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ORIGINAL RESEARCH

Immobilisation and clinical effects of four drug combinations used to chemically capture white rhinoceros (*Ceratotherium simum*)

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Etorphine is an ultra-potent opioid used to immobilise white rhinoceros (*Ceratotherium simum*) in the field, but it can also cause extensive metabolic and cardiorespiratory derangements. To potentially reduce the severity of these derangements, etorphine is combined with synergistic drugs including medetomidine (an alpha-2 adrenoreceptor agonist), midazolam (a benzodiazepine agonist), or azaperone (a butyrophenone drug). The potentiation effects of these synergistic drugs are believed to reduce induction times and excitement, induce muscle relaxation, and improve immobilisation quality and safety.

To test the benefits of these synergistic drugs, eight healthy, wild-caught boma-housed white rhinoceros (sub-adult males) were used in a repeated-measures randomised cross-over study. Each rhinoceros was immobilised with four treatments: etorphine only (control), etorphine + azaperone (azaperone), etorphine + medetomidine (medetomidine), and etorphine + midazolam (midazolam). Butorphanol was administered intravenously after 12 min of immobilisation in all treatments. First signs of drug effects, immobilisation induction times, immobilisation quality, tremor scores, rectal temperature, heart and respiratory rates, and peripheral arterial oxygen-haemoglobin saturation, were compared between and within treatments.

All four treatments effectively immobilised rhinoceros, however the addition of azaperone, medetomidine and midazolam were associated with significantly shorter induction times compared to the etorphine-only.

All treatments initially caused severe muscle tremors, hypopnea, hypoxaemia, and tachycardia (except for medetomidine). The administration of butorphanol partly corrected most of these derangements.

Our study shows that the synergistic drugs effectively speed up induction into immobilisation. However, they provide little other physiological or clinical benefit over etorphine-only. Butorphanol remains an important ancillary treatment when administering these drug combinations in rhinoceros.

Keywords: cardiopulmonary, etorphine, azaperone, midazolam, medetomidine

Introduction

Different drug combinations are used in the immobilisation of white rhinoceros (*Ceratotherium simum*) for the purposes of research, treatment, translocation, identification, and dehorning - efforts that are geared toward the conservation and management of this near-threatened species (Emslie et al. 2020). The choice of immobilising drugs is guided by several factors, which include the induction time, dart volume, drug efficacy, adverse physiological effects, and the ability to be antagonised (Kock & Burroughs 2021; Kreeger et al. 2023). White rhinoceros are typically immobilised with drug combinations that include etorphine, an ultra-potent opioid (Wenger et al. 2007; Kock & Burroughs 2021; Kreeger et al. 2023). Although etorphine is highly effective, especially for field immobilisations, it causes metabolic and cardiorespiratory derangements that can result in hypoxaemia, hypercapnia, acidaemia, tachycardia, hypertension, hyperthermia, muscle tremors, and hypermetabolism, potentially resulting in morbidity and mortality (Wenger et al. 2007; Boardman et al. 2014; Miller et al. 2013; De Lange et al. 2017; Buss et al. 2016 & 2018). In combination with etorphine, sedative or tranquilising drugs (*viz.* from alpha-2 adrenoreceptor agonists, benzodiazepine agonists, and butyrophenone drug classes) are sometimes added to the dart because it is thought that they might potentially reduce the necessary etorphine dose, mitigate deleterious physiological responses, enhance immobilisation, reduce excitement, and induce muscle relaxation (Kock et al. 1995). These central nervous system modulating drugs have been combined with etorphine with the intention of reducing morbidity and mortality risks associated with immobilisation in white rhinoceros.

Azaperone, a butyrophenone derivative, is a dopamine receptor antagonist and potentiates opioid-induced immobilisation

in rhinoceros (Swan 1993; Kock & Burroughs 2021; Posner 2018; Gaudio et al. 2020; Wenker et al. 1997). In immobilised white rhinoceros, it also antagonises alpha-1 adrenoreceptors resulting in peripheral vasodilation, thereby reducing etorphine-induced hypertension, but not tachycardia (Buss et al. 2016). Medetomidine, a potent alpha-2 adrenoreceptor agonist, has analgesic, sedative, and muscle relaxant effects, which are speculated to enhance induction and immobilisation quality (Meuffels et al. 2022). Midazolam, a benzodiazepine agonist, in some species also causes sedation and muscle relaxation (Muir et al. 2007; Langhout et al. 2016). When combined with potent opioids, midazolam is postulated to reduce muscle rigidity and improve ventilation during immobilisation, consequently reducing the risk of capture myopathy (Plumb 2018; Langhout et al. 2016).

Despite the perceived beneficial effects of combining etorphine with azaperone, medetomidine or midazolam in the immobilisation of white rhinoceros, there have been a few studies on these combinations aimed at comparing their immobilisation effects as well as assessing other physiological and clinical outcomes (De Lange et al. 2017; Nasr et al. 2021; Buss et al. 2022). There are also conflicting anecdotal opinions as to which combination offers the fastest induction and safest immobilisation with the least physiological disruptions (Swan 1993). The immobilising and clinical effects of the different combinations have not yet been evaluated in a properly controlled comparative study.

Therefore, this study evaluated the immobilising, physiological and clinical effects of four drug combinations: etorphine, etorphine-azaperone, etorphine-medetomidine, and etorphine-midazolam. The aim was to determine which drug combination provided the fastest and best immobilisation in terms of immobilisation induction, quality, and safety based on key clinical, physiological, metabolic, cardiovascular and respiratory-related measurements. Additionally, we assessed the effects of butorphanol administration, a cardiorespiratory support treatment commonly given once white rhinoceros are immobilised, on clinical outcomes when the different drug combinations were used.

Materials and methods

Animals and environment

Eight healthy white rhinoceros (sub-adult males) were used in this study. The study was conducted considering animal research reporting for *in vivo* experiments (ARRIVE guidelines 2.0). They were randomly selected and captured from the wild (convenience sample), then brought into purpose-built holding facilities (bomas) where they were held for 120 days at Veterinary Wildlife Service, Skukuza, Kruger National Park, South African National Parks (SANParks), Republic of South Africa (-24.98984 S 31.59263 E, altitude 317 M). The study was conducted between June and August 2021, with animal immobilisations from 06:30 to 13:00 hours. Ambient temperatures during immobilisations ranged from 17.6 to 27.2 °C (mean 22.5 ± 3.2 °C). Rhinoceros body mass ranged from 1104 to 1438 kg (mean 1312 ± 117 kg) across the trial period. The study was approved by the Research and Animal Ethics Committees of the University of Pretoria (REC011-21) and SANParks Animal Use and Care Committee (011-20).

