

## REFERENCE CARDIOPULMONARY PHYSIOLOGIC PARAMETERS FOR STANDING, UNRESTRAINED WHITE RHINOCEROSES (*CERATOTHERIUM SIMUM*)

Scott B. Citino, D.V.M., Dipl. A.C.Z.M. and Mitchell Bush, D.V.M., Dipl. A.C.Z.M.

**Abstract:** Chemical restraint is an important tool for the management and medical care of both captive and free-ranging rhinoceroses. Current anesthetic protocols for the white rhinoceros (*Ceratotherium simum*) are reported to cause varying degrees of hypertension, tachycardia, muscular stiffness and fasciculation, acidosis, and, most importantly, respiratory depression with resulting hypoventilation, hypoxia, and hypercapnea. To assist in the assessment and development of new and improved anesthetic techniques for the white rhinoceros, the following cardiopulmonary reference parameters for standing, unrestrained white rhinoceroses were generated (mean  $\pm$  standard error [minimum – maximum]): heart rate =  $39 \pm 0.8$  beats/min (32–42), respiratory rate =  $19 \pm 0.6$  breaths/min (16–23), corrected indirect systolic blood pressure =  $160 \pm 2.9$  mm Hg (146–183), corrected indirect diastolic blood pressure =  $104 \pm 2.3$  mm Hg (88–117), corrected indirect mean blood pressure =  $124 \pm 2.2$  mm Hg (108–135), end tidal CO<sub>2</sub> =  $45.1 \pm 0.7$  mm Hg (41.7–48.0), rectal temperature =  $36.8 \pm 0.1^\circ\text{C}$  (36.6–37.2), arterial blood pH =  $7.391 \pm 0.007$  (7.346–7.431), arterial partial pressure of oxygen =  $98.2 \pm 1.4$  mm Hg (90.2–108.6), arterial partial pressure of CO<sub>2</sub> =  $49.0 \pm 0.9$  mm Hg (44.4–53.7), base excess =  $3.5 \pm 0.4$  mmol/L (1.9–5.9), bicarbonate =  $29.3 \pm 0.4$  mmol/L (27.3–32.2), and arterial hemoglobin oxygen saturation (S<sub>a</sub>O<sub>2</sub>) =  $97.2 \pm 0.1\%$  (96.6–98.0).

**Key words:** Arterial blood gases, blood pressure, *Ceratotherium simum*, normal cardiopulmonary values, physiology, white rhinoceros.

### INTRODUCTION

Chemical restraint is an important tool for the management and medical care of both captive and free-ranging rhinoceroses. Current anesthetic protocols for anesthesia in rhinoceroses generally involve the use of a potent opioid (e.g., etorphine) in combination with the butyrophenone tranquilizer azaperone or an  $\alpha$ -2-adrenoceptor agonist such as xylazine or detomidine.<sup>2,6,9,12–16</sup> These anesthetic combinations are reported to cause varying degrees of hypertension, tachycardia, muscular stiffness and fasciculation, acidosis, and, most importantly, respiratory depression with resulting hypoventilation, hypoxia, and hypercapnea.<sup>2–7</sup> Of all the rhinoceros species, the white rhinoceros (*Ceratotherium simum*) seems to suffer the most from these physiologic alterations during anesthesia.<sup>2–4,6</sup> These physiologic alterations are accentuated during field anesthesia of free-ranging white rhinoceroses, because higher doses of anesthetic agents are required to shorten induction times and overcome the excitement of these procedures.<sup>2–4,6</sup>

To improve anesthetic techniques in the white rhinoceros, it would be helpful to have reference

ranges for cardiopulmonary parameters so that comparisons could be made. The purpose of this study was to generate reference ranges for cardiopulmonary parameters in standing, unrestrained white rhinoceroses.

### MATERIALS AND METHODS

#### Animal management

The group of white rhinoceroses used in this study was maintained at White Oak Conservation Center, a large, private conservation center located on the eastern Florida–Georgia border. The study group consisted of 12 (five males and seven females) white rhinoceroses ranging from 2 to 31 yr old. Seven of the 12 rhinoceroses were founder animals imported from the Republic of South Africa. The females and young were housed in a 2.4-ha natural, cable-enclosed pen with grass substrate and five 130-m<sup>2</sup> pipe-enclosed catch corrals with walk-through chutes. The adult bulls were either housed with the breeding herd in the large enclosure, or they were housed singly in 130-m<sup>2</sup> pipe enclosed pens. Animals were fed a concentrate pellet (Mazuri® ADF #16 Herbivore, Mazuri, St. Louis, Missouri 63166, USA) and coastal Bermuda hay, and they had free access to graze grass in their enclosures. All animals were considered in good body condition and health.

