluraneanesthetized garden dormice Eliomys quercinus undergoing laparotomy

Myriam Mugnier; Hanna Rauch-Schmuckling; Sylvain Giroud; Anna Haw; Attilio Rocchi ...

AMER ASSOC ZOO VETERINARIANS
https://hdl.handle.net/10539/46463
WOS

Downloaded from a DSpace repository. https://dspace.org/

BioOne DIGITAL LIBRARY

COMPARISON OF THREE SEDATION COMBINATIONS IN ISOFLURANE-ANESTHETIZED GARDEN DORMICE (ELIOMYS QUERCINUS) UNDERGOING LAPAROTOMY

Authors: Mugnier, Myriam, Rauch-Schmücking, Hanna, Giroud, Sylvain, Haw, Anna, Rocchi, Attilio, et al.

Source: Journal of Zoo and Wildlife Medicine, 56(2) : 410-419

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2024-0043

The BioOne Digital Library (https://bioone.org/) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (https://bioone.org/subscribe), the BioOne Complete Archive (https://bioone.org/archive), and the BioOne eBooks program offerings ESA eBook Collection (https://bioone.org/esa-ebooks) and CSIRO Publishing BioSelect Collection (https://bioone.org/csiro-ebooks).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commmercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

DOI: 10.1638/2024-0043 *Journal of Zoo and Wildlife Medicine* 56(2): 410–419, 2025
Copyright 2025 by American Association of Zoo Veterinarians

COMPARISON OF THREE SEDATION COMBINATIONS IN ISOFLURANE-ANESTHETIZED GARDEN DORMICE (*ELIOMYS QUERCINUS*) UNDERGOING LAPAROTOMY

Myriam Mugnier, DVM, Hanna Rauch-Schmücking, DVM, Sylvain Giroud, MSc, PhD, Anna Haw, BVSc, MBA, PhD, Attilio Rocchi, DVM, Dipl ECVAA, Géraldine Jourdan, DVM, MSc, PhD, and Friederike Pohlin, DVM, MSc, PhD, Dipl ECVAA

Abstract: The garden dormouse (*Eliomys quercinus*) is commonly used as model species for studies on hibernation, which may involve surgery. Similar to laboratory rodents, inhalational anesthesia, which does not provide analgesia, is often performed for surgical procedures. We retrospectively compared cardiorespiratory effects between ketamine-butorphanol-medetomidine (KBMed), ketamine-butorphanol-midazolam (KBMid) and butorphanol-medetomidine-midazolam (BMM) administered SC in 48 garden dormice undergoing laparotomy for bio-logger implantation plus tissue (liver, brown fat) biopsy surgery ($n = 48/48$ [28 females, 20 males] ~5 wk old) and bio-logger explantation surgery ($n = 42/48$ [24 females, 18 males], ~1 yr old). Doses were ketamine (40 mg/kg), medetomidine (0.2 mg/kg), midazolam (1 mg/kg), and butorphanol (0.2 mg/kg). Anesthesia was supplemented with isoflurane (1.29 ± 0.53%) in 100% oxygen via facemask; meloxicam and lactated Ringer's solution were administered SC; and a splash block using lidocaine was performed. Sedation score and recovery time were recorded. The pedal withdrawal reflex, pulse rate, RR, SpO_2, and temperature were monitored throughout the laparotomy. The effects of group and time were tested using linear mixed-effect models, with individuals as random factor. Sedation score was the deepest with KBMed. Pulse rate, RR, and temperature remained within physiological ranges for KBMid, but were decreased with KBMed and BMM ($P < 0.001$). SpO_2 remained >96% in all groups. Recovery time was shortest with KBMid (20.8 ± 18.1 min); KBMed and BMM required reversal with atipamezole after 60 min, otherwise recovery time would have been prolonged. All combinations allowed for appropriate intraoperative analgesia, cardiorespiratory stability, and adequate postoperative wound healing.

INTRODUCTION

The garden dormouse (*Eliomys quercinus*) is a native European nocturnal rodent species of the Gliridae family.[17] It is listed as "near threatened" by the International Union for Conservation of Nature Red List of Threatened Species due to a significant and ongoing population decline.[5] During winter (October–March), the garden dormouse hibernates in a state characterized by regular phases of torpor or metabolic depression with hypothermia and arousal, associated with eumetabolism and euthermic body temperature.[22,24] Hibernation can be artificially induced in this species by controlling temperature and food availability, making it an ideal laboratory model for hibernation research.[16,23] The garden dormouse therefore commonly undergoes anesthesia for experimental and surgical procedures. For example, intra-abdominal body temperature recording is a prerequisite to adequately track hibernating patterns during winter, and hence bio-logging implantation is a necessity for hibernation research. In laboratory rodents, inhalant anesthetics (such as isoflurane) are commonly the only drugs used to provide anesthesia for such procedures.[14,32,40] Although isoflurane provides hypnosis, amnesia, and muscle relaxation, it lacks any analgesic effects.[15,32] Induction of anesthesia with this drug is associated with a stress response and dose-dependent decrease in cardiac output, blood pressure and respiratory function.[32] These disadvantages can be minimized by providing

From the Research Institute of Wildlife Ecology, Department of Interdisciplinary Life Sciences, University of Veterinary Medicine Vienna, Savoyenstrasse 1, Vienna 1160, Austria (Mugnier, Rauch-Schmücking, Giroud, Haw, Pohlin); Clinical Unit of Anesthesiology and Perioperative Intensive-Care Medicine, University of Veterinary Medicine Vienna, Veterinärplatz 1, Vienna 1210, Austria (Rocchi, Pohlin); and Department of Anesthesia, Ecole Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31300 Toulouse, France (Mugnier, Jourdan). Present addresses: Energetic Lab, Department of Biology, Northern Michigan University, 1401 Presque Isle, Marquette, MI 49855-5301, USA (Giroud); University of the Witwatersrand, Brain Function Research Group, School of Physiology, Medical School, Parktown, South Africa (Haw); Shenzhen Mindray Animal Medical Technology Co., Ltd., Tower 4, Yesun Intelligent Community III, No. 1301-88 Guanguang Road, Longhua District, Shenzhen 518110, China (Rocchi); RESTORE Research Center, INSERM, CNRS, EFS, ENVT, Université P. Sabatier, Toulouse, France (Jourdan). Correspondence should be directed to Dr. Pohlin (friederike.pohlin@vetmeduni.ac.at).

