# Compared to etorphine–azaperone, the ketamine–butorphanol–medetomidine combination is also effective at immobilizing zebra (*Equus zebra*)

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## Abstract

**Objective:** To compare immobilization efficacy of a nonpotent opioid drug combination, ketamine–butorphanol–medetomidine (KBM) to the preferred etorphine–azaperone (EA) combination in zebras.

**Study design:** Randomized crossover trial.

**Animals:** A group of ten adult zebra (six females and four male).

**Methods:** KBM and EA were administered once to the zebras in random order by dart, 3 weeks apart. Once a zebra was recumbent and instrumented, physiological parameters were measured and recorded at 5-minute intervals until 20 minutes. Antagonist drugs were administered at 25 minutes. KBM was antagonised using atipamezole (7.5 mg $mg^{-1}$ medetomidine dose) and naltrexone (2 mg $mg^{-1}$ butorphanol dose). EA was antagonized using naltrexone (20 mg $mg^{-1}$ etorphine dose). Induction and recovery (following antagonist administration) times were recorded. Physiological parameters, including invasive blood pressure and blood gas analysis, were compared between combinations using a general linear mixed model. Data are reported as mean ± standard deviation or median (interquartile range).

**Results:** The doses of KBM and EA administered were 3.30 ± 0.18, 0.40 ± 0.02 and 0.16 ± 0.01 mg $kg^{-1}$; and 0.02 ± 0.001 and 0.20 ± 0.01 mg $kg^{-1}$, respectively. KBM and EA induction times were 420 (282–564) and 240 (204–294) seconds, respectively ($p$ = 0.03). Zebras

remained recumbent throughout the study procedures. Systolic blood pressure (226 ± 42 and 167 ± 42 mmHg) and oxygen partial pressure (64 ± 12 and 47 ± 13 mmHg) were higher for KBM compared to EA ($p < 0.01$). Recovery time, after administering antagonists, was 92 (34–1337) and 26 (22–32) seconds for KBM and EA, respectively ($p = 0.03$).

**Conclusions and clinical relevance:** Compared to EA, KBM also immobilized zebras effectively. Systemic hypertension and moderate hypoxaemia are clinical concerns of KBM and severe hypoxaemia is a concern of EA. This occurrence of hypoxaemia highlights the importance of oxygen administration during immobilization.

**Keywords:** butorphanol; *Equus zebra;* immobilisation; ketamine; medetomidine; zebra

## Introduction

The use of etorphine in horses paved the way for its use in zebras and other wild equids (Plotka et al. 1987). Subsequently, etorphine in combination with the tranquilizer azaperone, or the sedative detomidine, have been used commonly to chemically capture zebras (Young and Penzhorn, 1972, Plotka et al., 1987, Senthilkumar et al., 2005, Kock et al., 2012). Also, ketamine has been added to etorphine combinations to improve the quality and reliability of immobilization (Adin et al. 2007).

Etorphine drug combinations are effective in immobilizing zebras; however, information on cardiorespiratory effects is limited and their safety is anecdotal (Kock et al. 2012). These drug combinations are known to cause tachycardia, hypertension, acidosis, hypercapnia and hypoxaemia in most immobilized animals (Alford et al., 1974, Kock et al., 2012, Zeiler and Meyer, 2017). Etorphine is the only clinically effective opioid in zebras; thiafentanil, fentanyl and carfentanil require excessively high doses for immobilization. In some countries, etorphine is not readily available and periodic shortages may be experienced (Allen, 1994, Matthews et al., 1995). Therefore, there is a requirement to establish an effective and safe nonpotent, opioid zebra-immobilizing drug combination.

The study aims were to: 1) determine if a ketamine–butorphanol–medetomidine (KBM) drug combination could effectively immobilize zebras; and 2) compare cardiovascular and respiratory parameters to an etorphine–azaperone (EA) drug combination. Comparing the two combinations determined if either combination offered benefits in terms of immobilization efficacy and cardiorespiratory safety.

## Materials and methods

### Study approval

The study was approved by the Animal Ethics Committees of the University of Pretoria (Protocol V071-17) and the South African National Parks (SANParks; #003/17). The study took place at the SANParks Veterinary Wildlife Services facilities, Kimberly, South Africa (28º46’22.6” S, 24º44’59.3” E; 1400 meters above sea level).

## Animals and housing

In total, five family groups comprising four to six free-ranging zebras (*Equus* zebra) were mass-captured and habituated to captivity in outdoor holding facilities (a boma) for 30 days prior to the study. The boma consisted of five individual adjoining pens that each housed a family group. A pen consisted of two compartments separated by a metal sliding gate: 1) an outdoor day section (8 × 8 m, no roof) and 2) an indoor night room (8 × 4 m, roofed). The zebras were darted and observed from elevated walkways above the outdoor section. Lucerne (*Medicago sativa*), hay (*Eragrostis curvula*) and drinking water were available *ad libitum*. Overall, ten adults (six female and four male) were required and indeed used, calculated based on the time to induction, in a two-treatment, randomized crossover design with a 3-week washout period (assumptions: normal distribution; alpha = 0.05; standard deviation = 2 minutes; margin of error = 2 minutes). Zebras were randomised to receive two treatments, once in a single block design (www.randomization.com) crossover study. The washout period was 28 days.