#### Data collection

Physiologic data on the rhinoceroses were collected from 26 August 2003 to 31 August 2005.

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From White Oak Conservation Center, 581705 White Oak Road, Yulee, Florida 32097, USA (Citino); and Conservation and Research Center, Smithsonian National Zoological Park, 1500 Remount Road, Front Royal, Virginia 22630, USA (Bush). Correspondence should be directed to Dr. Citino (scottc@wogilman.com).

**Table 1.** Individual mean  $\pm$  SD of cardiopulmonary physiologic parameters from 12 healthy, standing, unrestrained white rhinoceroses (*Ceratotherium simum*).

Animal accession no.	Sex <sup>a</sup>	Date of birth	Heart rate (bpm) <sup>b</sup>	Respiration rate (bpm) <sup>c</sup>	Indirect systolic pressure (mm Hg)	Indirect diastolic pressure (mm Hg)	Indirect mean pressure (mm Hg)
Y34301	M	1 Jan 1974 (estimated)	35 $\pm$ 5 <i>n</i> = 6	20 $\pm$ 10 <i>n</i> = 6	140 $\pm$ 8 <i>n</i> = 4	89 $\pm$ 9 <i>n</i> = 4	113 $\pm$ 18 <i>n</i> = 4
980330	M	9 Nov 1990 (estimated)	40 $\pm$ 13 <i>n</i> = 6	17 $\pm$ 7 <i>n</i> = 6	157 $\pm$ 33 <i>n</i> = 6	85 $\pm$ 33 <i>n</i> = 6	130 $\pm$ 37 <i>n</i> = 6
980331	F	9 Nov 1988 (estimated)	39 $\pm$ 9 <i>n</i> = 5	16 $\pm$ 5 <i>n</i> = 5	141 $\pm$ 11 <i>n</i> = 5	79 $\pm$ 14 <i>n</i> = 5	106 $\pm$ 8 <i>n</i> = 5
980332	F	9 Nov 1992 (estimated)	37 $\pm$ 4 <i>n</i> = 6	20 $\pm$ 5 <i>n</i> = 6	131 $\pm$ 11 <i>n</i> = 6	70 $\pm$ 10 <i>n</i> = 6	102 $\pm$ 6 <i>n</i> = 6
980333	F	9 Nov 1992 (estimated)	41 $\pm$ 7 <i>n</i> = 6	17 $\pm$ 9 <i>n</i> = 6	127 $\pm$ 14 <i>n</i> = 4	82 $\pm$ 7 <i>n</i> = 4	97 $\pm$ 8 <i>n</i> = 4
980334	F	1 Jan 1988 (estimated)	32 $\pm$ 2 <i>n</i> = 5	16 $\pm$ 2 <i>n</i> = 5	131 $\pm$ 2 <i>n</i> = 4	79 $\pm$ 3 <i>n</i> = 4	96 $\pm$ 3 <i>n</i> = 4
980335	F	1 Jan 1997 (estimated)	41 $\pm$ 8 <i>n</i> = 4	20 $\pm$ 4 <i>n</i> = 4	141 $\pm$ 1 <i>n</i> = 4	80 $\pm$ 7 <i>n</i> = 4	100 $\pm$ 4 <i>n</i> = 4
Y14016	F	4 Jul 2001	38 $\pm$ 3 <i>n</i> = 5	20 $\pm$ 6 <i>n</i> = 5	139 $\pm$ 34 <i>n</i> = 4	87 $\pm$ 26 <i>n</i> = 4	105 $\pm$ 30 <i>n</i> = 4
Y24021	M	3 Oct 2002	39 $\pm$ 3 <i>n</i> = 6	23 $\pm$ 5 <i>n</i> = 6	130 $\pm$ 11 <i>n</i> = 4	75 $\pm$ 11 <i>n</i> = 4	93 $\pm$ 10 <i>n</i> = 4
Y34001	M	8 Jan 2003	39 $\pm$ 5 <i>n</i> = 6	19 $\pm$ 3 <i>n</i> = 6	122 $\pm$ 14 <i>n</i> = 6	66 $\pm$ 13 <i>n</i> = 6	92 $\pm$ 8 <i>n</i> = 6
Y34002	F	10 Jan 2003	40 $\pm$ 4 <i>n</i> = 6	19 $\pm$ 5 <i>n</i> = 6	125 $\pm$ 12 <i>n</i> = 6	67 $\pm$ 13 <i>n</i> = 6	93 $\pm$ 13 <i>n</i> = 6
Y34008	M	30 May 2003	42 $\pm$ 5 <i>n</i> = 5	16 $\pm$ 3 <i>n</i> = 5	133 $\pm$ 19 <i>n</i> = 4	80 $\pm$ 15 <i>n</i> = 4	98 $\pm$ 17 <i>n</i> = 4