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

sedation prior to induction of anesthesia.[32] The most widely used injectable agent is the dissociative anesthetic ketamine alone or in combination with an α-2 adrenergic agonist (e.g., xylazine or medetomidine) or a benzodiazepine (e.g., midazolam).[14,15,40] The addition of butorphanol has been shown to positively influence the analgesic quality and reduce isoflurane requirements in laboratory mice.[4] However, information regarding general anesthesia in dormice is sparse and only few reports exist. Fisher et al. (2018) recommend isoflurane inhalation anesthesia for blood sample collection and examination of free-ranging garden dormice.[13,21] Similarly, Kastenmayer et al. (2010) used isoflurane for phlebotomy in African dormice (*Graphiurus kelleni*),[26] whereas Bieber et al. (2014) and Watts et al. (2020) induced surgical anesthesia by SC injection of ketamine and xylazine in adult edible dormice (*Glis glis*) and garden dormice, respectively.[6,44] In the current retrospective study, we compared cardiorespiratory effects between ketamine-butorphanol-medetomidine (KBMed), ketamine-butorphanol-midazolam (KBMid), and butorphanol-medetomidine-midazolam (BMM) administered SC in garden dormice undergoing laparotomy for bio-logger implantation and explantation plus tissue (liver, brown fat) biopsy surgery maintained with isoflurane in 100% oxygen.

MATERIALS AND METHODS

Garden dormice were born in May 2020 from a breeding colony at the Research Institute of Wildlife Ecology, Vetmeduni Vienna, Austria. The dormice were housed in groups of two to four and same sex in species-specific designed enclosures that included nests, environmental enrichment, and *ad libitum* fresh water and food. As part of a larger study, 48 dormice (28 females, 20 males) underwent general anesthesia for abdominal bio-logger implantation and tissue (liver, brown fat) biopsy surgery in July 2020 (~5 wk old; males, ~61 g; females, ~55 g); 42 of these dormice (24 females, 18 males) underwent bio-logger explantation surgery in May 2021 (~1 yr old; males, ~105 g; females, ~82 g), resulting in 90 anesthesia monitoring records that were retrospectively analyzed for this study. All procedures and experiments were approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna, in accordance with the university's guidelines for good scientific practice and authorized by the Austrian Federal Ministry of Education, Science, and Research (approval no. BMBWF-68.205/0175-V/3b/2018) in accordance with the current legislation. In the past, the garden dormice at our facility were anesthetized with ketamine, xylazine or medetomidine, and isoflurane.[25] However, due to the invasiveness of this procedure (i.e., liver and brown fat biopsy), analgesia and muscle relaxation required improvement; therefore, butorphanol and midazolam, respectively, were added to the protocol. These adaptations took place gradually, resulting in three drug combinations used that were retrospectively analyzed for this study: KBMed, KBMid, and BMM. The following doses were used: ketamine (Ketamidor® [100 mg/ml], Richter Pharma, Wels, 4600, Austria; 40 mg/kg SC), medetomidine (Narco Start® [1 mg/ml], Richter Pharma; 0.2 mg/kg SC), butorphanol (Butomidor® [10 mg/ml], Richter Pharma; 0.2 mg/kg SC), and midazolam (Dormicum® [5 mg/ml], Roche Austria GmbH, Vienna, 1211, Austria; 1 mg/kg SC).

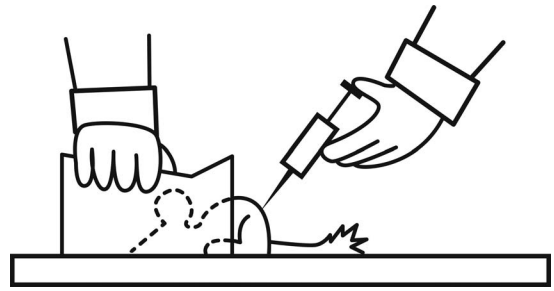


Figure 1. Image showing the technique used for manual restraint and subcutaneous drug injection in a garden dormouse (*Eliomys quercinus*). The dormouse is carefully restrained in a breathable fabric bag located on a table with the examiner wearing a leather animal handling glove. The caudal end of the dormouse is exposed outside of the bag, allowing for careful subcutaneous drug injection with the other hand or by a second examiner.

Before surgery, a veterinarian examined and weighed each dormouse for anesthetic dose calculation. The drug combination (KBMed, KBMid, or BMM) was then injected SC into a loose skin fold over the flank of the dormouse by using a disposable insulin syringe and needle while the dormouse was manually restrained in a fabric bag (Fig. 1). After the injection, the dormouse was placed into the bag and left unstimulated for 10 min ($t = 10$). A sedation score was assigned to each dormouse after this time (Table 1), and the dormouse was transferred to a surgical table (Fig. 2) specifically designed for rodents and heated to 40°C (Combi-vet® System

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

Table 1. Scoring system used for monitoring sedation in garden dormice (*Eliomys quercinus*) administered SC ketamine-butorphanol-medetomidine, ketamine-butorphanol-midazolam, or butorphanol-medetomidine-midazolam.[a]

Score 1	Immobilized within 10 min, no reaction to nonpainful (touch) and painful (PWR) stimuli, start isoflurane immediately on maintenance concentration
Score 2	Deep sedation, no reaction to nonpainful stimuli, reactive to painful stimuli, isoflurane via facemask required for 1 min
Score 3	Moderate sedation, reaction to nonpainful and painful stimuli, isoflurane via facemask required for 3 min
Score 4	No to only mild sedation, isoflurane via induction chamber

[a] PWR, pedal withdrawal response.

