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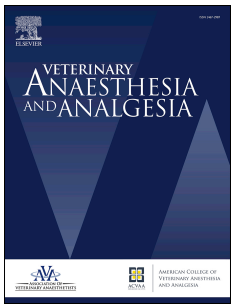

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RESEARCH STUDY

Immobilization of African buffalo (*Syncerus caffer*) using etorphine-midazolam compared with etorphine-azaperone.

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Running title: Potent opioid immobilization in buffalo

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Author contribution: JFG, MAM and GEZ responsible for study design and involved with execution thereof. LLL and PEB responsible for logistical support. All authors were involved in collection of data. JPR responsible for management of animals as well as all darting procedures. JFG processed the experimental data while GEZ performed the statistical analysis. JFG and GEZ wrote the initial drafts of the manuscript with input and comments from all authors.

The authors declare conflict of interest as drugs and animals were sponsored by Wildlife Pharmaceuticals Pty (Ltd).

Abstract

Objective To compare induction times and physiological effects of etorphine-azaperone with etorphine-midazolam immobilization in African buffalo.

Study design Randomized crossover study.

Animals A group of 10 adult buffalo bulls (mean body weight 353 kg).

Methods Etorphine-azaperone (0.015 and 0.15 mg kg^{-1}, respectively) and etorphine-midazolam (0.015 and 0.15 mg kg^{-1}, respectively) were administered once to buffalo, 1 week apart. Once in sternal recumbency, buffalo were instrumented and physiological variables recorded at 5-minute intervals, from 5 minutes to 20 minutes. Naltrexone (20 mg mg^{-1} etorphine dose) was administered intravenously at 40 minutes. Induction (dart placement to recumbency) and recovery (naltrexone administration to standing) times were recorded. Arterial blood samples were analysed at 5 and 20 minutes. Physiological data were compared between treatments using a general linear mixed model and reported as mean ± standard deviation. Time data were compared using Mann-Whitney-U test and reported as median (interquartile range) with $p \leq 0.05$.

Results Actual drug doses administered for etorphine, azaperone and midazolam were 0.015 ± 0.001, 0.15 ± 0.01 and 0.16 ± 0.02 mg $kg^{-1,}$ respectively. Induction time for etorphine-azaperone was 3.3 (3.6) minutes and not different to 3.2 (3.2) minutes from etorphine-midazolam. The overall mean arterial blood pressure for etorphine-azaperone (102 ± 25 mmHg) was significantly less than etorphine-midazolam (163 ± 18 mmHg) ($p < 0.001$). The PaO_2 for etorphine-azaperone (37 ± 12 mmHg; 5.0 ± 1.6 kPa) was not different from etorphine-midazolam (43 ± 8 mmHg; 5.8 ± 1.1 kPa). Recovery time was 0.8 (0.6) minutes for etorphine-azaperone and did not differ from 1.1 (0.6) minutes in etorphine-midazolam.

Conclusion and clinical relevance Etorphine-midazolam was equally as effective as etorphine-midazolam immobilization in this study. However, systemic arterial hypertension was a concern with etorphine-midazolam and both combinations produced clinically relevant hypoxaemia. Supplemental oxygen administration is recommended with both drug combinations.

Keywords benzodiazepines, butyrophenones, hypertension, hypoxaemia, opioids

Introduction

African buffalo (*Syncerus caffer*) are one of world's most dangerous animals (McNamee 2011). They are highly sought after in wildlife conservancy and breeding programmes, especially if the individual has a disease-free status or is a hunting-trophy animal. Chemical capture (immobilization) is required to complete various management procedures such as translocation, horn morphometry, testing for disease and general health evaluation.

A variety of drugs or drug combinations have been reported in textbooks and in descriptive studies for the immobilization of African buffalo; however, comparative studies are not available (Hattingh et al. 1984; Oosthuizen et al. 2009; Kock et al. 2012; Napier & Armstrong 2014; Wolfe 2015; Couch et al. 2017). In Africa, etorphine alone or, more commonly in combination with a sedative, is a popular choice to immobilize African buffalo (Oosthuizen et al. 2009; Kock et al. 2012).

Etorphine hydrochloride is a potent μ-opioid receptor agonist that also possesses κ- and δ-opioid receptors activity. The combination of etorphine-azaperone is currently a widely used drug combination in African buffalo (Curro 2007; McMahon & Bradshaw 2008; Kock et al. 2012; Wolfe 2015). Azaperone is a short-acting butyrophenone, which mainly antagonises dopamine (D2) and α_1-adrenoceptors, among others (Posner 2018). The benzodiazepine agonist midazolam (an imidazobenzodiazepine) is another sedative drug to combine with etorphine (Kock et al 2012). Midazolam binds to a specific benzodiazepine receptor binding site on gamma-aminobutyric acid A ($GABA_A$) receptors (Posner & Burns 2013; Rankin 2015). Binding produces a positive allosteric modulation of the $GABA_A$ receptor, enhancing the effect of GABA. This leads to central muscle relaxation, anxiolysis and hypnotic sedation. Benzodiazepine agonists are used in domestic and free-ranging ruminants either as premedication prior to general anaesthesia or as co-induction drugs (Caulkett 2003; Stegmann 2004; Dugdale 2010; Nain et al. 2010; Malik et al. 2011; Kumar et al. 2014; Bodh

et al. 2015). Midazolam's traditional vial concentration of 5.0 mg mL^{-1} requires delivery volumes that exceed projectile dart capacity. A concentrated formulation (50.0 mg mL^{-1}) is currently under investigation, which allows a therapeutic dose to be delivered *via* projectile dart in large animals. Theoretically, midazolam produces fewer adverse cardiovascular effects and provides better muscle relaxation compared with azaperone, which could prove advantageous in etorphine-based immobilization protocols.