Capture, boma housing, and study design

The animals were captured in the field by helicopter darting, loaded into custom-made crates, and transported by truck to the holding facilities. During the capture, a physical examination was performed, and blood samples were collected for haematology and serum biochemistry analysis to determine the health status of the animals. A long-acting tranquiliser, 60–80 mg zuclopenthixol acetate (Clopixol-Acuphase® 50 mg/ml, H. Lundbeck Pty. Ltd., North Riding, South Africa), was administered to each rhinoceros intramuscularly (IM) before transport. The rhinoceros were housed individually within the holding facilities in adjacent compartments and cared for according to SANParks Standard Operating Procedure for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife. The enclosures were constructed with vertically spaced wooden poles supported by a metal framework, and each compartment was 25 × 50 m in size. Ample shade was available and fresh water was provided *ad lib*. The animals were fed a mixture of 50% teff (*Eragrostis tef*) and 50% lucerne (*Medicago sativa*). A standardised scoring system for appetite, faecal consistency, faecal volume, and behaviour was used to assess boma adaptation (Miller et al. 2016). Health status and body condition scores were assessed and recorded daily. Once the rhinoceros adapted to the bomas (over 4–6 weeks), they were enrolled into the prospective repeated-measures randomised cross-over study where each animal was immobilised four times with different drug combinations (treatments – see below), in a random order, with a two-week rest and washout period between treatments.

Drugs used and chemical immobilisation

Treatments consisted of four immobilising drug combinations: etorphine only (Captivon® 9.8 mg/ml, Wildlife Pharmaceuticals, Nelspruit, South Africa) referred to hereafter as the 'control', etorphine plus azaperone (Stresnil® 40 mg/ml, Janssen Pharmaceuticals, Halfway House, South Africa) referred to as 'azaperone', etorphine plus medetomidine (40 mg/ml, Wildlife Pharmaceuticals, Nelspruit, South Africa) referred to as 'medetomidine', and etorphine plus midazolam (Sedolam® 50 mg/ml, Wildlife Pharmaceuticals, Nelspruit, South Africa) referred to as 'midazolam'. Drugs were administered intramuscularly (IM) by dart (darting – see below).

The etorphine dose was 2.5 mg (1000–1250 kg) or 3 mg (1250–1500 kg) depending on body mass, to achieve a target dose of approximately 0.002 mg/kg, as described by Buss et al. 2018. Azaperone and midazolam were given at 5-times the etorphine dose in mg (target dose of 0.01 mg/kg) and medetomidine at 2.5-times the etorphine dose in mg (target dose of 0.005 mg/kg). Treatment administration was randomised using an online random number generator (https://www.randomizer.at/).

Twelve minutes after the animals became recumbent, following the administration of a treatment, they were given butorphanol (Butanil®, 50 mg/ml Wildlife Pharmaceuticals, Nelspruit, South Africa) at a dose of 10-times the etorphine dose in mg (target dose of 0.02 mg/kg) intravenously (IV). To account for possible

poor dart administration, animals that did not become immobilised within 15 minutes of darting were antagonised with naltrexone (40 mg/ml, Kyron Laboratories, Benrose, South Africa) at 20-times the dose of etorphine in mg, IM, excluded from that trial, and re-immobilised with this treatment after a two-week washout period as per the treatment randomisation plan.

Darting

Immobilising drugs were administered using 3 ml darts with a 2 x 60 mm non-collared needle fired by a carbon dioxide compressed gas powered dart gun (Dan-Inject, International S.A., Skukuza, South Africa). Darting of rhinoceros in the holding facility was done by an experienced veterinarian, and all darts were fired into the nuchal hump trapezius muscle. In the immobilised animal, eye ointment was applied to prevent desiccation, a blindfold was placed over the eyes, and then it was rolled into lateral recumbency for instrumentation.

Clinical monitoring and data collection

The induction time was defined as the time from darting to when the animal could be safely placed into lateral recumbency. The time to the onset of the first signs of drug effects (occurrence of ataxia) was recorded and data were collected at T_{10}, T_{15}, T_{20}, T_{30} and T_{40} minutes. An experienced veterinarian scored the quality and level of immobilisation using a published scoring system (Table I) (Haw et al. 2015). Body position was alternated between left and right lateral recumbency for each immobilisation.

Table I: Scoring system used to assess the quality of the immobilisation

Level	Description
Level 1	No effect
Level 2	Cannot handle safely (standing)
Level 3	Can handle safely (standing)
Level 4	Recumbent, ear movements, tail curled
Level 5	Recumbent, fully relaxed, no ear movements, tail relaxed
Level 6	Recumbent, excessive depth, dilated pupils, bradycardia, respiratory rate < 3 breaths per minute

Muscle tremors were scored using a published scale (Buss et al. 2018) (Table II). Rectal temperature was measured using a calibrated rectal thermometer modified to include a protective probe sheath (HI 98509 Checktemp 1, Hanna Instruments, Woonsocket, USA) inserted 9–10 cm into the rectum against the rectal wall. Heart rate was monitored by auscultation of the thorax. A Nonin pulse oximeter with a transflectance probe (Nonin PalmSAT 2500A, Kyron Laboratories, Johannesburg, South Africa) placed under the third eyelid was used to determine pulse rate and peripheral arterial oxygen haemoglobin saturation (SpO_2). Respiratory rate was monitored by counting thoracic and abdominal excursions and air movement at the nares.

At the conclusion of data collection, after all the instruments were removed, the rhinoceros was stimulated to stand and guided into a crate. The crate was then suspended from a hanging scale (Crane scale, White River, Mpumalanga, South Africa) to determine the body mass of the rhinoceros. Thereafter, the effects of etorphine were fully antagonised with naltrexone (40 mg/ml IM, Kyron Laboratories, South Africa) at 20-times the etorphine dose in mg and those rhinoceros that were administered with medetomidine were given atipamezole (50 mg/ml IM, Vet Tech [Pty] Ltd, Midrand, South Africa) at five-times the medetomidine dose in mg). The rhinoceroses were returned to their respective compartments within the holding facility.

Table II: Scoring system used to rank tremor intensity based on visual observations

Observation	Score
No visible tremors	1
Slight tremors – fine trembling of the feet and legs	2
Mild tremors – fine trembling of the legs, feet, shoulder, and chest	3
Moderate tremors – gross trembling of the legs, feet, shoulder and chest	4
Severe tremors – gross trembling of the whole body and head	5

Data analysis

Physiological data, collected over the study time points (T_{10}, T_{15}, T_{20}, T_{30}, T_{40}) and between treatments, were compared using a linear mixed effects model. The physiological data, which consisted of rectal temperature, heart rate, respiratory rate, and peripheral arterial oxygen-haemoglobin saturation (SpO_2), were designated as response variables. Time and drug combination were designated as fixed effects, and rhino ID was designated as a random effect. A temporal autocorrelation term was included in the model. The residuals were calculated for each variable, and a Shapiro-Wilk test and a Kolmogorov-Smirnov test were used to confirm that the residuals were normally distributed. If the data was not normally distributed it was log transformed. Significant values were identified after a Bonferroni correction for multiple pairwise comparisons.