3 surgery table, Rothacher Medical GmbH, Heitenried, 1714, Switzerland).

Anesthesia was maintained with isoflurane (Vetflurane® [1,000 mg/g], Virbac Austria GmbH, Vienna, 1180, Austria) in 100% oxygen via a facemask (Combi-vet Mask system for mice or rats, Rothacher Medical GmbH). Isoflurane concentrations used for induction were 2–3% and for initial maintenance of anesthesia 1.29 ± 0.53%. Concentrations were titrated down during the procedure according to response to surgical stimulation, regardless of the drug combination used (Fig. 3). Meloxicam (Metacam® [2 mg/ml], Boehringer Ingelheim Vetmedica GmbH, Ingelheim, 55216, Germany; 2 mg/kg) and Ringer's lactate solution (Ringer-Lactat®, Braun, Melsungen, 34209, Germany; 10 ml/kg) were administered SC. Eye ointment (Vit-A-Vision®, Omnivision GmbH, Puchheim, 82178, Germany) was applied, and the dormouse was positioned in dorsal recumbency. A 1 × 2-cm abdominal area, 3 mm below the xiphoid, was clipped and aseptically prepared for surgery (Fig. 2). A 1-cm cutaneous incision was made, laparotomy was performed, and a bio-logger (built in-house; size, 16 × 12 × 8 mm; weight, 2.5 g; capacity, ~105,000 datapoints) was placed free-floating into the abdominal cavity. For the explantation, the bio-logger was removed instead of inserted. In addition, a liver biopsy was performed just before bio-logger implantation, but not during explantation. The incision was then closed with simple interrupted IM sutures (Surgicryl®, PGA, USP 3-0, 75 cm, TP19, SMI, Steinerberg, 4780, Belgium), followed by a splash block with 1 mg/kg lidocaine (Xylanaest® purum 1%, Gebro Pharma GmbH, Fieberbrunn, 6391, Austria), and continuous ID sutures (Surgicry®, PGA PLUS, USP 4-0, 75 cm, DS19, SMI). The dormice were then positioned in sternal recumbency to perform a brown fat biopsy from between the shoulder blades as described by Rauch et al. (2021).[35]



Figure 2. Photography of a garden dormouse (*Eliomys quercinus*) on a surgical table specifically designed for mice, rats, and reptiles (Combi-vet System 3 surgery table, Rothacher Medical GmbH). Note the facemask used for maintenance of anesthesia with isoflurane in oxygen and the pulse oximeter probe placed on the left hind paw of the dormouse to continuously monitor pulse rate and SpO_2. The abdomen is clipped and aseptically prepared to allow for laparotomy.

During surgery, the following clinical variables were continuously monitored and recorded every 5 min: pulse rate (PR) and SpO_2 were measured by a pulse oximeter attached to a hind paw

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

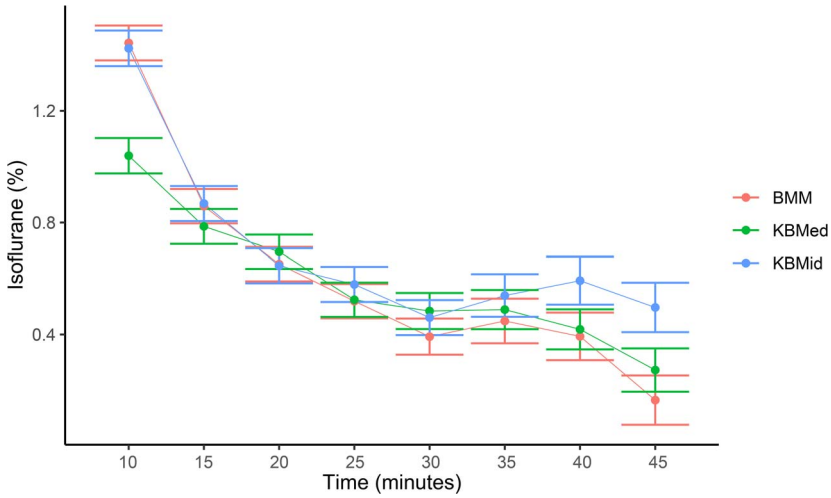


Figure 3. Concentration of isoflurane (%) used for induction and maintenance of anesthesia in garden dormice (*Eliomys quercinus*, $n = 83$), measured from 10 to 45 min after sedation (mean ± SEM). Dormice were sedated with either butorphanol-medetomidine-midazolam (BMM, $n = 28$), ketamine-butorphanol-medetomidine (KBMed, $n = 28$), or ketamine-butorphanol-midazolam (KBMid, $n = 27$).

(Root™ Radical-7, Masimo Corporation, Irvine, CA 92618, USA) (Fig. 2); RR was measured by counting the number of thoracic excursions; and body temperature was measured by using a rectal thermometer (HS digital veterinary thermometer, Henry Schein Animal Health, Vienna, 1120, Austria) at the beginning and end of the procedure. Because of the small size of the dormice, arterial blood pressure measurements were not performed. Appropriate intraoperative depth of anesthesia was ensured by a negative pedal withdrawal response (PWR) and negative response to surgical stimulus.