The aim of this study was to compare the commonly used etorphine-azaperone and novel etorphine-midazolam combination for the immobilization of African buffalo bulls. It was hypothesized that the time course of the immobilization and physiological variables over time during immobilization would not differ between treatments.

Materials and methods

This study was approved by the Animal Ethics Committee of The University of Pretoria (V050-18). This study took place at the (Wildlife Pharmaceuticals Wildlife Research Facility (Registration number: RF17/15918), Ngongoni Game Farm, Tipperary Conservancy, Nelspruit, RSA (25°31'29.75" S**,** 31°6'56.55" E**;** altitude was 829.0 meters above sea level)**.**

Animals, housing, and drug combinations

A group of 10 free-ranging African buffalo bulls [mean ± standard deviation (SD): 47 ± 19 months old; 353 ± 97 kg; 2.5/5 body condition score] were captured and translocated to an outdoor holding facility (boma) 2 weeks prior to commencement of the study to allow habituation (Burrow 2019). The sample size was based on induction time using the following assumptions: normal distribution; alpha = 0.05; standard deviation = 2 minutes; margin of error = 2 minutes, similar to previous research experience using a similar study design in

zebra (*Equus zebra*) (Stemmet et al. 2019). The boma consisted of five adjoining pens, each connected *via* a lockable sliding gate. A total of nine buffalo were housed in the two largest pens which consisted of a roofed (15 x 4 metres) and unroofed (15 x 5 metres) sections. The largest buffalo was housed alone in a smaller adjoining pen (8 x 5 metres) to prevent conflict with smaller bulls but had continuous visual contact with the others through a pole-wall division. Lucerne (*Medicago sativa*), hay (*Eragrostis curvula*) and water were available *ad libitum* and supplementary concentrate cubes (Alzu GP100 cubes; Alzu, RSA) were provided twice daily. Food and water were withheld 36 hours and 12 hours respectively prior to immobilization.

All buffalo were given both drug combinations once (none were excluded from completing the study), in a random (balanced, one-block design: http://www.randomization.com) order at a 1-week interval, as follows:

- EA - etorphine (0.015 mg kg^{-1}; Captivon 9.8 mg mL^{-1}; Wildlife Pharmaceuticals, RSA), azaperone (0.15 mg kg^{-1}; 100.0 mg mL^{-1}; Wildlife Pharmaceuticals, RSA).
- EM - etorphine (0.015 mg kg^{-1}; Captivon 9.8 mg $mL^{-1,}$ Wildlife Pharmaceuticals, RSA), midazolam (0.15 mg kg^{-1}; 50.0 mg mL^{-1}; Wildlife Pharmaceuticals, RSA)

Drug doses were based on an estimated body weight determined by experienced wildlife veterinarians. The primary investigator was blinded to the drug combination administered. Each drug was drawn up into separate syringes and injected sequentially into a 3 mL plastic dart (Dan-inject 3 mL; 2.0 mm x 40 mm collard needle; Dan-inject International, Denmark) using a 20-gauge 75 mm spinal needle (BD Medical; NJ, USA). The dart was filled to capacity with sterile water (Kyron Laboratories Ltd., RSA).

Pre-immobilization phase

Buffalo were darted in the shoulder muscles from a 5-8 metre distance using a carbon dioxide-powered injection rifle (Model JM; Dan-inject International, Denmark). Once the buffalo was darted, the induction time (time from dart placement and drug discharge to when the buffalo became recumbent without attempts to stand) was recorded and quality was scored using a subjective descriptive scale (Table 1). Once recumbency was achieved, the remaining buffalo were moved to the adjacent pen. Visual evaluation of the recumbent buffalo was used to subjectively determine when approach and handling were considered safe for the personnel involved e.g., the animal would not charge. When deemed safe, the immobilized buffalo was approached, blindfolded, and placed into sternal recumbency on a purpose-built stretcher. A 5-minute period was allowed to instrument and prepare the buffalo for data collection.

Immobilization phase

The interval from drug administration (dart placement and discharge) to the time when the buffalo were first handled was recorded. Time of first handling was defined as T0 for subsequent data collection. Animal response at first handling was used to evaluate quality of immobilization (Table 1). The buffalo were instrumented to measure the following physiological variables: heart rate (HR), invasive systolic (SAP), diastolic (DAP), mean (MAP) arterial blood pressure, respiratory rate (f_R) and expired partial pressure of carbon dioxide ($P_{expired}CO_2$) using a multiparameter monitoring machine (GE Datex Ohmeda CardioCap 5 Multiparameter; GE Healthcare, IL, USA). The physiological variables were continuously monitored and recorded every 5 minutes (T5, T10, T15 and T20, respectively) from T0. Immobilization quality was subjectively scored at T20 (Table 1).

An auricular artery was aseptically cannulated (22-gauge, 25 mm, Jelco IV Catheter; Smiths Medical, UK) and an electronic strain gauge transducer (Medex Abbott; Biometrix, The Netherlands) was attached and zeroed to atmospheric air pressure at the level of the right scapulohumeral joint for invasive measurement of arterial blood pressure. A polyvinyl chloride cuffed endotracheal tube (8 mm internal diameter; Kruuse, Denmark) was placed into the ventral nasal meatus to measure f_R and PexpiredCO_2 (peak value of CO_2 recorded during the capnograph tracing) from the nasopharyngeal region via the side-stream respiratory gas analyser (200 mL minute^{-1} sampling rate). f_R was confirmed by visual observation, over 1 minute, of abdominal excursion and turbulent air flow at nares. The HR was measured with a stethoscope (Littmann Classic; 3M, MN, USA) and correlated with the value obtained from the multiparameter monitor arterial pulse waveform. Rectal temperature was measured in degrees Celsius (°C) using an electronic thermometer (Thermoval Rapid; Hartmann, RSA).