Differences between treatments in the doses of etorphine and butorphanol (after the animal was weighed the actual dosage could be calculated), and differences in times from darting to showing first signs of drug effects and times from darting to recumbency (induction times) were determined using a one-way ANOVA and Tukey's post-hoc test. The difference in muscle tremors and immobilisation scores between treatments at each time point, and over time, was analysed using Friedman multiple comparison and Dunn's tests. Statistical analyses were performed using GraphPad Prism version 9.00 (GraphPad Software, San Diego, California, USA), with differences considered to be statistically significant if the *p*-values were less than or equal to 0.05 (i.e. $p \leq 0.05$).

Results

The mean ± SD drug doses administered were: etorphine 0.0022 ± 0.0001 mg/kg; azaperone 0.011 ± 0.0004 mg/kg; medetomidine 0.005 ± 0.0003 mg/kg; midazolam 0.011 ± 0.0004

mg/kg; and butorphanol 0.021 ± 0.0009 mg/kg. There were no differences in the dose of etorphine or butorphanol between the four treatments (p = 0.99). Mean body mass was 1312 ± 117 kg. The time from darting to showing first signs of drug effects was 371 ± 82 s for the etorphine-only control, 292 ± 55 s for the azaperone treatment, 295 ± 29 s for the medetomidine treatment and 307 ± 51 s for the midazolam treatment. There were no differences between the times to first signs of drug effects between treatments, except for the azaperone treatment, where they occurred earlier in the azaperone treatment compared to the control (p = 0.048) (Fig. 1A).

Azaperone (506 ± 84 s, p = 0.0001), medetomidine (604 ± 113 s, p = 0.002), and midazolam (637 ± 152 s, p = 0.008) treatments all produced significantly faster induction times compared to the control (855 ± 135 s), but there were no significant differences in induction times between the azaperone, medetomidine and midazolam treatments (Fig. 1B).

The level (p = 0.728) and quality (p = 0.999) of the immobilisation did not differ between the treatments, and it did not change over time, except in the azaperone treatment where the immobilisation level was greater at T_{20} (p = 0.026) and T_{30} (p = 0.026) compared to T_{10} (Fig. 2A).

At all the time points, there was no significant difference in tremor scores between the treatments (p > 0.05). However, in all treatments, tremor scores were initially higher at T_{10}, thereafter they decreased significantly compared to T_{10} (Fig. 2B): control treatment (T_{20} p = 0.005, T_{30} p = 0.002, T_{40} p = 0.001), azaperone treatment (T_{20} p = 0.002, T_{30} p = 0.001, T_{40} p = 0.001), medetomidine (T_{40} p = 0.029) and midazolam treatment (T_{20} p = 0.005, T_{30} p = 0.002, T_{40} p = 0.001).

The rectal temperatures of the rhinoceros ranged from 36.3 to 39.0 °C. Mean rectal temperature was 37.5 ± 1 °C for the control, 37.4 ± 0.8 °C for the azaperone treatment, 37.6 ± 0.8 °C for medetomidine treatment, and 37.2 ± 0.3 °C for midazolam treatment. There were no differences in the rectal temperatures between the treatments (p > 0.05) or within the treatments over time (p > 0.05).

In all the treatments, heart rate tended to decrease over time. Compared to T_{10}, this decrease in heart rate was significant from T_{15} to T_{40} in the azaperone treatment (T_{15} p = 0.024, T_{20} p =0.002, T_{30} p = 0.0002, T_{40} p = 0.001), and T_{20} to T_{40} in the midazolam treatment (T_{20} p = 0.0004, T_{30} p = 0.001, T_{40} p = 0.001) and in the control (T_{20} p = 0.0001, T_{30} p = 0.0001, T_{40} p = 0.0001), but there was no significant decrease from T_{10} in the medetomidine treatment (T_{15} p = 0.138, T_{20} p = 0.094, T_{30} p = 0.104, T_{40} p = 0.178) (Fig. 3A). Heart rates during the medetomidine treatment were

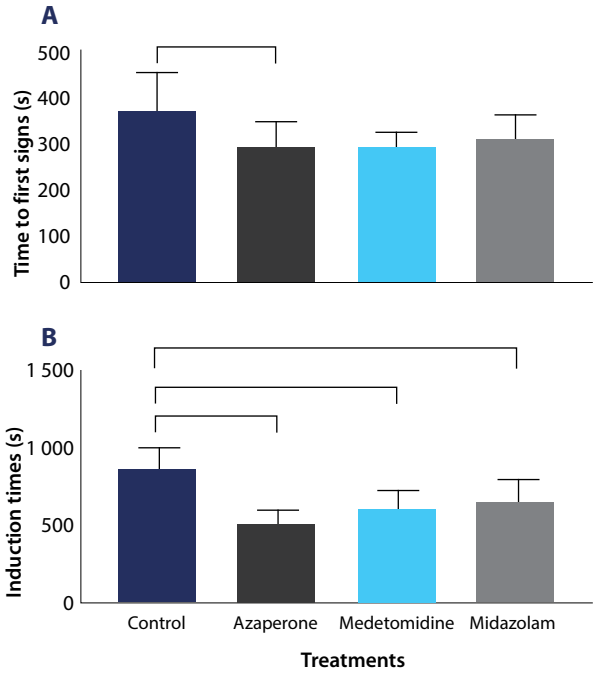

Figure 1: Graphs of the (A) time (mean and SD) from darting to showing first signs of drug effects (Time to first signs), and (B) time (mean and SD) from darting to sternal recumbency (Induction time) for eight white rhinoceros immobilised with etorphine only (control), etorphine + azaperone (azaperone treatment), etorphine + medetomidine (medetomidine treatment) and etorphine + midazolam (midazolam treatment) in a randomised repeated-measures cross-over study. Brackets above bars indicate where significances (p < 0.05) occurred between the treatments.