Each dormouse was placed in an individual type II rodent cage (Polycarbonate Mice Cage, PLEXX B.V. Lab Products Inc., 6662 PW Elst, The Netherlands) to recover from anesthesia. Body temperature was maintained by an adjustable heating pad below the cage, and flow-by oxygen (0.5 L/min) was provided until fully recovered. The time from the end of surgery to spontaneous movement was recorded as the recovery time. If a dormouse did not recover within 1 h, atipamezole 0.1 mg/kg (Narco Stop® [5 mg/ml], Richter Pharma) (KBMed, BMM) or flumazenil 0.01 mg/kg (Anexate® [0.5 mg/5ml], Roche Austria GmbH) (KBMid) was administered SC. Each dormouse was returned to its enclosure and, for 3 d following surgery, each dormouse was weighed daily, 1 mg/kg meloxicam was administered SC, and the surgical wound was evaluated using a wound evaluation score (Table 2).[25] To avoid unnecessary handling and stress, dormice were left unhandled if the wound score was 0 for 2 d in a row.

Statistical analyses were performed using RStudio® 3.6.2.[34] The effects of group on sedation score were tested using an ordinal logistic regression (function: polr, package MASS).[43] Because one dormouse escaped during application of the drugs, 89 of 90 recordings were included in this analysis.

Anesthesia monitoring data were assessed for normality and homoscedasticity of the model's residuals. The effects of group on rectal temperature were tested using Kruskal–Wallis and Wilcoxon rank sum tests; 62 of 90 recordings were available for this analysis because only measurements taken at exactly $t = 10$ min were included.

Table 2. Scoring system used for evaluating wound healing in garden dormice (*Eliomys quercinus*) for 3 d following laparotomy for bio-logger implantation and explantation plus tissue (liver, brown fat) biopsy surgery.

Score 0	Optimal wound healing, good wound contraction, no edema, erythema and/or exudate
Score 1	Good wound healing, mild occurrence of edema, erythema wound and/or exudate
Score 2	Moderate occurrence of wound exudate or erythema or opening of a skin suture
Score 3	Wound healing complication (e.g., opening of abdominal sutures due to manipulation of the animals)

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

Because the end-of-surgery times differed in length, only body temperature measured at 30 min ($t = 30$) was included in further statistical analysis. Changes over time and between sedation combinations for PR, RR, and body temperature were tested with ANOVA and post hoc tests by using linear mixed-effect models (nlme package) with group (KBMed, KBMid, BMM), time, and surgery (implantation, explantation) as fixed factors and dormouse as random factor.[33] To visualize the effect of these variables over time, the computed marginal means of each group at every time point were plotted (ggplot2). In total, 83 of 90 (PP and RR) and 19 of 90 (body temperature at 10 and 30 min) recordings were used for these analyses because some dormice had to be excluded due to incomplete anesthetic records. Because of heteroscedasticity of residuals and because data were not normally distributed, SpO_2 recordings were not included in the statistical analyses. However, all SpO_2 values were within a clinically acceptable range of ≥96%.[10]

The effects of group on postoperative changes in body weight were tested using linear mixed-effect models with the individuals as random factors. The effects of group, type of surgery, and duration of surgery on postoperative wound healing scores were tested using an ordinal logistic regression. The effects of group on recovery time were tested using Kruskal–Wallis and Wilcoxon rank rum tests. Three dormice were excluded from these analyses (two were administered a top-up dose and one had to be reopened due to logger issues), resulting in 87 of 90 recordings being used. Differences were considered significant when $P \leq 0.05$.

RESULTS

In 37 of 90 anesthetic events, dormice were sedated with KBMed; in 26 of 90 events, dormice were sedated with BMM; and in 27 of 90 events, dormice were sedated with KBMid. No dormouse received the same drug combination twice. The duration of the procedures was 31.8 ± 6.05 min for implantations and 20.8 ± 4.03 min for explantations.

The sedation score with KBMed (1.32 ± 0.784) was significantly deeper than that for BMM (2.69 ± 1.01) and KBMid (2.33 ± 1.07) ($P < 0.001$); there were no differences between KBMid and BMM. Body temperature at $t = 10$ and $t = 30$ was higher with KBMid (37.1 ± 0.8 and 37.6 ± 0.2°C, respectively) than with KBMed (35.9 ± 2.2°C, $P = 0.024$ and 36.1 ± 0.5°C, $P = 0.026$, respectively) and BMM (35.9 ± 0.8°C, $P = 0.001$ and 36.6 ± 0.2°C, $P = 0.022$, respectively). Time had no effect on body temperature. However, PR decreased over time in all groups ($P < 0.001$), but was higher ($P < 0.001$) and more stable with KBMid (223 ± 52 beats/min) than BMM (158 ± 43 beats/min) and KBMed (173 ± 34 beats/min) (Fig. 4). Similarly, RR decreased over time ($P = 0.015$) and was higher ($P = 0.030$) with KBMid (79 ± 4 breaths/min, $t = 15$) than with KBMed (56 ± 5 breaths/min, $t = 15$) from 15 to 45 min of anesthesia. At 30 and 45 min, RR was higher ($P < 0.035$) with KBMid than with BMM (75 ± 4 and 90 ± 7 breaths/min vs 63 ± 4 and 65 ± 6 breaths/min, respectively) (Fig. 5).

Recovery was smooth in all dormice. The shortest recovery time was noted with KBMid as 20.8 ± 18.1 min ($P < 0.001$), which was the only group in which none of the dormice required the administration of an antagonist. In the other two groups, recovery was prolonged and antagonists were administered after 60 min. Among the 26 BMM cases, 9 cases received atipamezole, 4 received flumazenil, and 3 received both drugs, resulting in an overall recovery time of 79.2 ± 50.2 min. Among the 35 KBMed cases, 3 cases were reversed with atipamezole, resulting in an overall recovery time of 66.8 ± 42.3 min.