## Drug combinations

The following two drug combinations were compared: 1) KBM – ketamine (1000 mg; Ketonil 200 mg $mL^{-1}$; Wildlife Pharmaceuticals, South Africa), butorphanol (120 mg; Butonil 50 mg $mL^{-1}$; Wildlife Pharmaceuticals), and medetomidine (48 mg; Metonil 40 mg $mL^{-1}$; Wildlife Pharmaceuticals); and 2) EA – etorphine (6 mg; Captivon 10 mg $mL^{-1}$; Wildlife Pharmaceuticals), and azaperone (68 mg; Zapnil 100 mg $mL^{-1}$; Wildlife Pharmaceuticals).

Drug doses were standardised for a 400 kg animal. Combination drugs were drawn up into separate syringes and injected sequentially into a dart via a 20 gauge 75 mm spinal needle. KBM was administered using a 7 mL dart (Type-P, 13 gauge, 38 mm wire barbed needle; Pneu-Dart, PA, USA) and EA was administered using a 1 mL dart (Type-P, 13 gauge, 38 mm wire barbed needle; Pneu-Dart). Both drug combinations filled the dart to capacity. The experimental procedures had three phases: induction (time from darting to recumbency without attempts at standing), immobilization (recumbency to antagonist administration) and recovery (antagonist administration to standing).

## Induction

The zebras were darted over 3–5 m, into the gluteus muscle group, using a carbon dioxide powered rifle ($CO_2$ Rifle, model J.M.ST.R.; Dan-Inject, Denmark). Once darted, induction time (time from darting until moving into recumbency without attempts to stand) was recorded and quality using a subjective descriptive scale (SDS) was evaluated (Table 1). A zebra not in sternal or lateral recumbency at 15 minutes after darting was administered an intervention to complete the induction by either redarting or injecting 300 mg ketamine intravenously. Once immobilized and recumbent, time zero (T0) was noted, the zebra was blindfolded, placed on a metal stretcher and instrumented within 5 minutes.

**Table 1.** Description of the simple descriptive scoring system used to categorize the quality of induction into recumbency in boma-habituated zebras immobilized using ketamine–butorphanol–medetomidine and etorphine–azaperone by remote dart delivery system

| Score | Description | Classifier |
|---|---|---|
| 1 | Slight ataxia observed followed by less than two attempts to move into recumbency. No signs of CNS excitation. No falling or stumbling. Smooth transition from sternal to lateral recumbency. Rapid time to reach sternal recumbency (≤4 minutes) and lateral recumbency (≤5 minutes). | Excellent |
| 2 | Moderate ataxia observed with more than two attempts required to move into recumbency. Minimal signs of CNS excitation. Moderate stumbling. Somewhat rapid time to reach sternal recumbency (>4 minutes) and lateral recumbency (>5 minutes). | Good |
| 3 | Severe ataxia with numerous attempts to move into recumbency. Moderate signs of CNS excitation. Severe stumbling and falling. Moderate risk of injury. Prolonged time to reach sternal recumbence (>8 minutes) and lateral recumbency (>10 minutes). | Fair |
| 4 | Severe ataxia without zebra becoming recumbent. Moderate to severe CNS excitation. Repeated stumbling and falling. High risk of injury. Time to reach sternal recumbency (>15 minutes) and lateral recumbency not achieved. Zebra requires redarting or hand bolus of ketamine to achieve reliable lateral recumbency. | Poor |

CNS, central nervous system.

## Immobilization

Physiological parameters were monitored continuously and recorded at 5, 10 and 15 minutes postrecumbency (T5, T10 and T15, respectively). Supplemental oxygen was not provided to zebras during immobilization.

The lateral metatarsal artery of the nondependent pelvic limb was aseptically cannulated (20 gauge, 25 mm, Jelco I.V. Catheter; Smiths Medical, UK) to allow continuous measurement of the blood pressure and intermittent blood sampling for blood gas analysis. Invasive systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure and pulse rate (PR) were measured using a patient-side pressure monitor (IntraTorr; IntraVitals, UK) that was connected with noncompliant tubing to an electronic strain-gauge transducer (zeroed to atmospheric air pressure at the level of the right atrium). Arterial blood samples (2 mL waste blood drawn prior to 1 mL sample each) were collected anaerobically via the arterial cannula into sodium-heparinized syringes at T5, T10 and T15. Once collected, the syringe was sealed using a rubber stopper to maintain an anaerobic environment and placed in a watertight plastic bag on ice. Once all the samples were collected, they were analysed within 10 minutes of final sample collection using a daily-calibrated bench-top analyser (RapidPoint 500; Siemens, South Africa). Peripheral oxygen haemoglobin saturation ($SpO_2$) was measured using a reflectance pulse oximeter probe (Veterinary Pulse Oximeter, Model 9847V; Nonin Medical, MN, USA) on the cheek mucosa. A multiparameter monitor (SurgVet Advisor V9201; Smiths Medical, UK) was used to measure respiration rate ($f_R$) and end-tidal carbon dioxide ($Pe´CO_2$): a shortened 10 mm internal diameter cuffed endotracheal tube was placed in the ventral meatus and connected to a side-stream sampling connector with a standard sampling line (sampling rate: 200 mL minute$^{-1}$). Rectal temperature (T) was measured using a digital thermometer (HI98509 Checktemp 1; Hanna Instruments, South Africa).