<sup>a</sup> M, male; F, female.<sup>b</sup> bpm, beats/min.<sup>c</sup> bpm, breaths/min.

Data collection occurred throughout the year to reduce seasonal and temperature effects on the data and between 1100 and 1400 hours of each collection day. Each rhinoceros was trained to line up within a pipe-enclosed chute with positive reinforcements of food treats and scratching for physiologic data collection. Animals were never locked in the chute, and they could leave whenever they wished. During the collection period, each rhinoceros was sampled on four to six separate occasions, so each rhinoceros had four to six values for each physiologic parameter at the end of the study.

Physiologic data that were collected included heart rate, respiration rate, indirect arterial blood pressure (systolic, diastolic, and mean), end tidal CO<sub>2</sub> (EtCO<sub>2</sub>), rectal temperature, and arterial blood pH, arterial partial pressure of oxygen (p<sub>a</sub>O<sub>2</sub>), arterial partial pressure of CO<sub>2</sub> (p<sub>a</sub>CO<sub>2</sub>), arterial hemoglobin oxygen saturation (S<sub>a</sub>O<sub>2</sub>), bicarbonate (HCO<sub>3</sub><sup>-</sup>), and base excess. Heart rate was determined by palpation of pulse at the auricular artery; respiration rate was determined by counting chest excursions; indirect blood pressure was determined

by the use of a portable multiparameter patient monitor (MDE Escort II, Model 20100, Medical Data Electronics, Arleta, California 91331-4309, USA) with the cuff (human wrist cuff) placed at the base of the tail; EtCO<sub>2</sub> was determined by the use of a portable multiparameter patient monitor with its mainstream sensor (Model 20020, Medical Data Electronics) attached to a 25.0-mm polyethylene tube placed in one nostril; and temperature was determined by the use of an electronic thermometer (FlashCheck Digital Veterinary & Laboratory Thermometer, Model 11026, DeltaTRAK, Inc., Pleasanton, California 94566, USA) placed in the rectum. Indirect blood pressures were corrected to heart level using the formula Corrected Blood Pressure = ([distance in cm from center of cuff on tail to heart base/1.36] + actual coccygeal blood pressure), with distance estimated as distance of the center of the cuff to the ground minus distance from the heart base to the ground.

Arterial blood was collected from an auricular artery on the inside of the ear using a heparinized 25-gauge butterfly set (Terumo SURFLO® Winged