Arterial blood samples were collected anaerobically from the auricular cannula (2 mL waste and 1 mL sample) at T5 and T20 in heparinized syringes (A-Line syringes; Becton Dickinson; NJ, USA), and analyzed immediately using an animal-side blood gas analyser (EPOC Blood Analysis System; Siemens Healthineers, Germany). The mean ± SD ambient temperature was 23.3 ± 2.4 °C and barometric pressure was 703 ± 2 mmHg, which were recorded from the blood gas analyser at each T5 analysis point. Arterial blood sample analysis provided values of oxygen partial pressure oxygen (PaO_2) and carbon dioxide partial pressure ($PaCO_2$), arterial haemoglobin oxygen saturation (SaO_2), pH, bicarbonate (HCO_3^-), base excess extracellular [BE(ecf)], base excess blood [BE(b)], sodium (Na^+), potassium (K^+), chloride (Cl^-), ionised calcium (iCa), haematocrit (Ht), haemoglobin concentration (Hb), glucose, lactate, creatinine. . The alveolar to arterial partial pressure of oxygen gradient was calculated using the following formula: $P(A\text{-}a)O_2 = PAO_2 – PaO_2$, where $PAO_2 = PIO_2 –$

($PaCO_2$ ÷ RQ) [RQ – respiratory quotient = 1.0 in ruminants] and $PIO_2 = FIO_2$ x ($PB - P_{H2O}$) [FIO_2 – fraction inspired oxygen, PB – mean ambient barometric pressure measured from the blood gas analyser, P_{H2O} – water vapour pressure was standardised at 47 mmHg] (Sarkar et al. 2017). The arterial oxygen content (CaO_2) was calculated using the formula: $CaO_2 = 1.34$ x Hb x SaO_2 + PaO_2 x 0.003 (1.34 – Huffner's constant; 0.003 = millilitres of oxygen carried by 1 dL of blood per 1 mmHg of partial pressure oxygen) (Haskins 2015).

At T20, after all variables had been recorded and samples taken, all instruments and cannulae were removed from the buffalo. The buffalo was then moved onto a rubber stretcher (of known mass) and weighed using a crane hanging scale (HIS; AE Adam, CT, USA). Then, horn morphometry and body measurements were taken for presale evaluations that were not relevant to the study.

Postimmobilization phase

At the end of all procedures, the buffalo was removed from the rubber stretcher and placed in sternal recumbency. The pure μ-opioid receptor antagonist naltrexone (20 mg mg^{-1} etorphine; Trexonil 40 mg mL^{-1}; Wildlife Pharmaceuticals, RSA) was administered intravenously at a standardised time of 40-minutes (T40). This time period allowed all procedures to be completed before recovering the animal. The recovery time was recorded as the time from administration of the antagonist until the buffalo began to walk. The quality of recovery was subjectively scored (Table 1). Food and water were provided once data collection had been completed on all buffalo. The buffalo were observed for any signs of renarcotization or other effects of the study procedures over a 24-hour period following immobilization.

Statistics

Table 1 Simple descriptive system used to score the quality of induction, immobilization and recovery of 10 boma-confined adult African buffalo darted with etorphine-azaperone, and etorphine-midazolam administered intramuscularly. Etorphine was dosed at 0.015 mg kg^{-1} and azaperone and midazolam were dosed at 0.15 mg kg^{-1}.

Score	Description	Classifier
Induction		
0	Animal does not become recumbent, although it can be approached and touched without running. No tail flicking observed. Head pressing may be observed.	Standing immobilization
1	Slight ataxia observed followed by < 2 attempts to lie down in sternal recumbence. No signs of CNS excitation observed without falling or stumbling. Smooth transition from sternal to lateral recumbence. Time to reach sternal recumbence (3-4 min) and lateral recumbence (up to 5 min).	Excellent
2	Moderate ataxia observed with > 2 attempts required to lie in sternal recumbence. Minimal signs of CNS excitation observed. Moderate stumbling observed. Time to reach sternal recumbence (> 4 minutes) and lateral recumbence (> 5 minutes).	Good
3	Severe ataxia with numerous attempts to lie down is observed. Moderate signs of CNS excitation are observed. Severe stumbling and falling is observed before the animal become recumbent. Moderate risk of injury. Time to reach sternal recumbence (> 6-8 minutes) and lateral recumbence (> 10 minutes).	Fair
4	Severe ataxia without animal becoming recumbent. Moderate to severe CNS excitation is observed. Repeated stumbling and falling is observed with animal requiring a second dart. Time to reach sternal recumbence (> 10-15 min) and lateral recumbence (> 30 minutes)	Poor
Immobilization		
1	Minimal immobilization achieved, animal posing a risk to ground crew and high risk of self-injury. No anaesthetic plane reached. Minimal immobilization achieved, animal posing a risk to ground crew and high risk of self-injury. No anaesthetic plane reached.	Limited effect

2	Animal struggles during manipulation, spontaneous motor activity noted. Animal responds to painful stimuli with both anal and palpebral reflexes still present.	Profound sedation
3	Reduced muscle rigidity to smooth complete relaxation. Anal reflex diminishes but retained slow palpebral. Loses pedal reflex. No reaction to painful stimuli. Animal can be handled safely. *Moderate level- no involuntary tail movements and tongue easily extractable.*	Light to moderate anaesthetic level
4	All characteristics of 3 present. Palpebral reflex and jaw tone diminished.	Surgical anaesthesia
5	Anaesthetic plane is too deep, all reflexes absent with evidence of hypoxia ARO cardiopulmonary depression.	Excessive level of anaesthesia
Recovery		
1	Transition from lateral to sternal occurs with minimal ataxic movements. Stands within 1 to 2 attempts, which are calm. Slight ataxia observed as start to walk. Recumbence to recovery < 10 minutes post reversal.	Excellent
2	Transition from lateral to sternal occurs with moderate ataxic movements. Standing requires > 2 attempts, which are relatively coordinated. Moderate ataxia observed as start to walk. Imbalance and in coordination are observed. Recumbence to recovery 10-15 minutes post reversal.	Good
3	Frequent and severely ataxic attempts to move from lateral to sternal. Numerous erratic attempts required to stand. Stumbling and falling is observed. Markedly ataxic gait when walking. Recumbence to recovery > 20 min post reversal.	Fair
4	Animal remains recumbent for > 30 minutes. No response to stimuli and no attempts to raise observed	Poor

CNS: central nervous system, ARO: as a result of.