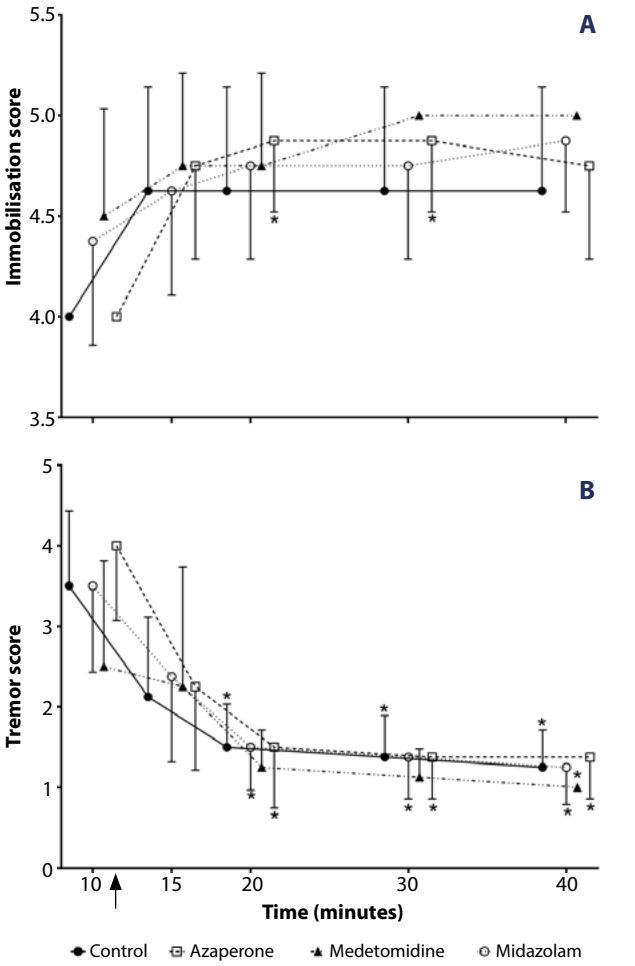

Figure 2: Graphs of the (A) immobilisation scores (median and range) and (B) tremor scores (median and range) of eight white rhinoceros immobilised with etorphine only (control), etorphine + azaperone (azaperone treatment), etorphine + medetomidine (medetomidine treatment) and etorphine + midazolam (midazolam treatment) in a randomised repeated-measures cross-over study. The asterisk (*) indicates a significant difference from T_{10} (p < 0.05) within a treatment. The black arrow indicates the time point (T_{12}) at which butorphanol was administered. The symbols are offset at each timepoint to allow for visual clarity.

significantly lower compared to the control (T_{10} $p = 0.016$, T_{15} $p = 0.001$, T_{20} $p = 0.007$, T_{30} $p = 0.004$), azaperone (T_{10} $p = 0.003$, T_{15} $p = 0.001$, T_{20} $p = 0.002$, T_{30} $p = 0.006$, T_{40} $p = 0.018$) and midazolam (T_{10} $p = 0.027$, T_{15} $p = 0.002$, T_{20} $p = 0.009$, T_{30} $p = 0.047$, T_{40} $p = 0.015$) treatments. There were no differences in the heart rates at any of the time points between the control, azaperone, or midazolam treatments.

The pulse rates measured by the pulse oximeter showed similar trends to the manual heart rates with significant differences between the medetomidine and other treatments ($p < 0.05$) i.e. T_{10} (azaperone vs. medetomidine $p = 0.046$) T_{15} (control vs. medetomidine $p = 0.011$, azaperone vs. medetomidine $p = 0.0004$, medetomidine vs. midazolam p = 0.011), T_{20} (control vs. medetomidine $p = 0.031$, azaperone vs. medetomidine $p = 0.002$, medetomidine vs. midazolam $p = 0.023$), T_{30} (control vs. medetomidine $p = 0.037$, azaperone vs. medetomidine $p = 0.007$, medetomidine vs. midazolam $p = 0.042$) and T_{40} (azaperone vs. medetomidine $p = 0.031$) (Fig. 3B)

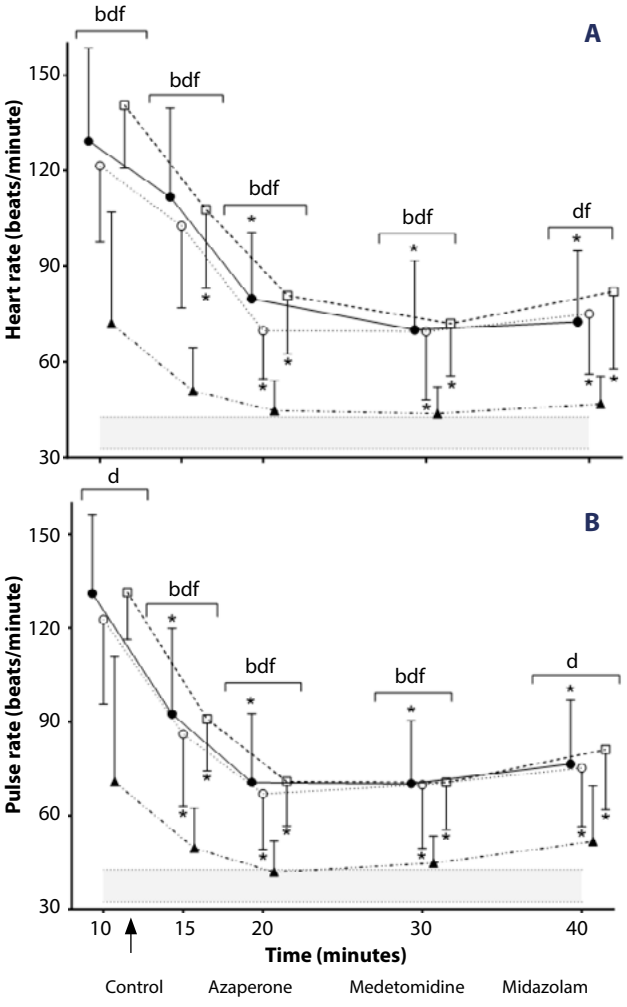

Figure 3: Graphs of the (A) heart rates (mean and SD) measured by auscultation of the thorax and B) pulse rate (mean and SD) measured by a pulse oximeter placed under the third eyelid, from eight white rhinoceros immobilised with etorphine only (control), etorphine + azaperone (azaperone treatment), etorphine + medetomidine (medetomidine treatment) and etorphine + midazolam (midazolam treatment) in a randomised repeated-measures cross-over study. The asterisk (*) indicates significant difference from T_{10} ($p < 0.05$) within a treatment. The black arrow indicates the time point (T_{12}) at which butorphanol was administered. The symbols are offset at each timepoint to allow for visual clarity. The letters indicate significant differences between the treatments as follows: b is control vs. medetomidine, d is azaperone vs. medetomidine, and f is midazolam vs. medetomidine. The grey shaded area represents "normal" heart rates in conscious and unrestrained white rhinoceros (Citino & Bush 2007).

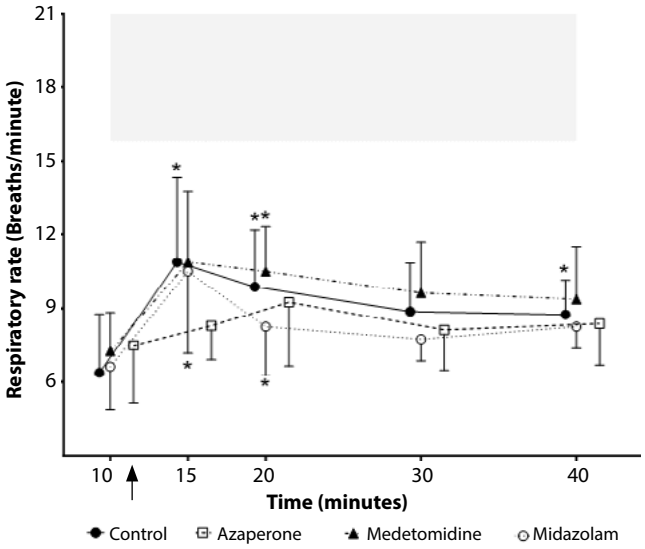

Figure 4: Respiratory rates (mean and SD) measured from the frequency of exhaled air at the nares and abdominal movement from eight white rhinoceros immobilised with etorphine only (control), etorphine + azaperone (azaperone treatment), etorphine + medetomidine (medetomidine treatment) and etorphine + midazolam (midazolam treatment) in a randomised repeated-measures cross-over study with a two-week washout period between treatments. The asterisk (*) indicates significant difference from T_{10} ($p < 0.05$) within a treatment. The black arrow indicates the time point (T_{12}) at which butorphanol was administered. The symbols are offset at each timepoint to allow for visual clarity. The grey shaded area represents the "normal" respiratory rate in conscious and unrestrained white rhinoceros (Citino & Bush 2007).

