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Cardiopulmonary Parameters and Arterial Blood Gases During Etorphine-Medetomidine-Midazolam Immobilization in Free-Ranging Black Rhinoceroses (*Diceros bicornis*) Undergoing Electro-Ejaculation—A Preliminary Study

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Conservation management interventions for the critically endangered black rhinoceros (*Diceros bicornis*) require immobilization, which offer opportunities for semen collection and cryopreservation to establish genetic reservoirs. In free-ranging rhinoceroses, a combination of the potent opioid etorphine and the tranquilizer azaperone is routinely used for chemical immobilization but is associated with muscle rigidity and severe cardiopulmonary changes. Additionally, azaperone inhibits semen emission. Seven free-ranging, male, sexually mature black rhinoceroses were immobilized with an alternative protocol consisting of 4.5 mg etorphine, 5 mg medetomidine, 50 mg midazolam and 2,500 IU hyaluronidase delivered remotely by darting from a helicopter. During the immobilization, electro-ejaculation was performed with a portable electro-ejaculator, and a species-specific rectal probe. Animals were observed for muscle tremors. Longitudinal changes in respiratory rate, heart rate and peripheral oxyhemoglobin saturation, measured at 5 min intervals, were assessed using a general mixed model. Non-invasive oscillometric blood pressure and arterial blood gas variables were measured at first handling and before reversal and compared using the Wilcoxon rank sum test. All animals were successfully immobilized, showed no muscle tremors, presented with normal heart rates and lactate concentration (<5 mmol/L), recovered uneventfully, but experienced acidemia, hypoxemia and hypercapnia. Induction time and total time in recumbency were 4.2 ± 0.41 and 38.4 ± 6.9 min, respectively. Electro-stimulation commenced after 11.7 ± 3.98 min and completed after 24.3 ± 6.65 min. Semen-rich fractions

were successfully collected from six animals. Our observations indicate that etorphine-medetomidine-midazolam provides a promising immobilization protocol for free-ranging black rhinoceroses, that allows for successful electro-ejaculation.

Keywords: black rhinoceros, immobilization, heart rate, medetomidine, midazolam, arterial blood gases, blood pressure, semen collection

INTRODUCTION

In a recent assessment of the black rhinoceros population (1), the species was again classified as "critically endangered" by the International Union for Conservation of Nature (IUCN) with just over 3000 individuals in the wild—a result of the extensive poaching for horn (2).

As the numbers of individuals in the wild decrease, the preservation of gametes and their use in assisted reproductive technologies is gaining importance as additional conservation tools (3). Semen collection and cryopreservation allow to effectively preserve viable gametes in large numbers for future use in artificial insemination or ovum pick-up and embryo transfer by minimally invasive techniques and can be performed opportunistically during chemical immobilization for other management purposes (4). The development and improvement of species-specific protocols and equipment require sufficient sound knowledge of the reproductive anatomy and physiology, which for most wildlife species is based on access to animals in captivity (4). Electro-ejaculation (EE) as the most commonly used method for semen collection in wildlife species has been criticized to have animal welfare implications when used in conscious domestic animal patients. However, in wildlife species chemical immobilization is required for this procedure (4). Heart rates and serum cortisol levels were significantly lower in anesthetized domestic animals compared to conscious animals during and following EE (5).

Immobilization protocols for free-ranging black rhinoceroses are usually based on a combination of the potent opioid etorphine and a tranquilizer and/or sedative. Potent opioids result in rapid induction times and therefore a reduced risk of capture myopathy and injury (6, 7). However, they are associated with muscle rigidity, respiratory depression, hypertension, acidemia and hyperlactatemia (6, 8, 9). Azaperone is the most commonly added tranquilizer, but is reported to inhibit semen emission (10). An alternative protocol is therefore needed where semen collection is performed in free-ranging rhinoceroses. Etorphine in combination with medetomidine and midazolam was used repeatedly for intensive medical management in a black rhinoceros in captivity (11) and resulted in reliable immobilization and rapid recovery. The specific α_2-adrenoceptor agonist medetomidine provides sedation, muscle relaxation and anxiolysis (12) and, additionally, was found to promote semen emission in other species (13, 14). Midazolam, a benzodiazepine receptor agonist, also provides sedation and muscle relaxation (15).