Immobilization quality was subjectively scored at T16 (Table 2) and, thereafter, all monitoring equipment was removed from the zebra. The zebra was weighed using a crane scale (Crane Scale, maximum weight 5000 kg; Punit Instruments, India) attached to the metal stretcher.

**Table 2**. Description of the simple descriptive scoring system used to categorize the quality of immobilisation in boma-habituated zebras immobilized using ketamine–butorphanol–medetomidine and etorphine–azaperone by remote dart delivery system

| Score | Description | Classifier |
|---|---|---|
| 1 | Minimal immobilization achieved and tries to stand repeatedly. Zebra poses a risk to inflict self-induced injury or injury to ground crew. No anaesthetic plane or state of immobilization achieved reached. | Limited effect |
| 2 | Zebra remains recumbent without attempts to stand. Continual muscle tremors or rigidity and spontaneous muscle activity may be observed. Mild autonomic and limb withdrawal response to arterial cannulation. Mild limb withdrawal response to algometer. Palpebral and anal reflex intact and vigorous. | Deep sedation |
| 3 | Zebra remains recumbent without attempts to stand. Reduced muscle rigidity to complete relaxation. No autonomic and limb withdrawal response to arterial cannulation. No limb withdrawal response to algometer. Palpebral and anal reflex intact, but sluggish.<br>Moderate level – no involuntary tail movements and tongue easily extractable. | Light to moderate anaesthetic level |
| 4 | Zebra remains recumbent without attempts to stand. All characteristics of 3 present. Palpebral and anal reflex intact but very sluggish to sometimes absent. | Surgical anaesthesia level |
| 5 | Zebra remains recumbent without attempts to stand. Anaesthetic plane is too deep, all reflexes absent with evidence of life-threatening cardiopulmonary depression. | Excessive level of anaesthesia |

## Recovery

At the end of each procedure (T20), the following antagonist drugs were administered. KBM: atipamezole – 7.5 mg $mg^{-1}$ medetomidine (atipamezole 50 mg $mL^{-1}$; Vtech, South Africa), split between intramuscular and intravenous administration; naltrexone at 2.0 mg $mg^{-1}$ butorphanol (Trexonil 40 mg $mL^{-1}$; Wildlife Pharmaceuticals), intravenously. EA: naltrexone – 20 mg $mg^{-1}$ etorphine (Trexonil 40 mg $mL^{-1}$; Wildlife Pharmaceuticals), intravenously.

After antagonist administration, time to standing (recovery time) was recorded and recovery quality scored using subjective criterion (Table 3). The zebra was kept under observation until fully recovered. Zebras were observed for any signs of renarcotization over a 24 hour period following immobilization.

**Table 3.** Description of the simple descriptive scoring system used to categorize the quality of recovery in boma-habituated zebras immobilized using ketamine–butorphanol–medetomidine and etorphine–azaperone by remote dart delivery system

| Score | Description | Classifier |
|---|---|---|
| 1 | Transition from lateral to sternal occurs with minimal ataxic movements. Stands within one or two attempts, which are calm and coordinated. Slight ataxia observed during initial ambulation attempt. Recumbency to standing in <10 minutes after administration of the antagonists. | Excellent |
| 2 | Transition from lateral to sternal occurs with moderate ataxic movements. Standing requires more than two attempts, which are relatively coordinated. Moderate ataxia observed during initial ambulation attempt. Imbalance and incoordination. Recumbency to standing in 10–15 minutes after administration of the antagonists. | Good |
| 3 | Frequent and severely ataxic attempts to move from lateral to sternal. Numerous erratic attempts required to stand. Stumbling and falling common. Markedly ataxic gait initial ambulation attempt. Recumbency to standing in >20 minutes after administration of the antagonists. | Fair |
| 4 | Zebra remains recumbent for >30 minutes after administration of the antagonists. No response to stimuli and no attempts to raise observed. A clinical examination is required and repeat dosing of the antagonists is considered. | Poor |

## Data analysis

Data from zebra that required an intervention during induction (redarting or a ketamine bolus) were excluded from the analysis. Data were assessed for normality by evaluating descriptive statistics, plotting of histograms and performing the Anderson–Darling test for normality. Quantitative serial data of normal distribution (PR, $f_R$, SAP, MAP, DAP, T, $SpO_2$, $Pe´CO_2$, $PaCO_2$, $PaO_2$, pH, $HCO_3^-$ and base excess) between the two combinations were compared using a general linear mixed model (fixed factors: time, combination; random factor: zebra). Body weights were compared between the two data collection periods using a paired *t* test. Induction durations and recovery times were compared between combinations using a Mann–Whitney *U* test. Subjective quality scores were compared between combinations using a Friedman test (response: quality score; treatment: combination; block: zebra). Data were reported as mean ± standard deviation or median (interquartile range), unless otherwise stated. Data were analysed using commercially available software (MiniTab 17.1.0; MiniTab Incorporated, PA, USA) and results interpreted at the 5% level of significance ($p < 0.05$).