**Table 1.** Extended.

EtCO <sub>2</sub> (mm Hg)	Rectal temp. (°C)	pH	p <sub>a</sub> O <sub>2</sub> (mm Hg)	p <sub>a</sub> CO <sub>2</sub> (mm Hg)	Base excess (mmol/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)	S <sub>a</sub> O <sub>2</sub> (%)
45.1 ± 2.4 <i>n</i> = 6	36.8 ± 0.2 <i>n</i> = 6	7.391 ± 0.023 <i>n</i> = 6	98.3 ± 4.8 <i>n</i> = 6	49.0 ± 3.1 <i>n</i> = 6	3.5 ± 1.4 <i>n</i> = 6	29.3 ± 1.4 <i>n</i> = 6	97.2 ± 1.0 <i>n</i> = 6
45.3 ± 6.7 <i>n</i> = 4	36.6 ± 0.1 <i>n</i> = 4	7.381 ± 0.012 <i>n</i> = 6	98.2 ± 10.8 <i>n</i> = 6	51.3 ± 3.1 <i>n</i> = 6	3.6 ± 1.8 <i>n</i> = 6	29.7 ± 2.0 <i>n</i> = 6	97.2 ± 1.0 <i>n</i> = 6
48.0 ± 8.5 <i>n</i> = 4	36.9 ± 0.4 <i>n</i> = 5	7.431 ± 0.019 <i>n</i> = 5	102.3 ± 13.2 <i>n</i> = 5	45.9 ± 1.6 <i>n</i> = 5	4.9 ± 1.9 <i>n</i> = 5	29.9 ± 2.0 <i>n</i> = 5	98.0 ± 0.6 <i>n</i> = 5
41.7 ± 5.2 <i>n</i> = 6	36.7 ± 0.5 <i>n</i> = 6	7.382 ± 0.013 <i>n</i> = 6	94.8 ± 3.6 <i>n</i> = 6	48.1 ± 4.0 <i>n</i> = 6	2.2 ± 2.1 <i>n</i> = 6	28.3 ± 2.1 <i>n</i> = 6	97.4 ± 0.6 <i>n</i> = 6
47.0 ± 3.6 <i>n</i> = 4	36.7 ± 0.4 <i>n</i> = 4	7.393 ± 0.042 <i>n</i> = 4	108.6 ± 25.8 <i>n</i> = 4	45.8 ± 4.5 <i>n</i> = 4	1.9 ± 1.3 <i>n</i> = 4	27.3 ± 1.1 <i>n</i> = 4	97.1 ± 2.3 <i>n</i> = 4
42.3 ± 5.7 <i>n</i> = 4	37.0 ± 0.6 <i>n</i> = 4	7.402 ± 0.028 <i>n</i> = 5	97.0 ± 13.7 <i>n</i> = 5	49.8 ± 5.1 <i>n</i> = 5	4.8 ± 4.6 <i>n</i> = 5	30.4 ± 4.9 <i>n</i> = 5	97.2 ± 0.8 <i>n</i> = 5
47.7 ± 6.5 <i>n</i> = 4	36.8 ± 0.2 <i>n</i> = 6	7.382 ± 0.013 <i>n</i> = 4	90.2 ± 14.8 <i>n</i> = 4	50.1 ± 4.2 <i>n</i> = 4	3.2 ± 1.8 <i>n</i> = 4	29.2 ± 1.6 <i>n</i> = 4	96.6 ± 0.7 <i>n</i> = 4
46.0 ± 4.6 <i>n</i> = 4	37.2 ± 0.3 <i>n</i> = 6	7.346 ± 0.012 <i>n</i> = 5	96.8 ± 16.5 <i>n</i> = 5	53.5 ± 2.1 <i>n</i> = 5	2.0 ± 0.1 <i>n</i> = 5	28.6 ± 0.4 <i>n</i> = 5	96.8 ± 1.4 <i>n</i> = 5
46.7 ± 3.8 <i>n</i> = 4	37.0 ± 0.2 <i>n</i> = 6	7.396 ± 0.024 <i>n</i> = 6	95.0 ± 10.4 <i>n</i> = 6	53.7 ± 4.3 <i>n</i> = 6	5.9 ± 2.6 <i>n</i> = 6	32.2 ± 2.8 <i>n</i> = 6	96.6 ± 1.2 <i>n</i> = 6
43.0 ± 5.0 <i>n</i> = 4	36.9 ± 0.2 <i>n</i> = 6	7.386 ± 0.027 <i>n</i> = 5	98.7 ± 10.6 <i>n</i> = 5	48.9 ± 6.9 <i>n</i> = 5	2.8 ± 1.3 <i>n</i> = 5	28.5 ± 2.3 <i>n</i> = 5	97.4 ± 0.8 <i>n</i> = 5
46.5 ± 2.1 <i>n</i> = 4	36.6 ± 0.4 <i>n</i> = 4	7.384 ± 0.010 <i>n</i> = 4	101.6 ± 5.2 <i>n</i> = 4	44.4 ± 8.8 <i>n</i> = 4	2.4 ± 1.0 <i>n</i> = 4	28.1 ± 1.1 <i>n</i> = 4	97.7 ± 0.8 <i>n</i> = 4
42.0 ± 2.0 <i>n</i> = 5	36.7 ± 0.0 <i>n</i> = 4	7.423 ± 0.012 <i>n</i> = 5	97.3 ± 9.4 <i>n</i> = 5	47.4 ± 2.5 <i>n</i> = 5	5.0 ± 0.7 <i>n</i> = 5	30.3 ± 1.0 <i>n</i> = 5	96.9 ± 1.2 <i>n</i> = 5