The aim of this study was to describe the changes in respiratory, cardiovascular and blood gas variables following immobilization of black rhinoceroses with an etorphine-medetomidine-midazolam combination for the purpose of EE.

MATERIALS & METHODS

Animals and Study Area

Seven free-ranging sexually mature (7–28 years) male black rhinoceroses (*Diceros bicornis*) were immobilized for ear-notching in a provincial nature reserve in the Eastern Cape, South Africa and were additionally subjected to electro-ejaculation.

Chemical Immobilization

Rhinoceroses were located by direct observation from either a helicopter or fixed-wing aircraft. A combination of 4.5 mg etorphine (etorphine hydrochloride 9.8 mg/mL, Captivon; Wildlife Pharmaceuticals, Karino, South Africa), 5 mg medetomidine (20 mg/mL, Kyron Laboratories, Benrose, South Africa), 50 mg midazolam (midazolam hydrochloride 50 mg/mL, Dazonil; Wildlife Pharmaceuticals) and 2,500 IU hyaluronidase (lyophilised hyalase, Kyron Laboratories) was delivered by dart gun (Pneu-Dart 389, Williamsport, Pennsylvania, USA) from a helicopter into the gluteal muscles using 2.0 mL aluminum darts with 2.5-inch uncollared needles (Pneu-dart Type C, Pneu-dart, Inc., Williamsport). Immobilized rhinoceroses were approached quietly, blindfolded and earplugs applied to reduce external stimuli. The animals were kept in sternal recumbency throughout the immobilization. Time from darting to recumbency (induction time) and total time in recumbency (down time) were recorded.

To reverse the effects of etorphine, 50 mg naltrexone HCl (Trexonil 50 mg/ml, Wildlife Pharmaceuticals) were injected in an auricular vein. Medetomidine was reversed with 10–20 mg atipamezole HCl (5 mg/mL mg; Antisedan, Zoetis, Sandton, South Africa). One and two thirds of the dose were injected intravenously (auricular vein) and intramuscularly in the nuchal hump, respectively. The time from reversal until the animal was standing was recorded as recovery time.

Monitoring, Blood Sampling, and Analyses

Blood samples (1 mL) were collected from the auricular artery into heparinized syringes (Arterial Blood Sampler Aspirator, Radiometer Medical ApS, Denmark) at first handling (t0) and before termination of immobilization (t_R) and promptly analyzed using a portable blood gas analyzer (i-STAT Portable Clinical Analyzer) with cartridges (i-STAT CG4+ and Chem8+ cartridges, Zoetis, Germany). Blood pH, partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$) and lactate (Lac) were measured and base excess (BE), bicarbonate (HCO_3^-) and hemoglobin oxygen saturation (SaO_2) calculated

by the analyzer at t0 and t_R. At t0, sodium (Na), potassium (K), chloride (Cl), ionized calcium (iCa), glucose (Glu), urea (BUN), creatinine (Crea), and hematocrit (Hct) were additionally measured. All blood gas results were interpreted at a fixed temperature of 37°C.

Animals were observed for the presence of muscle tremors. At 5-min intervals from 5 min after recumbency (t_5) until reversal of the immobilization, respiratory rate (f_R) was determined by counting exhalation from the nostrils, and heart rate (HR) and peripheral oxygen hemoglobin saturation (SpO_2) recorded with a pulse-oximeter (SunTech Vet30E, Morrisville, NC, USA) using a Y-lingual sensor (AccuVet) applied on a scarified edge of an ear. Attention was paid to not cause bleeding at the site of attachment. The same device (AccuVet), which is validated for indirect non-invasive measurement of blood pressure in horses, was used to measure mean (MAP), systolic (SAP) and diastolic (DAP) arterial blood pressures in the coccygeal artery as previously described (16) at t_0 and t_R. The 17 × 25 cm cuff was fitted around the tail, approximately 20 cm below the tail base and 20 cm above the right atrium and a single reading at each time point recorded.

Electro-Ejaculation

EE was performed with a portable, battery-powered electro-ejaculator (El Toro 3, Electronic Research Group, South Africa) and a specifically designed rectal probe with three electrodes, which were positioned dorsal to the prostate as previously described (17). HR and f_R were recorded immediately before the start, at least 5 min after commencement and after completion of electro-stimulation. EE took a maximum of six sets of stimuli. Each set consisted of 8–10 stimulations lasting 3 s, with a 2-s pause in between. The voltage was slowly increased with each set of stimuli to reach a maximum of 10 V and 277 mA.