## Results

Of the zebras studied, one died 6 days after receiving KBM during the first trial as a result of maladaption to the boma and was completely excluded from the study. The remaining nine zebras received both combinations. Data were excluded from two of the KBM immobilizations because they required an intravenous ketamine bolus to complete the induction. Data from nine EA and seven KBM immobilizations were analysed. The mean weight of the zebras was 300 ± 16 kg in the first week and was no different (299 ± 16 kg) 3 weeks later.

For KBM, the final doses of ketamine, butorphanol and medetomidine were 3.30 ± 0.18, 0.40 ± 0.02 and 0.16 ± 0.01 mg $kg^{-1}$, respectively. For EA, the final doses of etorphine and azaperone were 0.02 ± 0.001 and 0.20 ± 0.01 mg $kg^{-1}$, respectively. The induction duration for KBM was 420 (282–564) seconds and was significantly slower [240 (204–294) seconds] than EA ($p = 0.03$). The induction score did not differ between combinations: 2 (from 0 to 2) for KBM and 2 (2) for EA but the behaviour of the zebras at the end of the induction period did differ. During KBM induction, they initially stood stationary, started to sway, and then stumbled about while trying to maintain their feet before eventually becoming recumbent. During EA induction they adopted a typical hackney gait, often pushing themselves into the pen wall before eventually losing their balance and falling over. All zebras appeared to strongly resist the transition from standing to recumbency and made every effort to maintain a standing position.

During the immobilization phase, zebras maintained recumbency without attempts to stand until the antagonists were administered, regardless of combination used. The immobilization score was 3 (3) for KBM and 2 (2–3) for EA. Marked muscle rigidity and tremors were frequently observed (four had tremors, four had muscle rigidity, and one had both tremors and muscle rigidity) in zebras immobilized with EA as well as spontaneous motor activity, including involuntary tail and limb movements. Palpebral and anal reflexes remained intact with both combinations; however, the palpebral reflex was more sluggish with KBM compared to EA.

Pulse rate in zebras immobilized using KBM were 45 ± 6 beats $minute^{-1}$ and was slower (69 ± 17 beats $minute^{-1}$) compared to EA ($p < 0.01$). The arterial blood pressures were profoundly elevated in all zebras (Table 4). The systolic arterial blood pressure of the KBM-immobilized zebras was 226 ± 42 mmHg, which was greater (167 ± 42 mmHg) compared to using EA (Fig. 1; $p < 0.01$). The respiratory rates were 17 ± 7 breaths $minute^{-1}$ in zebras immobilized using KBM, which was faster than (9 ± 2 breaths $minute^{-1}$) using EA ($p < 0.01$). Arterial blood gas analysis revealed normocapnia and a normal blood pH balance in all zebras. However, zebras were less hypoxaemic [partial pressure oxygen ($PaO_2$): 64 ± 12 mmHg (8.5 ± 1.6 kPa)] when the KBM combination was used compared to EA [$PaO_2$: 47 ± 13 mmHg (6.3 ± 1.7 kPa)] (Fig. 2; $p < 0.01$).

**Table 4.** Physiological parameters of ketamine–butorphanol–medetomidine (KBM) and etorphine–azaperone (EA) immobilized zebras after induction of recumbency, over a 15 minute period. Parameters are reported as mean ± standard deviation (SD); (range) at 5 minute intervals