There were no differences in body weight changes between the different groups and days after surgery. However, group BMM had an overall higher body weight (81 ± 4 g) than that of the other two groups (70 ± 4 g for KBMed and 72 ± 4 g for KBMid), already before surgery. There were no differences in wound healing scores between the groups or associated with day, type, and duration of surgery. According to our classification, 65 (75%) of 87 dormice had a wound score of 0, 14 (16%) of 87 had a wound score of 1, 7 (8%) of 87 had a wound score of 2, and 1 (1%) of 87 had a wound score of 3 on the day after surgery. Only one dormouse manipulated its skin and abdominal suture, resulting in an intestinal hernia. This dormouse was reanesthetized to clean and replace the prolapsed omentum and replace the sutures. This individual, and only three other dormice with a wound score of 2, received postoperative antibiotics consisting of 10 mg/kg enrofloxacin for 3 d (Baytril® [25 mg/ml], Bayer Austria GmbH, Vienna, 1160, Austria). In these dormice, the skin was cleaned and sutures were replaced by a tissue adhesive (Surgibond tissue adhesive, SMI suture materials, SMI, St. Vith, 4780, Belgium).

DISCUSSION

All combinations, KBMed, KBMid, and BMM, allowed for moderate sedation with a stress-free

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

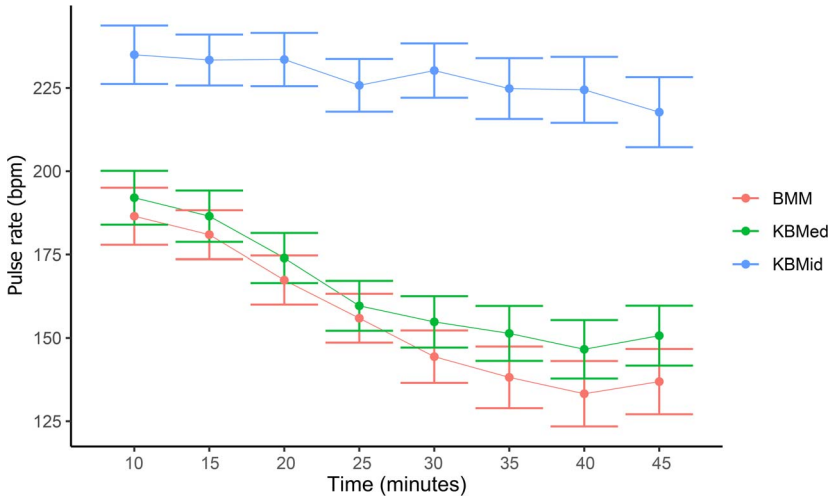


Figure 4. Effect of the three different drug combinations on the pulse rate (beats/min) during the surgical procedure in garden dormice (*Eliomys quercinus*, $n = 83$), measured from 10 to 45 min after induction of anesthesia (mean ± SEM). Dormice were sedated with butorphanol-medetomidine-midazolam (BMM, $n = 28$), ketamine-butorphanol-medetomidine (KBMed, $n = 28$), or ketamine-butorphanol-midazolam (KBMid $n = 27$), and anesthesia was maintained with isoflurane in oxygen.

induction of anesthesia, cardiorespiratory stability, and adequate postoperative wound healing. KBMed showed the deepest sedation score, providing the smoothest induction of anesthesia, but it was associated with a decreased PR and RR. PR, RR, and body temperature were closest to physiological ranges with KBMid, the combination that also had the fastest recovery time. These characteristics could be important for certain research questions requiring intraoperative clinical variables to be close to normal. Postoperative wound scores were low in all dormice, and there were no differences in the trend of body weight changes over time after surgery, indicating that adequate analgesia was likely provided intra- and postoperatively.

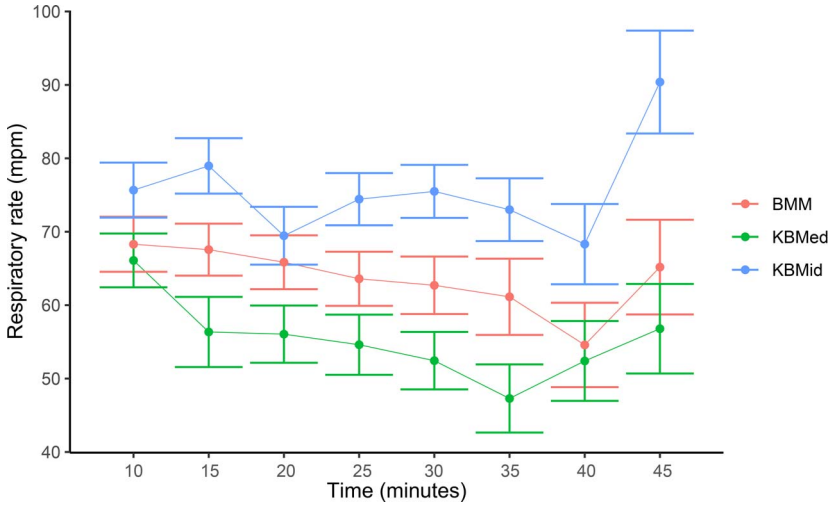


Figure 5. Effect of the three different drug combinations on the RR (breaths/min) during the surgical procedure in garden dormice (*Eliomys quercinus*, $n = 83$), measured from 10 to 45 min after induction of anesthesia (mean ± SEM). Dormice were sedated with butorphanol-medetomidine-midazolam (BMM, $n = 28$), ketamine-butorphanol-medetomidine (KBMed, $n = 28$), or ketamine-butorphanol-midazolam (KBMid, $n = 27$), and anesthesia was maintained with isoflurane in oxygen.