Data Analyses

Statistical analysis was performed with the software R version 3.6.1 (18). Data were assessed for normality by calculating descriptive statistics and plotting of histograms. Median (range), were calculated for each parameter and time point (**Tables 1**, **2**). A generalized linear model (fixed factor: time; random factor: individual rhinoceros) was used to assess the effect of time on HR, f_R, and SpO_2. Due to a small sample size, nonparametric analyses were used to compare arterial blood gas variables and non-invasive oscillometric blood pressure measurements at the beginning and end of immobilization, and HR and f_R before the onset, 5 min after commencement and after completion of EE, using the Wilcoxon rank sum test. Differences were considered significant when $P \leq 0.05$.

RESULTS

All animals were successfully immobilized, showed no muscle tremors, and recovered uneventfully. One animal needed a second dart with 2 mg etorphine, 5 mg medetomidine and 25 mg midazolam, presumably because of incomplete discharge of the first dart. His induction time was excluded from the induction time calculations. Mean induction time was 4.2 ± 0.4 min. Electro-stimulations started after 11.7 ± 4.0 (7–17) mins and were completed after 27.4 ± 8.9 (14–39) mins. The time from darting to reversal was 40.8 ± 7.5 (34–54) mins and total time in recumbency was 38.4 ± 6.9 (33-52) mins. Recovery after reversal was 1.7 ± 0.5 (1, 2) mins. Semen was successfully collected from six animals (86%) and first semen rich fractions were recovered as early as 40 s after the start of stimulation.

TABLE 1 | Heart rate (HR), respiratory rate (f_R) and partial pressure of oxygen (SpO_2) measured by pulse oximetry from the first measurement (t5) until maximum 50 mins (t50) and non-invasive oscillometric mean arterial (MAP), systolic (SAP) and diastolic (DAP) blood pressures at first handling (t0) and at reversal (t_R).

Time	*n*	Variable (Unit)		
		HR (beats/minute)	**f_R (breaths/minute)**	**SpO_2 (%)**
t5	*7*	60 (50–67)	4 (4–5)	80 (70–88)
t10	*7*	61 (50–67)	6 (4–7)	91 (79–97)
t15	*7*	56 (51–75)	6 (4–10)	92 (82–95)
t20	*7*	56 (52–80)	5 (4–7)	92 (83–96)
t25	*7*	57(52–77)	6 (4–8)	95 (90–97)
t30	*7*	58 (49–71)	5 (4–7)	90 (84–96)
t35	*4*	54 (32–66)	6 (4–6)	94 (79–95)
t40	*2*	59, 61	5	88, 94
t50	*1*	59	6	93
		MAP (mmHg)	**SAP (mmHg)**	**DAP (mmHg)**
t_0		67 (32–178)	90 (54–226)	63 (28–169)
t_R		161 (103–173)	199 (167–219)	147 (84–166)

Data presented as median (min-max) and n = number of animals.

Descriptive analyses of variables measured in this study, are presented in **Tables 1, 2**.

Time had no effect on HR ($P = 0.854$) and f_R ($P = 0.661$). Median HR was 58 (50–67) before the start of stimulations, 61 (51–80) after at least 5 mins (6.7 ± 1.37 mins) and 60 (50–66) bpm when stimulation was ended. f_R was 5 (4–6), 6 (5–10) and 6 (4–7) breaths per minute during the same time points. HR and f_R immediately before stimulation did not differ from measurements after commencement ($P = 0.173$ and $P = 0.157$) and completion of stimulation ($P = 0.395$ and $P = 0.335$). Time had a significant effect on SpO_2 ($P = 0.008$), which increased over time.

The pH, PaO_2, SaO_2 and BE were higher at t_R ($P = 0.018$, 0.018, 0.027 and 0.063 respectively) and $PaCO_2$ and lac lower ($P = 0.028$ and 0.018) compared to t0. Lac was < 5 mmol/L in all but one animal (**Table 2**). MAP, SAP and DAP did not differ significantly between t5 and t_R (MAP and DAP: $P = 0.091$; SAP: $P = 0.063$). SpO_2 and SaO_2 did not differ between t0 and t_R ($P = 0.207$; $P = 0.916$).