| Variable | Combination | 5 minutes | | 10 minutes | | 15 minutes | |
|---|---|---|---|---|---|---|---|
| | | Mean ± SD | Range | Mean ± SD | Range | Mean ± SD | Range |
| Pulse rate (beats minute$^{-1}$) | KBM | 45 ± 7* | (34–56) | 46 ± 5* | (36–52) | 44 ± 7* | (32–51) |
| | EA | 69 ± 18* | (52–104) | 69 ± 19* | (52–104) | 68 ± 16* | (48–96) |
| Respiration rate (breaths minute$^{-1}$) | KBM | 19 ± 6* | (11–28) | 16 ± 10* | (8–32) | 16 ± 5* | (11–24) |
| | EA | 9 ± 3* | (6–16) | 8 ± 2* | (6–12) | 9 ± 2* | (6–12) |
| Temperature (°C) | KBM | 38.0 ± 0.4 | (36.8–38.9) | 38.1 ± 0.6 | (37.6–38.6) | 38.5 ± 0.6 | (37.7–39.6) |
| | EA | 37.9 ± 0.6 | (36.8–38.9) | 37.9 ± 0.6 | (36.6–38.7) | 37.9 ± 0.5 | (36.9–38.6) |
| SAP (mmHg) | KBM | 222 ± 37* | (180–260) | 220 ± 55* | (136–286) | 235 ± 36* | (187–274) |
| | EA | 187 ± 53* | (93–268) | 154 ± 36* | (94–227) | 158 ± 31* | (93–195) |
| MAP (mmHg) | KBM | 174 ± 18* | (152–200) | 172 ± 27* | (118–201) | 181 ± 13* | (165–200) |
| | EA | 125 ± 41* | (58–196) | 97 ± 18* | (76–136) | 103 ± 17* | (77–115) |
| DAP (mmHg) | KBM | 153 ± 14* | (136–174) | 151 ± 20* | (109–172) | 156 ± 9* | (148–170) |
| | EA | 86 ± 22* | (40–112) | 75 ± 15* | (49–100) | 77 ± 13* | (55–97) |
| Pe´$CO_2$ (mmHg) | KBM | 41 ± 4 | (32–46) | 36 ± 8* | (27–47) | 39 ± 8* | (29–48) |
| | EA | 42 ± 9 | (27–56) | 44 ± 10* | (29–54) | 45 ± 11* | (29–56) |
| (kPa) | KBM | 5.5 ± 0.5 | (4.3–6.1) | 4.8 ± 1.1* | (3.6–6.3) | 5.2 ± 1.1* | (3.9–6.4) |
| | EA | 5.6 ± 1.2 | (3.6–7.5) | 5.9 ± 1.3* | (3.9–7.2) | 6.0 ± 1.5* | (3.9–7.5) |
| pH | KBM | 7.41 ± 0.01* | (7.38–7.42) | 7.41 ± 0.03* | (7.38–7.44) | 7.42 ± 0.03* | (7.39–7.45) |
| | EA | 7.33 ± 0.04* | (7.27–7.3)1 | 7.34 ± 0.05* | (7.38–7.41) | 7.38 ± 0.12* | (7.27–7.66) |
| $HCO^{-}_3$ (mmol L$^{-1}$) | KBM | 23.2 ± 3.0 | (18.2–27.2) | 22.8 ± 2.5 | (18.2–25.0) | 24.2 ± 2.3 | (19.7–27.0) |
| | EA | 21.6 ± 3.9 | (17.1–28.3) | 21.8 ± 3.9 | (17.8–29.3) | 21.0 ± 3.9 | (17.1–28.6) |
| $PaO_2$ (mmHg) | KBM | 71 ± 13* | (56–90) | 61 ± 11* | (47–77) | 60 ± 9* | (48–72) |
| | EA | 43 ± 11* | (20–59) | 50 ± 17* | (22–86) | 48 ± 11* | (25–61) |
| $PaO_2$ (kPa) | KBM | 9.5 ± 1.7* | (7–12) | 8.1 ± 1.4* | (6.3–10.3) | 8.0 ± 1.2* | (6.4–9.6) |
| | EA | 5.7 ± 1.5* | (3–8) | 6.7 ± 2.2* | (2.9–11.5) | 6.4 ± 1.4* | (3.3–8.1) |
| $PaCO_2$ (mmHg) | KBM | 37 ± 6* | (26–45) | 36 ± 5* | (28–43) | 37 ± 5* | (30–44) |
| | EA | 43 ± 6* | (35–54) | 42 ± 7* | (29–53) | 40 ± 8* | (31–56) |
| $PaCO_2$ (kPa) | KBM | 4.9 ± 0.8* | (3–6) | 4.8 ± 0.6* | (3.7–5.7) | 4.9 ± 0.6* | (4.0–5.9) |
| | EA | 5.7 ± 0.8* | (5–7) | 5.6 ± 0.9* | (3.9–7.1) | 5.3 ± 1.1* | (4.1–7.5) |
| BE (mmol L$^{-1}$) | KBM | −1.9 ± 4.0* | (−8.6 to 3.7) | −2.3 ± 3.2* | (−8.2 to 1.1) | −0.5 ± 3.1* | (−6.5 to 3.0) |
| | EA | −3.0 ± 5.3* | (−9.5 to 5.9) | −3.2 ± 5.4* | (−10.3 to 6.3) | −4.2 ± 5.4* | (−9.7 to 6.7) |

BE, base excess; DAP, diastolic arterial pressure; MAP, mean arterial pressure; $PaCO_2$, partial pressure of carbon dioxide; $PaO_2$, partial pressure of oxygen; Pe´$CO_2$, end-tidal carbon dioxide; SAP, systolic arterial pressure.

*Statistically different between combinations ($p < 0.05$).