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

KBMed induced deep sedation within 10 min of SC administration, after which time no reaction to nonpainful and painful stimuli was observed. Ketamine, absent in BMM, is a dissociative anesthetic that functionally disorganizes the CNS by noncompetitive antagonism at the central *N*-methyl-D-aspartate receptor, causing amnesia, analgesia, light sleep, and immobilization.[15,36] Medetomidine, absent in KBMid, is an α-2 adrenergic agonist that potentiates the sedative effects of ketamine by decreasing presynaptic norepinephrine release, providing sedation and anxiolysis.[15] Therefore, this combination proved the most effective for sedation, eliminating the need for isoflurane to diminish the PWR. By contrast, dormice sedated with BMM showed the poorest sedation scores, requiring isoflurane to achieve a surgical plane of anesthesia. Higher doses of BMM (5 mg/kg butorphanol, 0.3 mg/kg medetomidine, 4 mg/kg midazolam) have reliably induced surgical anesthesia in laboratory mice.[27,29] Further studies are required to determine the minimal effective doses of this drug combination to induce surgical anesthesia in garden dormice while minimizing undesired side effects such as bradycardia, hypoxemia, and hypothermia.[4,28,32]

For the purpose of comparison, we assumed that the PR measured via pulse oximetry corresponds to the HR. An awake, unstressed garden dormouse active at 37°C has a HR of ~350 beats/min.[37] With KBMed and BMM, PR was reduced, consistent with findings in laboratory mice by using similar drug combinations containing α-2 adrenergic agonists.[4,25,28] Peripherally, these drugs induce vasoconstriction, leading to hypertension and reflex bradycardia.[1] Centrally, they decrease sympathetic nervous system tone, thereby reducing HR and inotropy.[12] Similar to Karbacher (2019), who compared ketamine-medetomidine and ketamine-xylazine in isoflurane-maintained garden dormice, PR decreased over time.[25] In laboratory mice, HR is lowest ~26 min after SC α-2 adrenergic agonist injection and gradually increases thereafter, corroborating our observations of the PR rising toward the end of the procedures.[9]

Although isoflurane has minimal direct effects on HR, it may cause a marked decrease in RR, tidal volume, and oxygenation.[20,32] All dormice showed bradypnea compared with reference ranges reported in laboratory mice (180 breaths/min),[14] with KBMid showing the RR closest to the physiological rate. Despite the decrease in RR, all dormice maintained a physiological SpO_2, with a FiO_2 of 100%.[10] Anesthetized mice are known to experience profound hypoxemia if oxygen is not supplemented.[7] In our dormice, oxygen supplementation was provided throughout anesthesia until the dormice were fully recovered, successfully mitigating this complication. The development of hypothermia is another main concern in anesthetized small mammals because of their large surface area:body mass ratio.[14] Therefore, active warming was provided intraoperatively. The average body temperature of an awake garden dormouse is 36.96 ± 0.39°C,[23] and KBMid-sedated dormice maintained their body temperatures closest to this range. Mild hypothermia (32–37°C), observed in the other two groups, likely reflected the lower PR and reduced metabolic heat production associated with the medetomidine[31] and is considered a key factor for prolonged recovery from anesthesia.[3,18] Thus, recovery times were shortest with KBMid, which did not contain this drug, and longest with BMM. The latter has been associated with a recovery time of only 11.33 ± 0.58 min in mice,[27] but 105.8 ± 17.4 min in rabbits.[29] Species-specific differences as well as potentiation effects of isoflurane might have played a role in the longer recovery time in our dormice and require further investigation. Generally, a shorter recovery time, with animals quickly regaining normal physiological function, is preferred.[2] However, experience with bio-logger implantation and explantation surgeries at our facility has shown that eager cleaning and licking of the surgical wound after a rapid recovery may contribute to poor wound healing in garden dormice.[25] None of the dormice included in this study experienced these complications. There were no differences between the three drug combinations concerning wound healing and the trend of postoperative body weight, which remained stable in all dormice. Impaired wound healing and weight loss after surgery commonly result from manipulation of the surgical site and decreased appetite, respectively, caused by discomfort or pain.

For laboratory rodent anesthesia, it has been reported that <40% are administered intraoperative analgesic drugs and <25% receive postoperative analgesia.[40] There are only few reports describing the use of multimodal analgesia, or the use of more than one analgesic agent targeting different nociception sources, in mice.[32,40] For moderate-to-severe pain, the combination of an opioid, a local anesthetic, and an NSAID is recommended.[32] Although buprenorphine is the analgesic agent most frequently administered to laboratory rodents,[14,40] we used butorphanol in

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

our dormice. The opioid has both agonist (at the κ receptor) and antagonist (at the μ receptor) properties. It is a nonrestricted narcotic drug in many countries, thereby facilitating its use in laboratory animals where a veterinarian might not always be present.[11] Butorphanol has also been shown to improve analgesia when combined with ketamine and medetomidine in mice.[4] We provided additional incisional analgesia by applying the local anesthetic lidocaine to the surgical site. Local anesthetics inhibit membrane depolarization, nerve excitation, and conduction by blocking voltage-gated sodium channels, thereby offering a simple and cost-effective method to reduce pain after abdominal surgery.[39] However, few reports exist in mice[19,40] and further studies are needed to evaluate the efficacy and safety of incisional local anesthesia in laboratory rodents. By contrast, NSAIDs, which reduce inflammatory pain by inhibiting prostaglandin synthesis, are the second most commonly administered analgesics in laboratory rodents.[14,40] We administered meloxicam because it has been shown to provide adequate postoperative analgesia for partial hepatectomy in mice—a procedure similar to the one performed in our dormice.[41] Although meloxicam maintained near-normal growth rates in rats postsurgery,[8] our dormice showed no weight loss, but did not sustain their normal growth rates. Body weight changes alone may not fully reflect postoperative pain, and other behavioral and physiological indicators (e.g., nesting, burrowing, grooming behavior, PR, RR, body temperature) might offer more comprehensive assessments.[42] Mouse and rat grimace scales have proven to be accurate, reliable, and valid tools.[30,38] In 2015, software called Rodent Face Finder® was created that automatically generates a pain score with mouse or rat photographs.[38] Future studies in garden dormice could validate and use these tools to better investigate postoperative analgesia in this species.