In all four treatment groups, lower respiratory rates occurred at the start of the immobilisation. Thereafter, the respiratory rates increased and were significantly higher at different time points compared to T_{10} in control (T_{15} $p = 0.003$, T_{20} $p = 0.002$, T_{40} $p = 0.025$), medetomidine (T_{20} $p = 0.032$) and midazolam (T_{15} $p = 0.023$, T_{20} $p = 0.029$) treatments, but not the azaperone treatment (Fig 4). There were no differences in respiratory rates between the treatments ($p > 0.05$).

In all four treatments, a low peripheral arterial oxygen haemoglobin saturation (SpO_2) occurred at the start of immobilisation. Thereafter the SpO_2 increased and was significantly higher at different time points when compared to T_{10} in the control (T_{20} $p = 0.0095$, T_{30} $p = 0.0091$, T_{40} $p = 0.0002$), azaperone (T_{20} $p = 0.0023$, T_{30} $p = 0.0003$, T_{40} $p = 0.0002$) and midazolam treatments (T_{15} $p = 0.0001$ T_{20} $p = 0.0003$, T_{30} $p = 0.0002$, T40 $p = 0.0003$), but not in the medetomidine treatment (Fig. 5). There were no differences in peripheral arterial oxygen haemoglobin saturation concentration between the treatments ($p > 0.05$).

Discussion

All four drug combinations (etorphine only, azaperone, medetomidine, and midazolam treatments) effectively immobilised rhinoceros. Rhinoceros became recumbent and

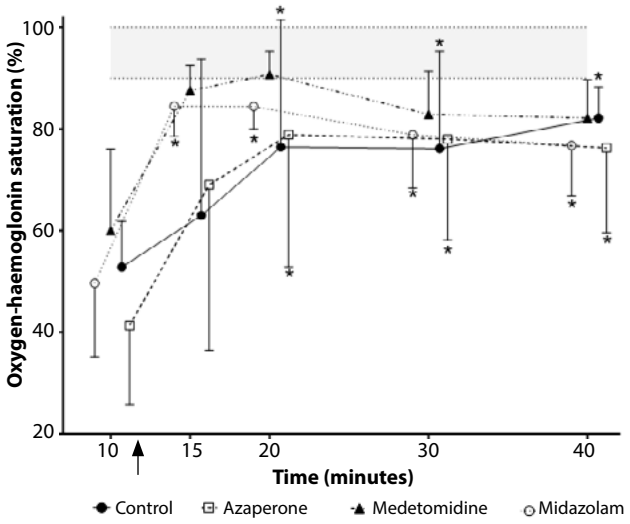

Figure 5: Peripheral arterial oxygen haemoglobin saturation (SpO_2) (mean and SD) measured by a Nonin PalmSAT pulse oximeter with a transflectance probe placed under the third eyelid of eight white rhinoceros immobilised with etorphine only (control), etorphine + azaperone (azaperone treatment), etorphine + medetomidine (medetomidine treatment) and etorphine + midazolam (midazolam treatment) in a randomised repeated-measures cross-over study with a two-week washout period between treatments. The asterisk (*) indicates significant difference from T_{10} ($p < 0.05$) within a treatment. The black arrow indicates the time point (T_{12}) at which butorphanol was administered. The symbols are offset at each time point to allow for visual clarity. The grey shaded area shows "normal" (SpO_2) in white rhinoceros (Mtetwa et al. 2024).

safe to handle, with no difference in immobilisation quality between the treatments, and with little change over time within each treatment. First signs of immobilisation occurred earlier in the azaperone treatment, and induction times were quicker in the azaperone, medetomidine and midazolam treatments compared to the control. Irrespective of treatment, the tremor intensity was highest at the beginning of the immobilisation and decreased over time, which was likely facilitated in part by the butorphanol administration. A key finding is that there were no differences in tremor scores between the treatments. There were no changes over time or differences in rectal temperatures between the treatments and temperatures were mainly within the normal range (36.8 to 37.8 °C, Citino & Bush 2007). Heart and pulse rates were initially elevated and decreased over time in all the treatments, with lower rates in the medetomidine treatment. While no apnoea occurred, hypopnoea was prevalent and severe hypoxaemia initially occurred in all the treatments. Physiological derangements were partially corrected by IV butorphanol administration at 12 min.

To limit variability a repeated measures study design, in a reasonably well-controlled environment which minimised capture-induced stress and other confounding factors associated with field captures, was used. However, the sample size of eight animals was conservative, and the outcome of the data analysis could still have been prone to a Type II error. Repeated measures and boma housing could induce chronic stress, which we tried to account for by randomising the treatments over time in the eight animals. Furthermore, to minimise stress and its confounding influence on the physiological effects of the four treatments, the animals were given a two-week rest and washout period between treatments. For safety and ethical reasons, we did not blind observers to the treatments used, and to eliminate inter-observer variation one experienced person on the team did the scoring.

Induction times were shortened when azaperone, midazolam, or medetomidine were combined with etorphine. This is an important result because reduced induction times help minimise the risk that animals, when darted in the field, encounter from environmental hazards that may pose a risk to their welfare. Reduced induction times may also translate into improved clinical outcomes because it usually is associated with reduced cardiopulmonary and metabolic derangements, which are often exacerbated by prolonged running after darting (Morkel 1994; Haw et al. 2015). Additionally, a shortened induction time may lead to reduced exposure of the rhinoceros to other environmental stressors induced by capture, therefore reducing the overall stress response.