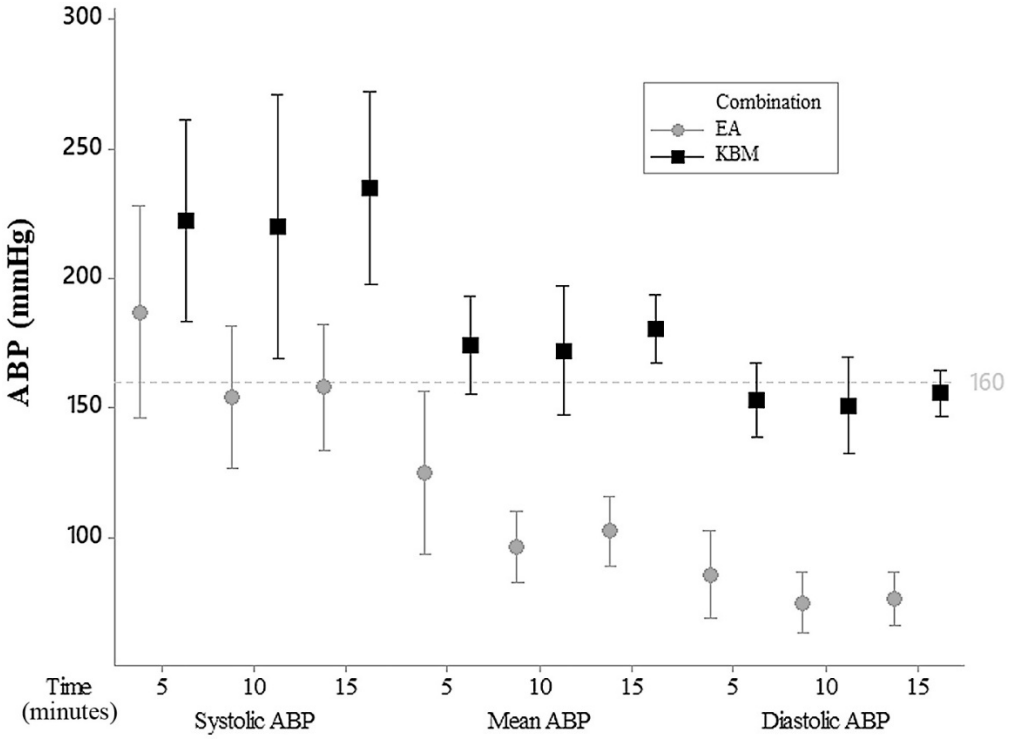


**Figure 1.** Systolic, mean and diastolic arterial blood pressure (ABP) over time in ketamine–butorphanol–medetomidine (KBM) and etorphine–azaperone (EA) immobilised zebras [All values were different between treatments ($p < 0.01$)]. The plot represents the mean and 95% confidence interval. Systemic hypertension is defined as a systolic ABP >160 mmHg, as represented by the reference line.

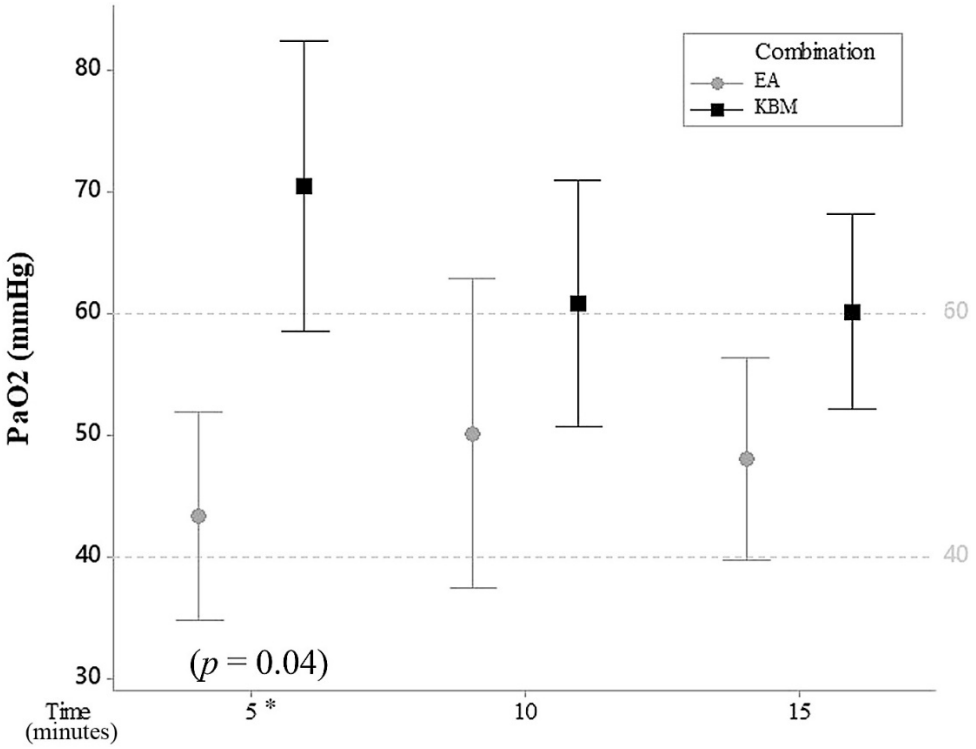


**Figure 2**. Partial pressure of oxygen ($PaO_2$) over time in ketamine–butorphanol–medetomidine (KBM) and etorphine–azaperone (EA) immobilized zebras ($p < 0.01$ between combinations; * indicates a significant time × combination effect). The plot represents the mean and 95% confidence interval. Severe hypoxaemia is defined as $PaO_2$ 40–60 mmHg (5.3–8.0 kPa) as represented by the reference lines.