Time and physiological data were normally distributed based on examination of histograms, descriptive statistics, and the Anderson-Darling test for normality. Physiological data were normally distributed and reported as mean ± SD. Whereas time data were non parametrically distributed and reported as median (interquartile range; IQR). Times to events (induction, first handling and recovery) were compared using Mann-Whitney-U test. Subjective quality scores during induction, immobilization and recovery were reported descriptively as median (IQR) values. Physiological data collected over time were compared between drug combinations using a general linear mixed model (Interactions: time, drug combination, drug combination x time; Fixed variables: time, drug combination; Random variable: buffalo). Model fits were assessed by visually inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Significant values were compared using Bonferroni correction for multiple pairwise comparisons. Physiological variables were averaged over time for each drug combination for textual reporting of an overall value. Commercially available software (MiniTab 18.1.0; MiniTab Incorporated, PA, USA) was used for analysis and the level of significance was set at $p \leq 0.05$.

Results

The drug doses were retrospectively corrected for body mass; total drug administered (mg) divided by body mass (kg). The administered calculated drug doses were etorphine (0.015 ± 0.001 mg kg^{-1}), azaperone (0.15 ± 0.01 mg kg^{-1}) and midazolam (0.16 ± 0.02 mg kg^{-1}). Induction time for etorphine-azaperone was 3.3 (3.6) minutes, which did not differ from 3.2 (3.2) minutes for the etorphine-midazolam treatment. Time to first handling for etorphine-azaperone was 5.1 (4.6) minutes and differ not from 6.0 (3.6) minutes for etorphine-midazolam. Induction score for etorphine-azaperone was 1 (2) and for etorphine-midazolam 2 (2). The two largest bulls had induction scores of 3 for both etorphine-azaperone and

etorphine-midazolam. Early signs of drug effect were salivation, ataxia, swaying, and drooping of the lower lip which were observed before the buffalo either stumbled and fell or calmly transitioned into sternal recumbency. No buffalo attempted to stand during the 40-minute immobilization phase with either drug combination. The immobilization score at T0 for etorphine-azaperone was 2 (1) and for etorphine-midazolam 3 (1). The overall immobilization score was 3 (1) for both combinations. Palpebral and anal reflexes were always present and none of the buffalo developed skeletal muscle rigidity. However, pronounced tail twitching (n = 1; etorphine-azaperone), thoracic limb tremors (n = 1; etorphine-midazolam), jaw trembling or chattering (n = 2; etorphine-azaperone and etorphine-midazolam) and reactivity to humans speaking in close proximity (n = 2; etorphine-azaperone) were observed.

The overall mean HR for etorphine-azaperone (113 ± 27 beats minute^{-1}) was significantly higher than etorphine-midazolam (79 ± 22 beats minute^{-1}) ($p < 0.001$: Fig. 1). The overall mean arterial blood pressure was significantly higher for etorphine-midazolam (163 ± 18 mmHg) compared with etorphine-azaperone (102 ± 25 mmHg) ($p < 0.001$: Table 2). The mean f_R for etorphine-azaperone (18 ± 4 breaths minute^{-1}) was significantly less than f_R values with etorphine-midazolam (23 ± 7 breaths minute^{-1}) ($p < 0.001$: Fig. 2). A shallow breathing pattern with intermittent deep breaths was observed frequently with both drug combinations. The $PexpiredCO_2$ for the etorphine-azaperone combination (60 ± 7 mmHg, or 8.1 ± 0.9 kPa) was significantly greater than the etorphine-midazolam treatment (56 ± 7 mmHg, or 7.6 ± 0.9 kPa) ($p = 0.03$). Rectal temperature for etorphine-azaperone (38.9 ± 0.7 $^{\circ}$C) was significantly less than the etorphine-midazolam (39.4 ± 0.5 $^{\circ}$C) ($p = 0.005$), although not clinically relevant.

Arterial blood gas analysis revealed severe hypoxaemia with both treatments etorphine-azaperone (PaO_2: 37 ± 12 mmHg,5 ± 1.6 kPa) and etorphine-midazolam (PaO_2: 43 ± 8