Sedative and tranquilising drugs are anecdotally believed to potentiate the immobilising effects of ultra-potent opioids and are therefore often termed "synergistic" drugs, (Kock & Burroughs 2021). Of these synergistic drugs, until now only azaperone has been shown to definitively potentiate the immobilising effects of etorphine by reducing induction times in rhinoceros (Buss et al. 2022). In contrast, this potentiation was not demonstrated in blesbok, where azaperone did not induce shorter immobilisation times when used with etorphine (Gaudio et al. 2020). How sedative and tranquilising drugs enhance opioid-induced immobilisation is not well understood. It has been postulated that azaperone potentiates the effects of opioids through its antagonistic effects on central dopamine receptors, where direct interactions between the pharmacological effects of etorphine and azaperone on their receptors result in reduced induction times and enhanced opioid-induced catatonia (Buss et al. 2022). For midazolam and medetomidine, their potentiation of etorphine may be the result of receptor-induced pharmacological effects which produce central muscle relaxation and sedation, effects which are likely additive to the catatonic and sedative effects of etorphine. Despite differences in induction times between the other treatments and the control treatment, there were no differences in immobilisation quality. Muscle tremors, which influence immobilisation quality, likely cause increased muscle metabolism, resulting in increased oxygen utilisation and carbon dioxide production, which can result in hypoxaemia and hypercapnia (Buss et al. 2018). Therefore, the need to reduce muscle tremors during immobilisation is important, and it is believed that sedative drugs with good muscle relaxant effects, like alpha-2 adrenergic drugs and benzodiazepines, are suitable for this purpose (Van Zijll Langhout et al. 2016; De Lange et al. 2017; Pohlin et al. 2020a). Alpha-2 adrenergic drugs are often combined with etorphine (Kock & Burroughs 2021, but their ability to reduce muscle tremors had yet to be shown in white rhinoceros immobilisations. The benzodiazepine midazolam is also often combined with etorphine, and this combination has been used in the immobilisation of white rhinoceros and purportedly reduces muscle tremors (Van Zijll Langhout et al. 2016). However, when midazolam was compared to azaperone

in etorphine-based drug combinations, there was no difference in the tremors seen initially in immobilised white rhinoceros (Nasr et al. 2021). These findings are similar to the present study, in which we did not see any differences between the midazolam and azaperone treatments. Interestingly, there were also no differences between midazolam and the control treatment, and the medetomidine treatment did not reduce tremors compared to the control and other treatments. Although there were no differences in tremor scores initially (at T_{10} and T_{15}) between the treatments, within each treatment, these scores decreased over time. These decreases were attributed to the partial opioid antagonist effects of butorphanol that we administered at $T_{12.}$ That there were no differences in the tremor scores between the treatments, and that butorphanol decreased them in all treatments, suggests that tremors are caused primarily by etorphine.

Butorphanol, a partial mixed opioid agonist-antagonist, has been shown to successfully reduce tremor intensity when administered to immobilised rhinoceros (De Lange et al. 2017; Buss et al. 2018; Buss et al. 2024; Boesch et al. 2025). Additionally, and in parallel to the reduction in tremors, butorphanol reduces metabolism (decreases oxygen consumption and carbon dioxide production) which benefits immobilised rhinoceros by reducing the risk of hyperthermia and improving their partial pressure of oxygen (PaO_2) and carbon dioxide in arterial blood ($PaCO_2$), thereby reducing hypoxaemia, tissue hypoxia and hypercapnia, and improving overall immobilisation safety (Buss et al. 2018).

The mean rectal temperature of the rhinoceros in all the treatments ranged from 36.3–37.8 ˚C with no difference between or within the treatments. The immobilisations were carried out in the cooler months and early mornings, which may explain why some animals had moderately lower than normal (< 36.6 °C) body temperatures. The drugs used for immobilisation often disrupt normal thermoregulation, through central depressant effects on the hypothalamus, making animals "poikilothermic" (Kock & Burroughs 2021). However, some of the animals also had moderately elevated (> 37.2 °C) body temperatures. These increases were unlikely associated with ambient conditions, but rather more likely related to the stress induced by capture (i.e. stress-induced hyperthermia), and, or hyperthermia caused by hypermetabolism from etorphine-induced tremoring. That the rhinoceros did not develop severe hyperthermia, which is often seen during field captures (Haw et al. 2015 and personal observations), could be explained by the fact that the stress of capture was limited by darting in the bomas and that the administration of butorphanol possibly also minimised heat production from etorphine-induced tremoring and hypermetabolism. Vascular effects of the drugs may have also played a role in the body temperatures; medetomidine can cause peripheral vasoconstriction and potential retention of body heat, whereas azaperone can induce vasodilation, facilitating the offloading body heat into the environment on cool days (Kock & Burroughs 2021). However, these effects were obviously not profound enough to create significant differences in body temperatures between the treatments.

While the "synergistic" drugs did not influence body temperatures, they did have effects on heart and pulse rates. When immobilised with the medetomidine treatment, heart rates throughout the immobilisation were mainly lower compared to the control and other treatments. The heart was also challenging to auscultate and could be described clinically as a "whispering heart rate". Alpha-2 adrenergic agonists are known to cause bradycardia and decrease contractility, as a reflex response to hypertension caused by drug-induced peripheral vasoconstriction, and centrally mediated reduction in sympathetic tone (West et al. 2024). Initially, the heart and pulse rates were elevated above normal (32–42 beats/minute, Citino & Bush 2007) in all the treatments and generally decreased from 15–20 minutes into the immobilisation. The initial elevation could be as a result of the stress induced by darting or the sympathetic effects of etorphine (Buss et al. 2016; Boesch et al. 2025), and the decrease could be ascribed to the administration of butorphanol, which has been shown to decrease heart rates in rhinoceros immobilised with etorphine and etorphine + azaperone (Bush et al. 2011; Buss et al. 2024). It could also be attributed to decreasing stress responses or metabolism of etorphine over the immobilisation period, which has been described in other species (Meyer et al. 2008; Laubscher et al. 2022).

In contrast to heart rates, initially, in all the treatments, the rhinoceros had lower than normal (range 16–23 breaths/minute, Citino & Bush 2007) respiratory rates. This hypopnoea was likely caused by etorphine depressing neurons in the brainstem's respiratory centres that regulate breathing and respiratory rhythm, leading to a reduction of breathing rate and depth (Buss et al. 2015; Portas 2004; Kock & Burroughs 2021). Opioids also depress respiratory rates and cause hypoventilation by increasing chest wall rigidity, which limits the expansion of the lungs and rib cage, leading to less negative intrathoracic pressure during inspiration in immobilised rhinoceros (Buss et al. 2015; Portas 2004). In all the treatments, except azaperone, respiratory rates increased transiently following intravenous butorphanol administration. In etorphine-immobilised rhinoceros butorphanol has been shown to transiently increase respiratory rates, by possibly reducing the mu-opioid receptor agonist effects of etorphine (Haw et al. 2015; Buss et al. 2015 & 2018; Boesch et al. 2025). Why butorphanol did not increase respiratory rates in the azaperone-treated rhinoceros is not clear and is in contrast to the findings of Buss et al. 2024 where a transient doubling of respiratory rates occurred after its administration.