A major limitation of this study is the different conditions encountered during the two surgical procedures, implantation and explantation. Explantation took less time, and the postsurgical housing conditions were slightly different. During the implantation surgeries, two garden dormice were housed in one enclosure with two separate nests, whereas for the explantation surgeries four dormice were housed in the same enclosure with two separate nests. In addition, during the implantation surgeries, dormice were only 5 wk old and considered of pediatric age, whereas for the explantation surgeries dormice were >1 yr of age and considered adults. We accounted for these factors in the statistical model by including surgery type as fixed effect. Nevertheless, it is not possible to directly distinguish the effects of surgery type, housing, and age from each other, which presents a main limitation. Despite these limitations, we believe that our results provide a valuable contribution to the current literature by comparing three sedation combinations that allow for appropriate intraoperative analgesia, cardiorespiratory stability, and adequate postoperative wound healing in isoflurane-anesthetized garden dormice undergoing invasive procedures.

Acknowledgments: The authors thank Gabrielle Stalder, Johanna Painer-Gigler, Ursula Teubenbacher, Joy Einwaller, and Antonia Rippel-Rachle for assisting with the coordination and conduction of clinical work and data entry. We thank Peter Steiger for his valuable help with dormouse care. This work was supported by the Austrian Science Fund (FWF; P 31577 to S.G.), the Austrian Agency for International Cooperation in Education and Research (Scientific and Technological Cooperation grant FR 09/2020), and the city of Vienna.

LITERATURE CITED

1. Ahmad AA, Douay G, Low MR, Fabbri S, Chen HC. Comparison of the effects of butorphanol–midazolam–medetomidine and butorphanol–azaperone–medetomidine in wild common palm civets (*Paradoxurus musangus*). Vet Anesth Analg. 2021;48(3):380–387.

2. Albrecht M, Henke J, Tacke S, Markert M, Guth B. Effects of isoflurane, ketamine-xylazine and a combination of medetomidine, midazolam and fentanyl on physiological variables continuously measured by telemetry in Wistar rats. BMC Vet Res. 2014;10(1):198.

3. Armstrong SR, Roberts BK, Aronsohn M. Perioperative hypothermia. J Vet Emerg Crit Care 2005;15(1):32–37.

4. Bauer C, Schillinger U, Brandl J, Meyer-Lindenberg A, Ott A, Baumgartner C. Comparison of pre-emptive butorphanol or metamizole with ketamine + medetomidine and s-ketamine + medetomidine anaesthesia in improving intraoperative analgesia in mice. Lab Anim. 2019;53(5):459–469.

5. Bertolino S. IUCN Red List of Threatened Species: *Eliomys quercinus*. IUCN Red List of Threatened Species. 2008 Jun 30 [accessed 2022 Oct 19]. https://www.iucnredlist.org/species/7618/12835766

6. Bieber C, Lebl K, Stalder G, Geiser F, Ruf T. Body mass dependent use of hibernation: why not

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

prolong the active season, if they can? Funct Ecol. 2014;28(1):167–177.

7. Blevins CE, Celeste NA, Marx JO. Effects of oxygen supplementation on injectable and inhalant anesthesia in C57BL/6 mice. J Am Assoc Lab Anim Sci. 2021;60(3):289–297.

8. Brennan MP, Sinusas AJ, Horvath TL, Collins JG, Harding MJ. Correlation between body weight changes and postoperative pain in rats treated with meloxicam or buprenorphine. Lab Anim. 2009;38(3): 87–93.

9. Burnside WM, Flecknell PA, Cameron AI, Thomas AA. A comparison of medetomidine and its active enantiomer dexmedetomidine when administered with ketamine in mice. BMC Vet Res. 2013;9(1):48.

10. Cantwell SL. Ferret, rabbit, and rodent anesthesia. Vet Clin N Am Exotic Anim Pract. 2001;4(1): 169–191.

11. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. 2010. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

12. Dugdale A, Beaumont G, Bradbrook C, Gurney M. Sedation and premedication: small animals. In: Veterinary anaesthesia: principles to practice. 2nd edition. Chichester (United Kingdom): John Wiley & Sons; 2020. p. 55–76.

13. Fischer D, Ziegler L, Hail K, Heckmann J, Reiners TE, Bauer C, Lierz M, Lang J. Clinical examination methods and investigation into the occurrence of endoparasites, Borna disease virus and genetic variability in the garden dormouse, *Eliomys quercinus*. Folia Zool. 2018;67(2):69.

14. Flecknell PA, Thomas AA. Comparative anesthesia and analgesia of laboratory animals. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA (eds.). Veterinary anesthesia and analgesia. 5th ed. Of Lumb and Jones. Ames (IA): Wiley Blackwell; 2015. p. 754–763.

15. Gargiulo S, Greco A, Gramanzini M, Esposito S, Affuso A, Brunetti A, Vesce G. Mice anesthesia, analgesia, and care. Part I: anesthetic considerations in preclinical research. J Inst Lab Anim Res. 2012;53(1):E55–E69.

16. Giroud S, Habold C, Nespolo RF, Mejías C, Terrien J, Logan SM, Henning RH, Storey KB. The torpid state: recent advances in metabolic adaptations and protective mechanisms. Front Physiol. 2021;11: 623665.

17. Giroud S, Zahn S, Criscuolo F, Chery I, Blanc S, Turbill C, Ruf T. Late-born intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to hibernation: consequences for ageing processes. Proc R Soc B Biol Sci. 2014;281(1797): 20141131.

18. Grahn DA, Heller MC, Larkin JE, Heller HC. Appropriate thermal manipulations eliminate tremors in rats recovering from halothane anesthesia. J Appl Physiol. 1996;81(6):2547–2554.

19. Grant GJ, Vermeulen K, Langerman L, Zakowski M, Turndorf H. Prolonged analgesia with liposomal bupivacaine in a mouse model. Reg Anesth. 1994;19(4):264–269.

20. Groeben H, Meier S, Tankersley CG, Mitzner W, Brown RH. Influence of volatile anaesthetics on hypercapnoeic ventilatory responses in mice with blunted respiratory drive. Br J Anaesth. 2004;92(5): 697–703.