DISCUSSION

Immobilization with etorphine-medetomidine-midazolam resulted in short induction times similar to those previously reported in free-ranging black rhinoceroses immobilized with other etorphine-combinations (6, 8, 9) and uneventful recoveries. No muscle tremors were observed and lac was normal in all

TABLE 2 | PH, partial pressure of carbon dioxide ($PaCO_2$), pressure of oxygen (PaO_2), base excess (BE), bicarbonate (HCO_3^-), hemoglobin oxygen saturation (SaO_2) and lactate (Lac) at first handling (t_0) and reversal (t_R) of seven black rhinoceroses anesthetized in this study with etorphine-medetomidine-midazolam; Blood chemistry parameters of seven black rhinoceroses at t_0: sodium (Na), potassium (K), chloride (Cl), ionized calcium (iCa), Glucose (Glu), urea (BUN), creatinine (Crea), and hematocrit (Hct).

Variable	Unit	Time	
		t_0	t_R
pH		7.23 (7.14–7.38)	7.33 (7.28–7.36)
$PaCO_2$	mmHg	56.8 (45–65)	49.6 (45–56)
PaO_2	mmHg	63 (35–84)	66 (49–86)
HCO_3^-	mmol/L	23.90 (15–29)	26.10 (22-28)
BE	mmol/L	−4 (−14–2)	0 (−5–2)
SaO_2	%	87 (53–92)	92 (82–95)
Lac	mmol/L	1.5 (0.6–12.5)	0.53 (0.4–6.6)
Na	mmol/L	131 (125–133)	
K	mmol/L	4.8 (3.5–4.5)	
Cl	mmol/L	98 (96–103)	
BUN	mg/dL	7 (4–11)	
CREA	mg/dL	0.60 (0.06–0.80)	
Glu	mg/dL	85 (74–101)	
iCa	mmol/L	1.55 (1.46–1.60)	
Hct	%PCV	46 (40–50)	

Data presented as median (range).

but one animals. However, rhinoceroses in this study initially presented with acidemia, hypoxemia and hypercapnia, which improved over the course of anesthesia. Indirect oscillometric blood pressure was hypertensive and the HR normal (normal heart rate range 32–42 bpm, reported from unrestrained standing white rhinoceroses) throughout the immobilization.

Capture of free-ranging rhinoceroses, especially when using helicopters, is linked with overexertion-related physiological changes including hyperthermia, hypoxemia, acidemia and hyperlactatemia. Potent opioids, although resulting in rapid induction, additionally are associated with severe side-effects like muscle rigidity, tachycardia, hypertension, respiratory depression and sequelae of the latter (6, 19). Hypoventilation resulting from μ-receptor-related effects of the etorphine including respiratory muscle rigidity, increased upper airway resistance and a central inhibitory effect on the respiration, as well as the pressure of the abdominal organs on the lungs and ventilation–perfusion mismatch, causes acidemia, hypoxemia and hypercapnia (9, 20–25). However, etorphine-induced pulmonary hypertension and its negative effects on gas exchange, was identified as main reason for the initial severe hypoxemia (26).

Hypoxemia and hypercapnia were comparable to etorphine-azaperone immobilized black rhinoceroses which were also positioned in sternal recumbency (6, 8). However, compared to rhinoceroses in those studies, our rhinoceroses had a lower f_R suggesting a higher tidal volume leading to a comparable alveolar minute ventilation and $PaCO_2$. This is possibly due to improved muscle relaxation induced by medetomidine and midazolam decreasing chest wall rigidity and enhancing respiratory excursions resulting in a decrease in global ventilation-to-perfusion mismatch and improved gas exchange. The improved muscle relaxation compared to etorphine-azaperone and lack of muscle tremors may have decreased oxygen consumption and therefore CO_2 production resulting in the comparable PaO_2 and $PaCO_2$ level (27). Further studies comparing the different anesthetic protocols under more standardized conditions are required to confirm these assumptions.