Infusion Set, Terumo Medical Corp., Elkton, Maryland 21921, USA) attached to a 3.0-ml heparinized plastic syringe (Monoject® 3-ml Syringe-Regular Luer Tip, Tyco Health Care Group LP, Mansfield, Maryland 02048, USA). Syringes containing arterial blood were capped with an air bubble removal device (Portex Filter-Pro® Air Bubble Removal Device, SIMS Portex, Inc., Keene, New Hampshire 03431, USA), all gas bubbles were removed, and the samples were stored on ice until analyzed. Blood gas and pH analyses on arterial blood samples were performed within 30 min of collection on a portable analyzer (AVL OPTI Critical Care Analyzer, AVL Scientific Corp., Roswell, Georgia 30077, USA).

Because each rhinoceros had four to six measured values for each physiologic parameter, means ± SD were first calculated for each physiologic parameter of each animal, and then descriptive statistics were performed on the means of each physiologic parameter using a commercial statistics software package (SigmaStat for Windows, version 3.0.1, SPSS Inc., Chicago, Illinois 60611, USA). The mean of the means, standard error of the mean, range, minimum and maximum values, and 25th and 75th percentiles are presented for each physiologic parameter. Physiologic values were com-

pared between rhinoceroses 2 to 4 yr old and rhinoceroses >4 yr old using the Mann-Whitney rank sum test for significant difference at  $P \leq 0.05$  (SigmaStat for Windows, version 3.0.1).

## RESULTS

Table 1 displays means ± SD for each physiologic parameter measured for each individual rhinoceros, so intra-animal variation for each parameter could be evaluated. Reference physiologic data for healthy, unrestrained white rhinoceroses are presented in Table 2. Reference arterial blood pH, p<sub>a</sub>O<sub>2</sub>, p<sub>a</sub>CO<sub>2</sub>, S<sub>a</sub>O<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and base excess data are presented in Table 3. No significant difference was found in any physiologic parameter between rhinoceroses 2 to 4 yr old and >4 yr old, so data were combined to determine reference values.

## DISCUSSION

Little information is available in the literature regarding normal cardiopulmonary physiologic parameters for unrestrained rhinoceroses. Heart rate has been reported as 64–67,<sup>18</sup> 70–140 (juveniles),<sup>10</sup> and 30–40<sup>9</sup> beats/min; respiratory rate has been reported as 12–16,<sup>18</sup> 20–40,<sup>10</sup> and 6–12<sup>9</sup> breaths/min; and temperature has been reported as 29.4–35.0,<sup>18</sup> 37.0–39.0,<sup>10</sup> and 34.5–37.5°C<sup>9</sup> in the rhinoceros.

**Table 2.** Reference physiologic data from 12 healthy, standing, unrestrained captive white rhinoceroses (*Ceratotherium simum*).

Physiologic parameter	Mean	SE	Range	Min.	Max.	25%	75%
Heart rate (bpm) <sup>a</sup>	39	0.8	10	32	42	38	41
Respiratory rate (bpm) <sup>b</sup>	19	0.6	7	16	23	17	20
Indirect systolic pressure (mm Hg) <sup>c</sup>	135	2.7	34	123	157	128	140
Indirect systolic pressure (mm Hg) <sup>d</sup>	160	2.9	37	146	183	155	167
Indirect diastolic pressure (mm Hg) <sup>c</sup>	78	2.2	23	66	89	73	84
Indirect diastolic pressure (mm Hg) <sup>d</sup>	104	2.3	29	88	117	100	111
Indirect mean pressure (mm Hg) <sup>c</sup>	102	3.1	38	92	130	95	106
Indirect mean pressure (mm Hg) <sup>d</sup>	124	2.2	27	108	135	119	129
EtCO <sub>2</sub> (mm Hg)	45.1	0.7	6.3	41.7	48.0	42.7	46.9
Rectal temp. (°C)	36.8	0.1	0.6	36.6	37.2	36.7	37.0

<sup>a</sup> bpm, beats/min.<sup>b</sup> bpm, breaths/min.<sup>c</sup> Coccygeal blood pressures uncorrected to heart level.<sup>d</sup> Coccygeal blood pressures corrected to heart level.