21. Hail K, Lang J, Lierz M, Fischer D, Ziegler L. Handling garden dormice (*Eliomys quercinus*) for research: methods, techniques and protocols. Proc. of the International Dormouse Conference. Liege, Belgium; 2017.

22. Heldmaier G, Ortmann S, Elvert R. Natural hypometabolism during hibernation and daily torpor in mammals. Respir Physiol Neurobiol. 2004;141(3): 317–329.

23. Huber N, Vetter S, Stalder G, Gerritsmann H, Giroud S. Dynamic function and composition shift in circulating innate immune cells in hibernating garden dormice. Front Physiol. 2021;12:620614.

24. Jastroch M, Giroud S, Barrett P, Geiser F, Heldmaier G, Herwig A. Seasonal control of mammalian energy balance: recent advances in the understanding of daily torpor and hibernation. J Neuroendocrinol. 2016;28(11):jne.12437.

25. Karbacher H. Surgical implantation of a free-floating intra-abdominal thermo-logger in garden dormice (*Eliomys quercinus*): anaesthesia, surgery, pre-, intra- and postoperative management. Diplomathesis, Vetmeduni Vienna; 2019.

26. Kastenmayer RJ, Moak HB, Jeffress EJ, Elkins WR. Management and care of African dormice (*Graphiurus kelleni*). J Am Assoc Lab Anim Sci. 2010;49(2): 173–176.

27. Kawai S, Takagi Y, Kaneko S, Kurosawa T. Effect of three types of mixed anesthetic agents alternate to ketamine in mice. Exp Anim. 2011;60(5): 481–487.

28. Kirihara Y, Takechi M, Kurosaki K, Kobayashi Y, Saito Y, Takeuchi T. Anesthetic effects of a three-drugs mixture: comparison of administrative routes and antagonistic effects of atipamezole in mice. Exp Anim. 2015;64(1):39–47.

29. Kirihara Y, Takechi M, Kurosaki K, Matsuo H, Kajitani N, Saito Y. Effects of an anesthetic mixture of medetomidine, midazolam, and butorphanol and antagonism by atipamezole in rabbits. Exp Anim. 2019;68(4):443–452.

30. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, LaCroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, Maagdenberg AMJMvan den, Ferrari MD, Craig KD, Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat Methods 2010;7(6):447–449.

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna

31. Looney DP, Buller MJ, Gribok AV, Leger JL, Potter AW, Rumpler WV, Tharion WJ, Welles AP, Friedl KE, Hoyt RW. Estimating resting core temperature using heart rate. J Meas Phys Behav. 2018;1(2): 79–86.

32. Navarro KL, Huss M, Smith JC, Sharp P, Marx JO, Pacharinsak C. Mouse anesthesia: the art and science. J Inst Lab Anim Res. 2021;62(1–2):238–273.

33. Pinheiro JC, Bates DJ, DebRoy S, Sakar D. The nlme package: linear and nonlinear mixed effects models, R version 3; 2012. https://CRAN.R-project.org/package=nlme

34. R: the R project for statistical computing. 2022 Nov 28 [accessed 2022 Nov 28]. https://www.r-project.org/

35. Rauch H, Rauch H, Pohlin F, Einwaller J, Heiderich E, Haw A, Giroud S, Stalder G, Painer J. A biopsy technique for sampling the interscapular brown adipose tissue in the garden dormouse (*Eliomys quercinus*). Proc. of the Joint AAZV/EAZWV Conference. Online; 2021.

36. Robertson SA. Analgesia and analgesic techniques. Vet Clin N Am Exotic Anim Pract. 2001;4(1): 1–18.

37. Rzeszowska-Tokarska G, Burlet C, Legait E. Histophysiological study of the splenic modifications during hibernation of the garden dormouse *Eliomys quercinus*. J Cell Res Microsc Anat. 1972;128:385–392.

38. Sotocina SG, Sorge RE, Zaloum A, Tuttle AH, Martin LJ, Wieskopf JS, Mapplebeck JC, Wei P, Zhan S, Zhang S, McDougall JJ, King OD, Mogil JS. The rat grimace scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. Mol. Pain 2011;7:1744-8069-7–55.

39. Steagall PVM, Benito J, Monteiro B, Lascelles D, Kronen PW, Murrell JC, Robertson S, Wright B, Yamashita K. Intraperitoneal and incisional analgesia in small animals: simple, cost-effective techniques. J Small Anim Pract. 2020;61(1):19–23.

40. Stokes EL, Flecknell PA, Richardson CA. Reported analgesic and anaesthetic administration to rodents undergoing experimental surgical procedures. Lab Anim. 2009;43(2):149–154.

41. Tubbs JT, Kissling GE, Travlos GS, Goulding DR, Clark JA, King-Herbert AP, Blankenship-Paris TL. Effects of buprenorphine, meloxicam, and flunixin meglumine as postoperative analgesia in mice. J Am Assoc Lab Anim Sci. 2011;50(2):185–191.

42. Turner PV, Pang DS, Lofgren JL. A review of pain assessment methods in laboratory rodents. Comp Med. 2019;69(6):451–467.

43. Venables WN, Ripley BD. Modern applied statistics with S. New York (NY): Springer; 2002 http://link.springer.com/10.1007/978-0-387-21706-2

44. Watts AJ, Logan SM, Kübber-Heiss A, Posautz A, Stalder G, Painer J, Gasch K, Giroud S, Storey KB. Regulation of peroxisome proliferator-activated receptor pathway during torpor in the garden dormouse, *Eliomys quercinus*. Front Physiol. 2020;11:615025.

Accepted for publication 7 March 2025

Downloaded From: https://bioone.org/journals/Journal-of-Zoo-and-Wildlife-Medicine on 10 Jun 2025
Terms of Use: https://bioone.org/terms-of-use Access provided by Veterinary Medical University Vienna