During recovery, the recovery time in zebras recovering from the KBM immobilization [92 (34–1337) seconds] was longer in duration compared to EA [26 (22–32) seconds] ($p$ = 0.03). However, despite the difference in times to standing, the recovery score of the zebras immobilized using KBM [3 (1–4)] was no different to EA [1 (1)] ($p$ = 0.06). Often, the zebras would stand within one or two attempts and were trembly and mildly ataxic. Only three of the zebras recovering from KBM immobilization initially rolled on to their backs before adopting a sternal position and two zebras experienced a prolonged recovery. No renarcotization was observed within the 24 hour observation period postreversal with either of the combinations.

## Discussion

KBM effectively induced a stable immobilization in all but two of the zebras and was comparable to the advocated EA combination. Systemic hypertension was profound and clinically relevant in KBM compared to EA immobilization. The zebras were normocapnic and had normal blood pH balance, regardless of the combination used. However, hypoxaemia was present in all zebras. Time to recovery was rapid and the recovery phase was mostly uneventful with the exception of three zebras recovering from KBM that rolled about during early attempts to move into sternal recumbency.

To the authors' knowledge, this is the first report of successfully immobilizing zebras using a combination that does not contain a potent opioid. KM combinations have been reported in wild Przwalski horses [*Equus* ferus; KM dose range: (from 1.8–2.6 to 0.07–0.10) mg $kg^{-1}$] and feral donkeys (*Equus asinus*; KM dose: 4.1–0.14 mg $kg^{-1}$) (Matthews et al., 1995, Woolnough et al., 2012). Despite the use of similar KM doses, the zebras immobilized using KBM had shorter induction times compared to Przewalski horses (11 minutes) and feral donkeys (9 minutes) (Matthews et al., 1995, Woolnough et al., 2012). This may be because butorphanol complimented the KM combination, although other factors could account for these differences such as: 1) species specific pharmacodynamic and pharmacokinetics; 2) habituation to captivity; and 3) differences in drug delivery (Kock et al. 2012). Overall, two zebras in our study were not immobilized with KBM and possible causes included: 1) poor dart placement and incorrect injection of the large volume of drugs into fascia or subcutaneous tissues during the injection phase; 2) incomplete injection; or 3) peak drug effect of the combination requires a longer duration than the 15 minute intervention limit that was based on an appropriate induction time for rapid field immobilization (Zeiler & Meyer 2017). The EA combination induction times were similar to the 4 minutes reported in Grant's zebras (Senthilkumar et al. 2005), but shorter than the 5–9 minute range reported in Cape Mountain zebras (Young & Penzhorn 1972).

As was observed in immobilization of other free-ranging equids (Matthews et al., 1995, Woolnough et al., 2012), all zebras resisted the transition from standing to recumbency. Despite the characteristics of the pre-recumbency struggle being different between the two combinations, the greatest risk to injury remains associated with the duration of induction (Kock et al. 2012). Thus, KBM could increase the risk of injury based on its significantly longer induction time compared to EA.

Both combinations induced a reliable state of immobilization without attempts to stand for at least 20 minutes after becoming recumbent. Therefore, KBM could be used effectively as an alternative to EA for immobilization during routine management procedures in captive situations. Our findings are unlike those reported in KM-immobilized wild equids, where sudden spontaneous arousal may occur (Matthews et al. 1995). We found that zebras immobilized using EA exhibited muscle rigidity and tremors, similar to previous reports (Plotka et al., 1987, Walzer et al., 2006). Therefore, the apparent superior muscle relaxation and suspected deeper plane of anaesthesia achieved with KBM should be further evaluated as a potential advantage of its use.

Systemic hypertension is defined as a systolic arterial pressure >160 mmHg (Grimm et al. 2015). Therefore, systemic hypertension was a consistent finding in all zebras, especially when undergoing KBM immobilization. Medetomidine and ketamine may have contributed to the hypertension. Medetomidine interacts with extrasynaptic $\alpha_2$-adrenoceptors which results in peripheral vasoconstriction, increased systemic vascular resistance and a rise in systemic blood pressure (Scheinin et al., 1989, Virtanen, 1989). The hypertensive effects of medetomidine could have been augmented by the indirect cardiovascular effects of ketamine (Grimm et al. 2015). Ketamine stimulated endogenous catecholamines release may lead to vascular postsynaptic $\alpha_1$-adrenoceptors activation, causing increased vasoconstriction; and a potential release of noradrenaline within the myocardium which would result in increased chronotropic and inotropic effect (Grimm et al. 2015). The hypertension and lower PR in KBM-compared to EA-immobilized zebra may be explained by a baroreceptor reflex (Scheinin et al. 1989). The systemic hypertension in EA-immobilized zebras was not profound. We speculate that etorphine-induced hypertension was as a result of increased sympathetic tone as described in horses when opioids are administered (LeBlanc et al. 1987). It is possible that azaperone reduced the systemic hypertension by decreasing systemic vascular resistance through its $\alpha_1$-adrenoceptor antagonistic properties (Kock et al. 2012). Sustained systemic hypertension can result in end-organ damage, which may include renal, ocular or neurological lesions (Brown et al. 2007). Therefore, currently we cannot recommend KBM for prolonged procedures, without correcting the hypertension; however, this effect may be more pertinent in compromised (such as animals suffering hypovolaemia or uncontrolled haemorrhage) compared to healthy individuals.