These ranges are disparate, and no information is given regarding how measurements were taken. The values generated in this study fall closest to those in the third grouping mentioned above. Field studies evaluating the physiologic response of white rhinoceroses to narcotic-tranquilizer/sedative combinations have shown markedly elevated heart rate and moderately reduced respiration rate in comparison with the values described in this study.<sup>2,5-7,13,19</sup>

Reference ranges for arterial blood pressure and EtCO<sub>2</sub> in the unrestrained white rhinoceros could not be found in the literature. Direct blood pressures in normal adult horses are reported as 126–168 mm Hg (systolic) and 85–116 mm Hg (diastolic), with a mean of 110–133 mm Hg.<sup>8</sup> Indirect resting coccygeal blood pressures in clinically normal horses corrected to heart level were reported as 149 ± 19.0/97.6 ± 14.0 mm Hg.<sup>11</sup> The corrected indirect coccygeal pressure values found for standing, unrestrained white rhinoceroses in this study fall within the ranges described for both direct and indirect blood pressure in the horse. These refer-

ence values verify that hypertension is often present in anesthetized white rhinoceroses.<sup>2,4,5,7,13</sup>

Reported values for P<sub>a</sub>O<sub>2</sub> in adult resting horses include 96.0 ± 8.0 mm Hg<sup>8</sup> and from 90.2 ± 2.2 to 101.7 ± 1.6 mm Hg.<sup>1</sup> The P<sub>a</sub>O<sub>2</sub> reference value from this study falls within the range of normal values for the horse, but as expected, it is much higher than those values reported for chemically restrained white rhinoceroses in the field.<sup>2-5</sup> The reference P<sub>a</sub>CO<sub>2</sub> value in white rhinoceros is slightly higher than the P<sub>a</sub>CO<sub>2</sub> in the adult horse, which is reported as ranging from 41.5 ± 1.0 to 43.0 ± 0.7 mm Hg,<sup>1</sup> and it is lower than P<sub>a</sub>CO<sub>2</sub> values reported for anesthetized white rhinoceroses.<sup>2-5,19</sup> Arterial blood pH reported for resting adult horses ranged from 7.404 ± 0.005 to 7.428 ± 0.007,<sup>1</sup> which is higher than the reference pH value for white rhinoceroses found in this study. Base excess and HCO<sub>3</sub><sup>-</sup> are useful measurements for evaluating acid-base status in animals. Base excess and HCO<sub>3</sub><sup>-</sup> values were reported as 2.6 ± 1.7 and 28.6 ± 1.9 mmol/L, respectively, in stabled standardbred horses,<sup>17</sup> which are lower but fall within the range for

**Table 3.** Reference arterial blood pH, p<sub>a</sub>O<sub>2</sub>, p<sub>a</sub>CO<sub>2</sub>, S<sub>a</sub>O<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, and base excess from 12 healthy, standing, unrestrained captive white rhinoceroses (*Ceratotherium simum*).

Arterial blood parameter	Mean	SE	Range	Min.	Max.	25%	75%
pH	7.391	0.006	0.085	7.346	7.431	7.382	7.399
p <sub>a</sub> O <sub>2</sub> (mm Hg)	98.2	1.3	18.4	90.2	108.6	95.9	100.2
p <sub>a</sub> CO <sub>2</sub> (mm Hg)	49.0	0.8	9.3	44.4	53.7	46.7	50.7
Base excess (mmol/L)	3.5	0.4	4.0	1.9	5.9	2.3	4.9
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	29.3	0.4	4.9	27.3	32.2	28.4	30.1
S <sub>a</sub> O <sub>2</sub> (%)	97.2	0.1	1.4	96.6	98.0	96.9	97.4

the reference values found in this study. Initial base excess and  $\text{HCO}_3^-$  values reported in field anesthetized white rhinoceroses in one study were  $-6.4$  and  $22 \pm 4$  mmol/L, respectively.<sup>2</sup> The reference blood gas, acid-base,  $\text{S}_a\text{O}_2$ , and  $\text{EtCO}_2$  values for the white rhinoceroses reported from this study help to verify the apparent marked hypoxemia, hypercapnea, and acidemia reported during field anesthesia in this species.<sup>2-4,6</sup>

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