Acidemia, present in rhinoceroses immobilized with etorphine-combinations, is believed to be both of respiratory and metabolic (lactic acid) origin (6). The elevated $PaCO_2$ concentrations at t0 indicate a respiratory acidemia in this study. No published lactate reference values for black rhinoceroses are available, however, five of the seven animals in this study had lactate concentrations that are within the normal range quoted for non-sedated white rhinoceroses (28). This suggests that lactate did not contribute to the acidemia in these six individuals. Only one animal showed a significantly elevated lactate concentration (12.49 mmol/L) together with the lowest recorded pH (7.136), but a normal $PaCO_2$ (45 mmHg). Hyperlactatemia is associated with intense muscle activity following prolonged induction times and increased anaerobic metabolism with lactate production (29). The overall low lactate concentrations of the remaining animals including the one that was darted twice, suggest efficient induction with the drug combination used. Lateral recumbency, resulting in increased dead-space ventilation and more severe hypoxemia, has been associated with higher lactate values in previous studies (8). Sternal positioning employed in this study appeared to have a positive effect on ventilation.

Overall, blood gas values improved in all animals from t0 to t_R, which suggests an improvement in ventilation during the immobilization. Similar results were obtained in other black rhinoceros studies that employed etorphine-based drug combinations (6). In a previous report on the repeated use of etorphine-medetomidine-midazolam in a black rhinoceros, immobilization and analgesia lasted for 34–78 mins without supplemental treatment (11). At t_R, the effects of the anesthetic drugs were probably less pronounced than at t0, resulting in increased cardiac output, decreased pulmonary hypertension and therefore an improved gas exchange through enhanced pulmonary perfusion (26).

Systemic hypertension is a known side-effect of etorphine (6, 19) and will initially be exacerbated by the peripheral vasoconstriction caused medetomidine (12, 30). In this study, indirect blood pressures recorded at t5 varied greatly amongst the individuals and were only increased above the reference ranges for unsedated white rhinoceroses in three of the seven rhinoceroses (15). At t_R, all rhinoceroses experienced hypertension, which could have been due to sympathetic activation caused by the EE. Unfortunately, continuous measurements during EE were not possible owing to the position of the probe handle close to the tail and thus the measuring cuff. Additionally, the method

used has not been validated for rhinoceroses and, given the short, conical tail, the correct positioning of the cuff is rather challenging. Furthermore, the pronounced peripheral vascular resistance associated with the medetomidine at t_0, may have resulted in incorrect readings and explain the large differences between individuals, no longer present t_R.

Tachycardia is another common finding in rhinoceroses immobilized with etorphine-combinations (22), which was suggested to be a result of etorphine-induced upregulated sympathetic activity (31). Medetomidine on the other hand, is known to cause bradycardia as response to vasoconstriction as well as through a reduced sympathetic tone and suppression of the cardiovascular center (30, 32). This drug therefore may have compensated for the sympathomimetic action of etorphine and explain why heart rates remained within normal limits quoted for rhinoceroses in all individuals in this study (16).

EE had no effect on the HR and all animals remained immobilized indicating that the present immobilization protocol provided sufficient immobilization and analgesia. Continuous invasive blood pressure monitoring together with the measurement of serum catecholamine concentrations are required in future studies to truly investigate the sympathetic nociceptive response to EE in etorphine-immobilized black rhinoceroses. Finally, semen was collected successfully from six of seven individuals despite the limited time available, and with acceptable blood gas and acid base variables.

Limitations

Data collection time points were pre-determined by the planned procedure as blood pressure measurements could not be repeated during the EE procedure. Additionally, the SunTech indirect blood pressure monitor has not been validated for the use in rhinoceroses. However, the equine settings were used and the paired measurements still provide information about the changes in blood pressure during the immobilization with etorphine-medetomidine-midazolam. The distance the animals moved from darting until recumbency was not estimated and may have influenced the results. Scarified SpO_2 readings have not been validated in rhinoceroses. However, SpO_2 readings were consistent with SaO_2.

CONCLUSIONS

The combination of etorphine-medetomidine-midazolam resulted in uncomplicated immobilizations, fast induction times and allowed for successful semen collection via electro-ejaculation. These preliminary results suggest that etorphine-medetomidine-midazolam is an acceptable immobilization protocol for use in free-ranging black rhinoceroses in general and for individuals undergoing EE.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Pretoria Animal Ethics and Research Committee (REC113-19).

AUTHOR CONTRIBUTIONS

JM, IL, and IL-B designed the experiment together with BT. BT took veterinary care of the rhinoceroses and collected the arterial blood samples. Data was collected and prepared by JM and analyzed by FP and JM. JM prepared the manuscript together with all co-authors. All authors approved the final manuscript.

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