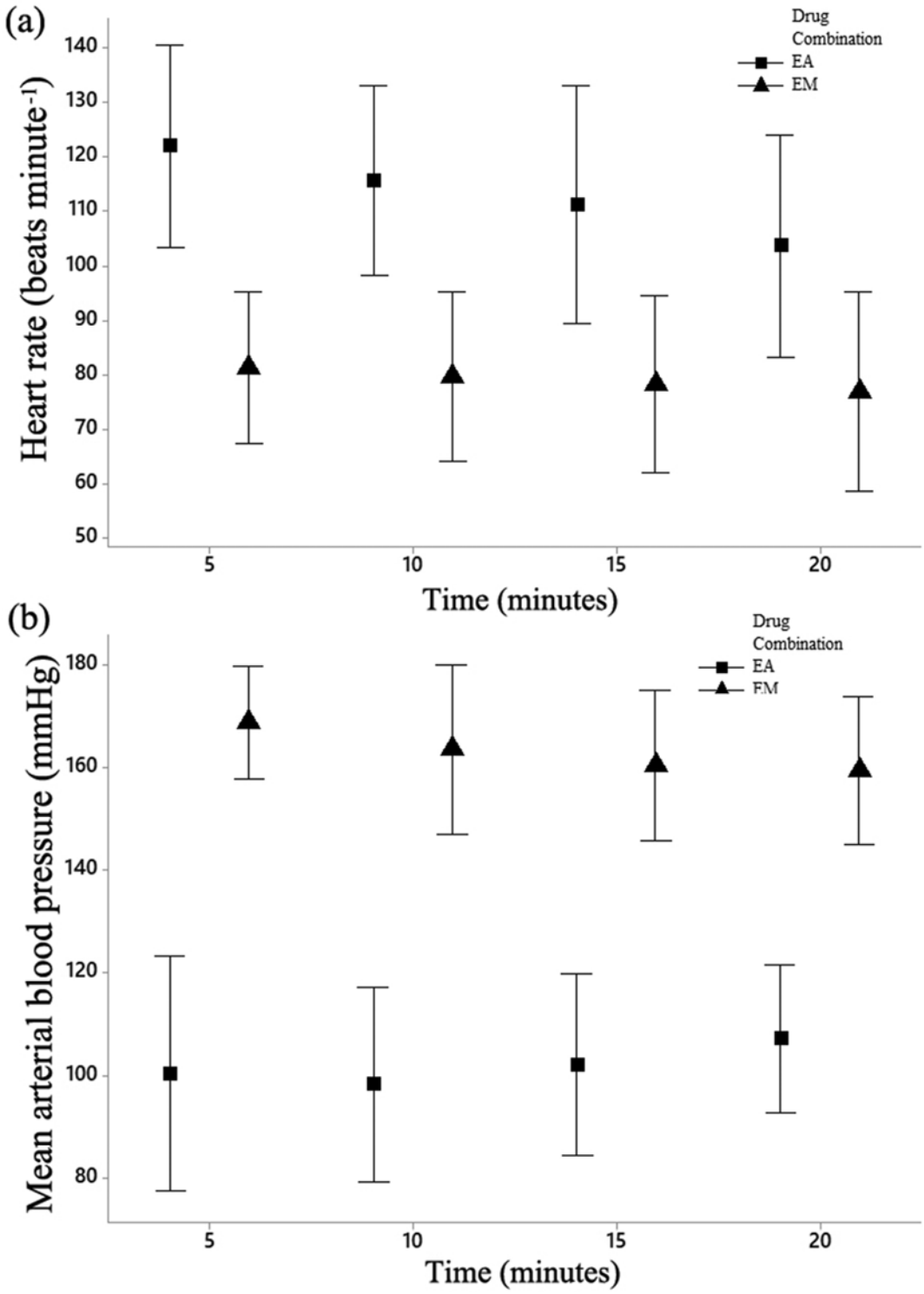

Figure 1. (a) Heart rate ($p < 0.001$ between treatments) and (b) mean arterial blood pressure ($p < 0.001$ between treatments) over time measured in 10 boma-confined adult African buffaloes immobilized with either etorphine–azaperone (EA) or etorphine–midazolam (EM) administered intramuscularly using a randomized crossover design. Etorphine was dosed at 0.015 mg kg^{-1}, and azaperone and midazolam were dosed at 0.15 mg kg^{-1}. The graph shows the mean values and 95% confidence interval over time

Table 2 Physiological variables measured at 5, 10, 15, and 20 minutes (T5, T10, T15 and T20) after the intramuscular injection of etorphine-azaperone or etorphine-midazolam administered to 10 boma-confined male African buffalo. For detailed legend see Table 1. Variables are reported as mean ± standard deviation (SD).

Variable	Drug combination	5 minutes		10 minutes		15 minutes		20 minutes	
		mean	± SD	mean	± SD	mean	± SD	mean	± SD
Heart rate	EA	122*	26	116*	24	111*	30	104*	29
(beats minute^{-1})	EM	81*	20	80*	22	78*	23	77*	26
SAP	EA	120*	27	117*	29	121*	26	127*	24
(mmHg)	EM	194*	21	187*	29	186*	28	189*	23
DAP	EA	86*	33	85*	27	88*	25	94*	19
(mmHg)	EM	15*	11	147*	17	141*	17	141*	16
MAP	EA	100*	30	98*	26	102*	25	107*	20
(mmHg)	EM	169*	13	163*	22	160*	19	159*	19
Respiratory rate	EA	18*	7	17*	4	18*	4	18*	3
(breaths minute^{-1})	EM	22*	6	24*	6	23*	7	22*	8
PexpiredCO_2	EA	61*	10	61*	6	61*	6	58*	6
(mmHg)	EM	58*	9	56*	6	56*	6	56*	6
(kPa)	EA	8.2*	1.4	8.2*	0.8	8.2*	0.8	7.8*	0.8
	EM	7.8*	1.2	7.6*	0.8	7.6*	0.8	7.6*	0.8
Rectal temperature	EA	39.2*	0.6	39.0*	0.6	38.9*	0.7	38.7**	0.8
(°C)	EM	39.4*	0.5	39.4*	0.5	39.4*	0.5	39.3*	0.6

SAP: systolic arterial blood pressure, DAP: diastolic arterial blood pressure, MAP: mean arterial blood pressure, PexpiredCO_2: partial pressure of peak expired carbon dioxide, °C: degrees Celsius, mmHg: millimetres of mercury, kPa: kilopascals, *: statistical difference between treatments, statistical difference reported as p-value ≤ 0.05.