Similar to the trends in respiratory rate, initially in all treatments the rhinoceros had low peripheral oxygen haemoglobin saturations (indicating severe hypoxaemia - SpO_2 < 70%), that mainly improved post-butorphanol administration at T_{15}–T_{20}. This improvement was sustained throughout the immobilisation, but although hypoxaemia improved substantially the animals mostly remained hypoxaemic (SpO_2 < 90%, Mtetwa et al. 2024). Severe hypoxaemia is a consistent finding in white rhinoceros immobilised with etorphine-based drug combinations. This hypoxaemia results from a myriad of drug and immobilisation-induced adverse effects, which include respiratory depression resulting in hypoventilation, hypermetabolism, and, or

intrapulmonary pathophysiology affecting gas exchange such as ventilation-perfusion mismatch, shunting and, or impairment of gas diffusion (Portas 2004; Wenger et al. 2007; Miller et al. 2013; Buss et al. 2015; Boesch et al. 2025;).

In etorphine-immobilised rhinoceros, butorphanol helps corrects hypoxaemia not only by transiently improving ventilation, but also mainly by reducing etorphine's sympathomimetic and hypermetabolic effects, and improving pulmonary gas exchange (Buss et al. 2018 & 2024; Boesch et al. 2025). Butorphanol, an opioid agonist-antagonist with antagonistic, or partial agonist, effects at mu-opioid receptors and agonist effects on kappa-opioid receptors, is used as a preferred drug to partly counteract the adverse respiratory effects and to improve hypoxaemia in etorphine-immobilised rhinoceros (Kock & Burroughs 2021; West et al. 2024).

White rhinoceros are particularly sensitive to the adverse effects of etorphine on their cardiorespiratory system, and mortalities associated with cardiorespiratory failure and severe hypoxaemia have been reported (Kock et al. 1995; Meyer et al. 2018). Little work has been done to understand how the "synergistic" drugs change rhinoceros' cardiorespiratory function when combined with etorphine, and how butorphanol affects these changes. Although, through our clinical measures in this study, we have shown some clinical effects, more in-depth monitoring and analysis are needed to better understand what these drugs do to rhinoceros, and if they have any other potential benefits or harms. These studies may benefit from including more rhinoceros using the information from our study to perform an *a priori* sample size calculation.

Conclusion

We found that all four drug combinations were effective in immobilising white rhinoceros. Azaperone, midazolam, and medetomidine all potentiated the immobilisation effects of etorphine, leading to reduced induction times when compared to the control of etorphine only. In field settings, shorter induction times induced by these "synergistic" drugs may reduce muscle overexertion and hypermetabolism, potentially reducing the risk of myopathy and other metabolic derangements. However, whether these shorter induction times and physiological benefits occur would need to be confirmed in free-living rhinoceros. In this study there were no differences in the induction times between the "synergistic" drug treatments. Furthermore, all the drug combinations caused equally severe tremors, hypopnoea, hypoxaemia, and tachycardia, except for the medetomidine treatment, which caused lower heart rates. Therefore, in general, none of the four drug combinations provided a better risk profile during immobilisation. Fortunately, butorphanol improved many of the physiological derangements induced by all the drug combinations. However, to better understand the effects and potential differences of these drug combinations, further in-depth cardiorespiratory and metabolic assessment should be done to better understand the risks and benefits of each combination.

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Competing interests

The authors have no personal or financial relationship that may influence the writing of this article.

Ethics

The study was approved by the Research and Animal Ethics Committees of the University of Pretoria (REC011-21) and SANParks Animal Use and Care Committee (011-20).

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References

Boardman, W.S., Caraguel, C.G., Raath, J.P., et al., 2014, Intravenous butorphanol improves cardiopulmonary parameters in game-ranched white rhinoceroses (Ceratotherium simum) immobilised with etorphine and azaperone, *Journal of Wildlife Diseases* 50(4), 849-57. https://doi.org/10.7589/2013-12-327.

Boesch, J.M., Gleed, R.D., Buss, P.E., et al., 2025, Etorphine induces pathophysiology in immobilised white rhinoceros through sympathomimesis that is attenuated by butorphanol, *Conservation Physiology* 13(1), coaf009. https://doi.org/10.1093/conphys/coaf009.

Bush, M., Citino, S.B., Lance, W.R., 2011, The use of butorphanol in anaesthesia protocols for zoo and wild mammals, *Fowler's Zoo and Wild Animal Medicine: Current Therapy*, 7, 596-603. https://doi.org/10.1016/B978-1-4377-1986-4.00077-9.

Buss, P., Olea-Popelka, F., Meyer, L., et al., 2015, Evaluation of cardiorespiratory, blood gas, and lactate values during extended white rhinoceros (Ceratotherium simum) immobilisation, *Journal of Zoo and Wildlife Medicine* 46(2), 224-233. https://doi.org/10.1638/2014-0089R.1.

Buss, P., Miller, M., Fuller, A., et al., 2016, Cardiovascular effects of etorphine, azaperone, and butorphanol combinations in chemically immobilised captive white rhinoceros (Ceratotherium simum), *Journal of Zoo and Wildlife Medicine* 47(3), 834-843. https://doi.org/10.1638/2015-0298.1.

Buss, P., Miller, M., Fuller, A., et al., 2018, Postinduction butorphanol administration alters oxygen consumption to improve blood gases in etorphine-immobilised white rhinoceros, *Veterinary Anaesthesia and Analgesia* 45(1), 57-67. https://doi.org/10.1016/j.vaa.2017.03.008.

Buss, P., Miller, M., Fuller, A., et al., 2022, Effect of azaperone on induction times in etorphine-immobilised white rhinoceros (Ceratotherium simum), *The Journal of Wildlife Diseases* 58(1), 245-247. https://doi.org/10.7589/JWD-D-21-00072.

Buss, P., Miller, M., Fuller, A., et al., 2024, Effects of butorphanol on respiration in white rhinoceros (Ceratotherium simum) immobilised with etorphine-azaperone, *The Journal of Wildlife Diseases* 60(2), 388-400. https://doi.org/10.7589/JWD-D-23-00034.

Citino, S.B., Bush, M., 2007, Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceroses (Ceratotherium simum), *Journal of Zoo and Wildlife Medicine* 38(3), 375-379. https://doi.org/10.1638/2006-0007R1.1.

De Lange, S.S., Fuller, A., Haw, A., et al., 2017, Tremors in white rhinoceroses (Ceratotherium simum) during etorphine-azaperone immobilisation, *Journal of*

the South African Veterinary Association 88(0), e1-e10. https://doi.org/10.4102/jsava.v88i0.1466.

Emslie, R., 2020, White rhino (Ceratotherium simum), The IUCN Red List of Threatened Species 2020: T4185A45813880. Available from: https://rhinoresourcecenter.com/wp-content/uploads/2025/06/1589193466.pdf. Accessed on 04 June 2025.

Gaudio, E., Laubscher, L.L., Pfitzer, S., et al., 2020, Immobilisation quality and cardiopulmonary effects of etorphine alone compared with etorphine azaperone in blesbok (Damaliscus pygargus phillipsi), *Veterinary Anaesthesia and Analgesia* 47, 258-536. https://doi.org/10.1016/j.vaa.2019.10.012.