Hypoxaemia is defined as a $PaO_2$ <80 mmHg (10.7 kPa) and is a consistent finding in all zebras (Grimm et al. 2015). Opioids frequently result in hypoventilation in immobilized animals, especially when combined with a tranquillizer or sedative that can result in hypoxaemia and hypercapnia (Kock et al. 2012). Minute ventilation was not determined in the immobilized zebras, and only respiratory rate, partial pressure of carbon dioxide ($PaCO_2$) and $Pe´CO_2$ were measured as ventilation indicators. Hypercapnia is a common sequala of immobilization of wildlife, regardless of the drug combinations used during capture (Gerlach et al., 2017, Zeiler and Meyer, 2017); therefore, it was unexpected that we found the $PaCO_2$ and $Pe´CO_2$ were within acceptable normocapnic limits for awake equids (Grimm et al. 2015). Thus, hypoxaemia was more likely to have resulted from mechanisms other than simple drug-induced hypoventilation. Medetomidine and etorphine are known to cause pulmonary hypertension (Kästner, 2006, Meyer et al., 2015). This hypertension could contribute to gas diffusion deficits through either pulmonary oedema or decreasing the capillary blood transit time decreasing the opportunity for less soluble gases, like oxygen, to

diffuse adequately through the alveolus–capillary junction (Meyer et al. 2015). Another mechanism, often overlooked, is systemic oxygen extraction. Etorphine can result in increased sympathetic tone and a rise in cellular metabolism with increased oxygen consumption (Buss et al. 2018). Medetomidine is known to decrease cellular metabolism and therefore decrease the oxygen extraction, and could explain why the $PaO_2$ was higher in KBM-immobilized zebras (Zeiler & Meyer 2017). Therefore, it is critical that veterinarians detect and respond to hypoxaemia by providing oxygen support, especially in diseased or injured animals where the metabolic rate might be increased.

Recovery times in the immobilized zebras following antagonist administration were rapid, regardless of the immobilizing drug combination used. KM-immobilized Przewalski horses and feral donkeys took 13 and 21 minutes, respectively, to stand after atipamezole was administered (intramuscularly in Przewalski horses and a combination of intravenous and subcutaneous in feral donkeys) (Matthews et al., 1995, Woolnough et al., 2012). The differences in recovery times could be explained by dissimilar atipamezole-to-medetomidine ratios and administration routes. Atipamezole was administered at 2.5 mg $mg^{-1}$ medetomidine intramuscularly compared to our study (Matthews et al., 1995, Woolnough et al., 2012). The prolonged recovery experienced by two of the zebras immobilized with KBM were likely due to: 1) injection of atipamezole into a fascial plane which delayed absorption; 2) individual difference in drug kinetics, especially medetomidine; and 3) differences in the compounded atipamezole drug formulation or its stability (Kock et al., 2012, Zeiler and Meyer, 2017). The KBM combination recoveries were mostly calm; however, three zebras rolled on to their backs, which could have potentially resulted in colon displacement and colic (Grimm et al. 2015). By comparison, the administration of naltrexone in EA-immobilized zebra resulted in recovery times of <1 minute with no evidence of renarcotization. Earlier reports suggest a high incidence of partial reimmobilization in opioid-immobilized zebras antagonised with diprenorphine (Allen 1990).

A potential limitation of incorporating KBM as a routine immobilization drug combination is that we had to use a large and heavy 7 mL dart to administer the combination. Despite the zebra having well-muscled areas to place a dart, these heavy darts could result in unnecessary soft tissue trauma. Furthermore, the authors do not recommend firing these darts at distances >10 m as it is anticipated that the kinetic energy required to propel the dart will lead to significant soft tissue trauma (Kock et al. 2012). Currently, the use of this combination may not be feasible in free-ranging settings and further investigations are required.

## Conclusion

A KBM combination provided effective immobilization in boma-habituated zebras compared to the preferred EA combination. Further evaluation of the efficacy of KBM in free-ranging zebras is required. Systemic hypertension and moderate hypoxaemia are clinical concerns of KBM and severe hypoxaemia is a concern of EA. This occurrence of hypoxaemia highlights the importance of oxygen administration during immobilization.

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## Author contributions

GPS, LCRM and GEZ: designed the study and were involved in the planning and execution thereof. AB, PB and DZ: translocation and management of animals in the boma facility as well as all darting procedures. All of the authors were involved in collection of data. GPS processed the experimental data while GEZ performed the statistical analysis. All authors were involved in the interpretation of the results. GPS, LCRM and GEZ wrote the initial draft of the manuscript with input and comments from all authors.

### Conflict of interest

The authors declare no conflict of interest for this study.

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