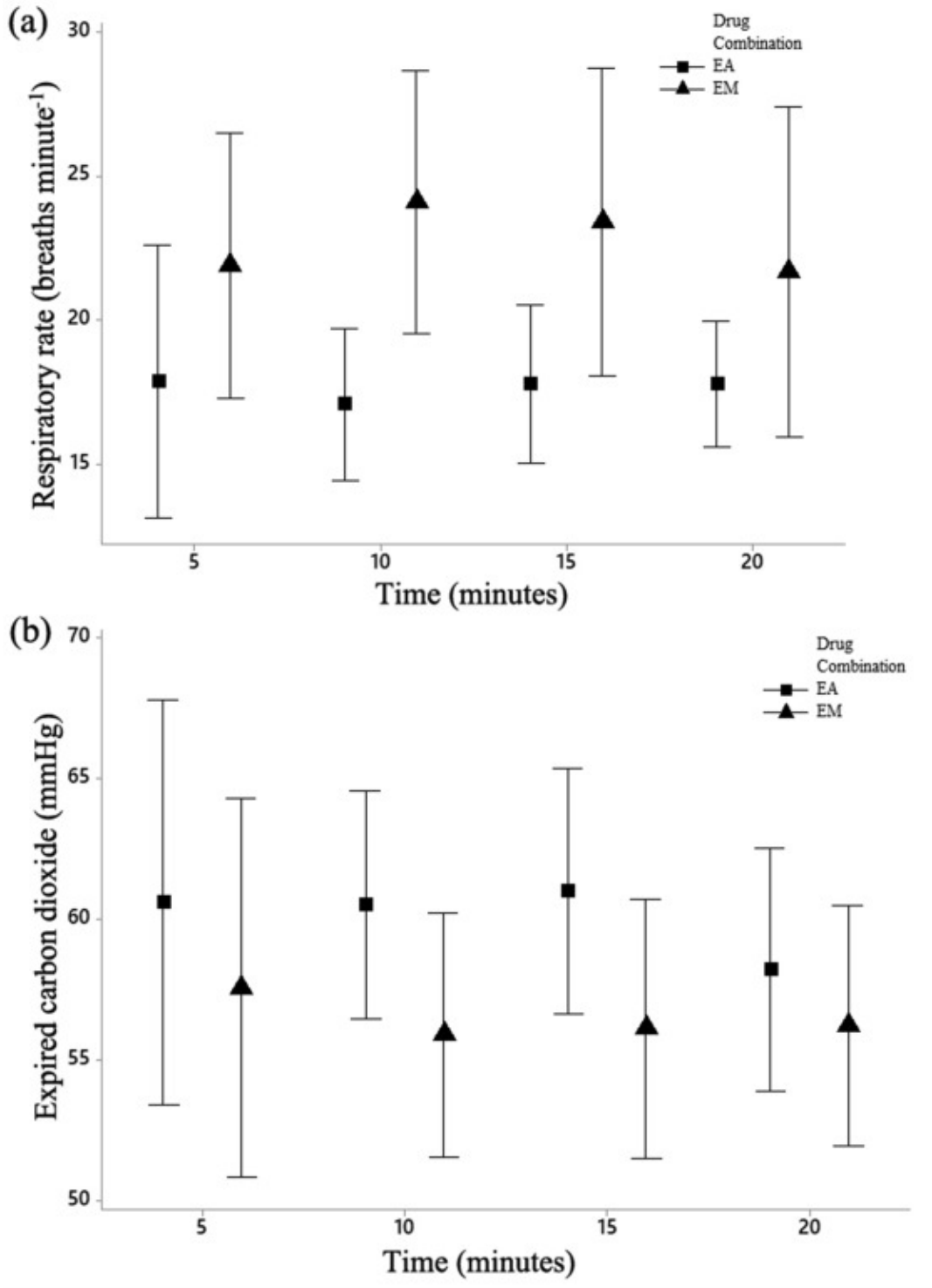

Figure 2. (a) Respiratory rate ($p < 0.001$ between treatments) and (b) expired partial pressure of carbon dioxide ($p = 0.03$ between treatments) over time measured in 10 boma-confined adult African buffaloes immobilized with either etorphine–azaperone (EA) or etorphine–midazolam (EM) administered intramuscularly using a randomized crossover design. Etorphine was dosed at 0.015 mg kg^{-1}, and azaperone and midazolam were dosed at 0.15 mg kg^{-1}. The graph shows the mean values and 95% confidence interval

Table 3 Measured arterial blood gas partial pressures and calculated alveolar to arterial gas differences obtained or derived from blood and expired gas samples taken at 5 minutes (T5) and 20 minutes (T20) after the intramuscular injection of etorphine-azaperone (EA) or etorphine-midazolam (EM) administered to 10 boma-confined male African buffalo. Variables are reported as mean ± standard deviation (SD).

Variable	Combination	5 minutes		20 minutes	
		mean	± SD	mean	± SD
$PaCO_2$	EA	57	6	57	3
(mmHg)	EM	56	4	55	3
(kPa)	EA	7.7	0.8	7.7	0.4
	EM	7.6	0.5	7.4	0.4
PaO_2	EA	37	10	37	14
(mmHg)	EM	42	9	45	7
(kPa)	EA	5	1.4	5	1.9
	EM	5.7	1.2	6.1	0.9
SaO_2 (%)	EA	63	15	70	11
	EM	71	14	76	10
Calculated gradient and arterial oxygen content					
$P(A\text{-}a)O_2$	EA	44	8	43	14
(mmHg)	EM	40	7	39	6
(kPa)	EA	6.0	1.1	5.8	1.9
	EM	5.4	1.0	5.3	0.8
CaO_2	EA	9.1	3.8	9.5	3.5
(mL dL^{-1})	EM	11.1	3.1	11.0	2.2

$PaCO_2$: arterial partial pressure of carbon dioxide, PaO_2: arterial partial pressure of oxygen, SaO_2: arterial haemoglobin oxygen, $P(A\text{-}a)O_2$ gradient: partial pressure gradient between alveolar and arterial oxygen, CaO_2: arterial oxygen content was calculated using the following equation $CaO_2 = 1.34 \times Hb \times SaO_2 + PaO_2 \times 0.003$ equation, where Hb is haemoglobin concentration

Table 4 Biochemical variables measured in arterial blood samples taken at 5 minutes (T5) and 20 minutes (T20) after the intramuscular injection of etorphine-azaperone (EA) or etorphine-midazolam (EM) administered to 10 boma-confined male African buffalo. For detailed legend see Table 1. Variables are reported as mean ± standard deviation (SD).