Haw, A., Hofmeyr, M., Fuller, A., et al., 2015, Butorphanol with oxygen insufflation improves cardiorespiratory function in field-immobilised white rhinoceros (Ceratotherium simum), *Journal of the South African Veterinary Association* 86(1), E1-E10. https://doi.org/10.4102/jsava.v86i1.1276.

Kock, M.D., & Burroughs, R., 2021, Chemical and physical restraint of African wild animals, Third Edition, Michael D. Kock, IWVS (Africa), Greyton, South Africa.

Kock, M.D., Morkel, P., Atkinson, M., et al., 1995, Chemical immobilisation of free-ranging white rhinoceros (Ceratotherium simum) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine, and detomidine, *Journal of Zoo and Wildlife Medicine* 26(2), 207-219.

Kreeger, T.J., Arnemo, J.M., Caulkett, N.A., et al., 2023, Handbook of wildlife chemical immobilisation. 6th edn, South Freeport, Maine, USA: CWS Press.

Langhout, M.V.Z., Caraguel, C.G., Raath, J.P., Boardman, W.S., 2016. Evaluation of etorphine and midazolam anaesthesia, and the effect of intravenous butorphanol on cardiopulmonary parameters in game-ranched white rhinoceroses (ceratotherium simum). *J Zoo Wildl Med* 2016 47(3), 827-33. https://doi.org/10.1638/2015-0167.1.

Laubscher, L.L., Meyer, L.C.R., Laurence, M., et al., 2022, A comparison of immobilisation quality and cardiorespiratory effects of etorphine-azaperone versus etorphine-midazolam combinations in blesbok, *Journal of the South African Veterinary Association* 93(1), 8-16. https://doi.org/10.36303/JSAVA.2022.93.1.491.

Meuffels, J., Bertschinger, H., Tindall, B., et al., 2022, Arterial blood gases and cardiorespiratory parameters in etorphine-medetomidine-midazolam immobilised free-ranging and game-farmed southern white rhinoceroses (Ceratotherium simum) undergoing electro-ejaculation, *Frontiers in Veterinary Science* 9, 862100. https://doi.org/10.3389/fvets.2022.862100.

Meyer, L.C.R., Hetem, R.S., Fick, L.G., et al., 2008, Thermal, cardiorespiratory and cortisol responses of impala (Aepyceros melampus) to chemical immobilisation with four different drug combinations, *Journal of the South African Veterinary Association* 79(3), 121-9. https://doi.org/10.4102/jsava.v79i3.258.

Meyer, L.C.R., Fuller, A., Hofmeyr, M., et al., 2018, Use of butorphanol and diprenorphine to counter respiratory impairment in the immobilised white rhinoceros (Ceratotherium simum), *Journal of the South African Veterinary Association* 89, 1-8. https://doi.org/10.4102/jsava.v89i0.1683.

Miller, M., Buss, P., Joubert, J., et al., 2013, Use of butorphanol during immobilisation of free-ranging white rhinoceros (Ceratotherium simum), *Journal of Zoo and Wildlife Medicine* 44(1), 55-61. https://doi.org/10.1638/1042-7260-44.1.55.

Miller, M., Kruger, M., Kruger, M., et al., 2016, A scoring system to improve decision making and outcomes in the adaptation of recently captured white rhinoceroses (Ceratotherium simum) to captivity, *Journal of Wildlife Diseases* 52, S78-S85. https://doi.org/10.7589/52.2S.S85.

Morkel, P., 1994, Chemical immobilisation of the black rhinoceros (Diceros bicornis), Proceedings of a South African Veterinary Association Symposium on Rhinos as Game Ranch Animals, Onderstepoort, South Africa (pp. 128-135).

Mtetwa, T.K., Snelling, E.P., Buss, P.E., et al., 2024, Reliability, clinical performance and trending ability of a pulse oximeter and pulse co-oximeter, in monitoring blood oxygenation, at two measurement sites, in immobilised white rhinoceros (Ceratotherium simum), *BMC Veterinary Research* 20, 319. https://doi.org/10.1186/s12917-024-04179-5.

Muir, W.W., Hubbell, J.A.E., Bednarski, R.M., et al., 2007, Handbook of Veterinary Anaesthesia. 4th edn, pp 35-36, Mosby Elsevier. Louis, Missouri, USA.

Nasr, M., Meyer, L.C.R., Buss, P., et al., 2021, Muscle tremors observed in white rhinoceroses immobilised with either etorphine-azaperone or etorphine-midazolam: An initial study, *Journal of the South African Veterinary Association* 92(0): e1-e3. https://doi.org/10.4102/jsava.v92i0.2142.

Plumb, D.C., 2018. Veterinary drug handbook. Pocket 9 edn. Iowa, USA: Wiley-Blackwell Publishing. pp. 624-626.

Pohlin, F., Buss, P., Hooijberg, E. H., et al., 2020a, midazolam alters acid-base status less than azaperone during the capture and transport of Southern White Rhinoceroses (Ceratotherium simum), *Animals* 10(8), 1323. https://doi.org/10.3390/ani10081323.

Portas, T., 2004, A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species, *Australian Veterinary Journal* 82(9), 542-549. https://doi.org/10.1111/j.1751-0813.2004.tb11196.x.

Posner, L.P., 2018, Sedatives and tranquillisers, in J.E. Riviere, M.G. Papich (eds.), Veterinary Pharmacology and Therapeutics, 10th Edition, pp. 324-368, John Wiley & Sons, Inc., Newark, USA.

Swan, G.E., 1993, Drugs are used for the immobilisation, capture, and translocation of wild animals, in McKenzie, A.A. (ed.) The Capture and Care Manual, Pretoria, South Africa: The South African Veterinary Foundation, pp. 3-59.

Van Zijll Langhout, M., Caraguel, C.G.B., Raath, J.P., et al., 2016, Evaluation of etorphine and midazolam anaesthesia, and the effect of intravenous butorphanol on cardiopulmonary parameters in game-ranched white rhinoceroses (Ceratotherium simum), *Journal of Zoo and Wildlife Medicine* 47(3), 827-833. https://doi.org/10.1638/2015-0167.1.

Wenger, S., Boardman, W., Buss, P., et al., 2007, The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field anaesthetised white rhinoceroses (Ceratotherium simum), *Journal of Zoo and Wildlife Medicine* 38(3), 380-387. https://doi.org/10.1638/2006-0038R.1.

Wenker, C.J., 1997, Anaesthesia of exotic animals, *The Internet Journal of Anaesthesiology* 2(3). Available from: http://ispub.com/IJA/2/3/3568. Accessed: 5 June 2025.

West, G., Heard, D. Caulkett, N., 2024, Zoo Animal and Wildlife Immobilization and Anesthesia3rd edn. Iowa, USA: Blackwell Publishing. pp. 991-1025. https://doi.org/10.1002/9781119539278.