Variable	Combination	5 minutes		20 minutes	
		Mean	± SD	Mean	± SD
pH	EA	7.359	0.038	7.365	0.022
	EM	7.354	0.046	7.376	0.021
HCO_3^-	EA	32.0	1.8	32.8	2.3
(mmol L^{-1})	EM	31.2	2.6	32.1	1.6
BE(ecf)	EA	6.5	1.9	7.4	2.6
(mmol L^{-1})	EM	5.7	3.2	7.0	1.6
BE(b)	EA	5.4	1.8	6.3	2.6
(mmol L^{-1})	EM	4.4	3.0	5.8	1.5
Na^+	EA	139	3	138	5
(mmol L^{-1})	EM	139	6	139	6
K^+	EA	3.8*	0.3	3.7*	0.3
(mmol L^{-1})	EM	4.2*	0.4	4.0*	0.4
iCa	EA	1.06	0.07	1.04	0.06
(mmol L^{-1})	EM	1.05	0.06	1.04	0.07
Cl^-	EA	99	2	98	4
(mmol L^{-1})	EM	99	5	99	5
Ht	EA	31	6	29	8
(%)	EM	34	6	31	5
Hb	EA	10.4	2.1	9.8	2.7
(g dL^{-1})	EM	11.5	2.1	10.6	1.6
Glucose	EA	5.7	1.2	5.5	1.1
(mmol L^{-1})	EM	5.9	1.5	5.6	1.5
Lactate	EA	2.79	1.64	2.12	1.05
(mmol L^{-1})	EM	3.57	2.30	2.54	1.74
Creatinine	EA	119	25	117	17
(μmol L^{-1})	EM	127	14	123	14

BE(ecf): base excess of the extracellular fluid, BE(b): base excess of the blood, Cl^-: chloride concentration, Hb: haemoglobin concentration, HCO_3^-: bicarbonate concentration, Ht: haematocrit, iCa: ionised calcium concentration, K^+: potassium concentration, Na^+: sodium concentration, *: statistical difference between treatments, statistical difference reported as *p*-value ≤ 0.05.

mmHg, 5.8 ± 1.0 kPa) (Table 3). The $P(A\text{-}a)O_2$ gradients were greater than 20 mmHg and did not differ between the treatments at T5 and T20. An overall moderate hypercapnia was observed with etorphine-azaperone ($PaCO_2$: 57 ± 6 mmHg, 7.7 ± 0.6 kPa) and did not differ from etorphine-midazolam ($PaCO_2$: 56 ± 3 mmHg, 7.5 ± 0.5 kPa). The CaO_2 (< 11.1 mL dL^{-1}) was similar for both drug combinations. The arterial biochemical variables are presented in Table 4.

Recovery time for etorphine-azaperone was 0.8 (0.6) minutes and did not differ from 1.1 (0.6) minutes for etorphine-midazolam. The recovery score was 1 (0) for both treatments. All buffalo were ataxic for the first few seconds after standing. With the etorphine-midazolam treatment, two buffalo fell after standing but stood up immediately. All buffalo recovered completely and no renarcotization occurred within 24 hours after naltrexone administration.

Discussion

Boma-confined buffalo were effectively immobilized with both etorphine-azaperone and etorphine-midazolam drug combinations. Physiological and immobilization characteristics were similar between the two drug combinations, although marked systemic hypertension was observed in buffalo given etorphine-midazolam compared with etorphine-azaperone. Clinically relevant hypoxaemia and hypercapnia were evident with both drug combinations.

A chemical capture (immobilization) technique should ideally produce a rapid (< 5 minutes) transition from dart placement to recumbency. A prolonged induction time when immobilizing free-ranging wild animals could result in an animal fleeing into the bush, succumbing to the cardiopulmonary effects of the drugs, or becoming susceptible to predation (Fowler 2008; Schumacher 2008). Effective immobilization ideally produces minimal excitation, excellent muscle relaxation, adequate analgesia, and minimal

cardiopulmonary depression (Schumacher 2008). Both drug combinations in this study provided a rapid induction with calm transition from standing to sternal recumbence. The overall immobilization quality for both drug combinations was adequate, with no attempts to stand and sufficient relaxation to allow manipulation of the buffalo. No additional drugs were administered during the immobilization phase. The two largest bulls had longer times to recumbency and first handling than the other buffalo. Possible explanations include underdosing of the drugs administered, or delayed onset due to injectate being delivered to areas with low perfusion such as fascial planes or subcutaneous tissue (Zeiler & Meyer 2017).

Rapid recovery with minimal ataxia after administration of the antagonists is important to minimise risk of injury and predation when using immobilization, especially in free-ranging animals. Recovery was rapid with both etorphine-azaperone and etorphine-midazolam, but the two buffalo that fell after standing following etorphine-midazolam immobilization were a concern. This may not be solely related to residual drug effect. The intravenous administration of midazolam produces approximately 20 minutes of sedation in buffalo, although the pharmacokinetics are unknown (Kock et al. 2012). Transition from recumbency to standing should be calm, rapid and excitement free (Schumacher 2008). Minimal ataxia and stumbling along with a rapid return of the swallowing reflex reduces the risk of injury and aspiration pneumonia, which decreases the risk of predation.

Systemic hypertension was observed with etorphine-midazolam treatment. Severe systemic hypertension is a common side effect from using potent μ-opioid agonists (Meyer et al 2015; Buss et al. 2018). Midazolam possesses minimal cardiovascular effects at clinically used doses, while azaperone reduces peripheral resistance (Jones et al. 1979; Posner 2018). This probably explains the observed differences in arterial blood pressure between etorphine-azaperone and etorphine-midazolam. The marked systemic hypertension with etorphine-

midazolam probably resulted in a baroreceptor reflex, which caused a reduction in HR compared to etorphine-azaperone. We speculate that the lower systemic arterial blood pressure observed with etorphine-azaperone probably resulted from α_1-adrenoceptor antagonist effect of azaperone, which triggered a baroreflex that increased HR to maintain systemic arterial blood pressure (Kirchheim 1976). The elevated HR with etorphine-azaperone could also be due to direct (drug) or indirect (hypoxaemia or hypercapnia) sympathetic stimulation (Muir 2015). It should be noted that persistent hypertension for prolonged procedures (> 45 minutes as with hoof trimming and bandage management) should be managed to prevent end-organ system damage (Stemmet et al. 2019).

Clinically relevant ventilatory effects were observed with both etorphine-azaperone and etorphine-midazolam. In this study, minute ventilation was not determined and so the indicators for ventilation were f_R and PexpiredCO_2. Even though the f_R was within expected ranges (18-30 breaths minute^{-1}) with both etorphine-azaperone and etorphine-midazolam, significant hypoventilation (decreased alveolar ventilation relative to metabolic rate) occurred, which probably resulted from a decrease in tidal volume. The rumen compressing the diaphragm, decreasing functional residual capacity or a decrease of total lung compliance may all have contributed to a presumed reduction in tidal volume, resulting in an increase in $PaCO_2$ (Hattingh et al. 1984; Wilson 2007; Riebold 2015). In white rhinoceros (*Ceratotherium simum*), etorphine increases metabolic rate, which is probably related to muscle tremors, and thus leads to an increase in CO_2 production and $PaCO_2$ (Buss et al. 2018). Respiratory muscle rigidity that reduces tidal volume has been reported in impala and is thought to result from the stimulation of the μ-opioid receptor in specific areas of the medulla and pons (Pattinson 2008; Zeiler & Meyer 2017). Hypercapnia ($PaCO_2$ or PexpiredCO_2 > 55 mmHg) was observed with both etorphine-azaperone and etorphine-midazolam, but was less marked with etorphine-midazolam, possibly because midazolam

produces minimal ventilatory depression and may also reduce ventilatory muscle rigidity (Haskins 2015; Rankin 2015). The $P_{expired}CO_2$ was sampled from the nasopharynx, therefore the gas sample could have been contaminated by ruminal CO_2 which could have falsely elevated its partial pressure (Riebold 2015). Endotracheal intubation was not attempted in this study but would have enabled more accurate sampling of alveolar respiratory gases. Ruminant species are not only prone to regurgitation and possible aspiration when endotracheal intubation is attempted in a light plane of anaesthesia, but arousal may also occur endangering personnel working with wild ruminant species (Riebold 2015).

Animals in this study breathed room air and hypoventilation contributed to the severe hypoxaemia (PaO_2 = 20-30 mmHg) observed with both etorphine-azaperone and etorphine-midazolam. However, a widened $P(A\text{-}a)O_2$ gradient was also observed (normal < 15 mmHg Grimm et al. 2015; Muir 2015) and combined with hypercapnia suggested that ventilation to perfusion mismatch occurred (Napier & Armstrong 2014). We speculate that this mismatch was related to both right-to-left intrapulmonary shunting and impairment of gas diffusion across the alveolocapillary membrane primarily rather than dead-space ventilation or hypoventilation (Zeiler & Meyer 2017). When the shunt fraction is greater than 50%, hypercapnia starts to develop (Sarkar 2017). Hypoxic pulmonary vasoconstriction is reportedly a strong pulmonary reflex in domestic cattle, and this may be true in wild bovids as well (Robinsons 2013). This could also have contributed to the ventilation to perfusion mismatch and blood gas derangements. Etorphine induces pulmonary hypertension in goats, which combined with right-to-left intrapulmonary shunting contributes to the severe hypoxaemia (Meyer et al. 2015). Pulmonary hypertension increases hydrostatic pressure, which in turn leads to oedema of the interstitium and alveoli. This increase in the diffusion

distance restricts the ability of less soluble gases such as oxygen to traverse the alveolocapillary membrane (Meyer et al. 2015).

Notable limitations of this study were that only buffalo bulls were immobilized, gas sampling occurred in the nasopharynx and not from the proximal lower respiratory tract, and minute ventilation was not measured; thus, we can only speculate on the degree of hypoventilation. The drug combinations we studied were given to boma-confined buffaloes, and further investigation in free-ranging buffaloes is required. The interval from darting to first handling was subjectively affected by personnel safety considerations. Differences in drug absorption due to dart placement may also alter the 'induction' interval. To prevent the variation from being reflected in all subsequent data collection intervals, time of first handling was selected to represent T0.

Conclusion

Etorphine-midazolam provided adequate immobilization of boma-confined buffalo bulls, similar in effect to the commonly used etorphine-azaperone combination. However, the systemic arterial hypertension was a clinical concern when the etorphine-midazolam combination was used. Hypoxaemia and hypercapnia were observed with both treatments. Therefore, oxygen insufflation and appropriate monitoring during immobilization are recommended with both etorphine-azaperone and etorphine-midazolam combinations.

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