A randomized clinical trial to compare ketamine butorphanol azaperone medetomidine and detomidine-etorphine-acepromazine for anesthesia of captive Przewalski horses (Equus przewalskii)

Title

A randomized clinical trial to compare ketamine-butorphanol-azaperone-medetomidine and detomidine-etorphine-acepromazine for anesthesia of captive Przewalski's horses (*Equus przewalskii*)

Author names and affiliations

Ellie L. Milnes VetMB, DVSc, DACZM, Alicia M. Skelding DVM, DVSc, DACVAA, Cédric B. Larouche DVM, DVSc, DACZM, Angelica Ferro DVM, Pauline Delnatte DMV, DVSc, DACZM, Christopher Dutton BVSc, MSc, DACZM & Neil E. Anderson BVSc, PhD

From the Toronto Zoo, Toronto, ON M1B 5K7, Canada (Milnes, Larouche, Delnatte, Dutton); The Royal (Dick) School of Veterinary Studies and the Roslin Institute, University of Edinburgh, Midlothian EH25 9RG, UK (Milnes, Ferro, Anderson); and Toronto Animal Health Partners Emergency and Specialty Hospital, Toronto, Ontario M3B 2R2, Canada (Skelding).

Corresponding author

Address correspondence to Dr. Milnes (emilnesdvm@yahoo.co.uk)

Abstract **Word Count 246**

Objective To compare ketamine-butorphanol-azaperone-medetomidine (KBAM) to detomidine-etorphine-acepromazine (DEA) for field anesthesia in captive Przewalski's horses (*Equus przewalskii*).

Animals 10 adult Przewalski's horses.

Procedures A prospective randomized crossover trial was conducted. Each horse was immobilized once with KBAM (200 mg ketamine, 109.2 mg butorphanol, 36.4 mg azaperone, 43.6 mg medetomidine) and once with DEA (40 mg detomidine premedication, followed 20 minutes later by 3.9-4.4 mg etorphine and 16-18 mg acepromazine). Both protocols were administered by IM remote dart injection with a washout period of 6 months between treatments. Selected cardiorespiratory variables and quality of anesthesia were recorded. Antagonists were administered IM (KBAM: 215 mg atipamezole and 50 mg naltrexone; DEA: 4 mg RX821002 and 100 mg naltrexone).

Results All horses were anesthetized and recovered uneventfully. Inductions (DEA 6.8 min, KBAM 11.6 min; $p = 0.04$) and recoveries (DEA 3.2 min, KBAM 19.6 min; $p < 0.01$) were faster with DEA compared to KBAM. Quality scores for induction and recovery did not differ between protocols, but maintenance quality was poorer for DEA ($p < 0.01$). Clinical concerns during DEA immobilizations included apnea, severe hypoxemia ($PaO_2 < 60$ mm Hg), muscle rigidity and tremors. Horses treated with KBAM were moderately hypoxemic, but arterial partial pressures of oxygen were higher compared to DEA ($p < 0.01$).

Clinical relevance Captive Przewalski's horses are effectively immobilized with KBAM, and this protocol results in superior muscle relaxation and less marked hypoxemia during the maintenance phase, but slower inductions and recoveries, compared to DEA.

Keywords anesthesia, etorphine, immobilization, ketamine, medetomidine, Przewalski's horse *(Equus przewalskii)*

The Przewalski's horse (*Equus przewalskii*) is an endangered species of nondomestic equid that became extinct in the wild in the mid-1960s, but has since been successfully reintroduced into parts of its former range.[1] Przewalski's horses survived because of *ex situ* breeding from a small population of founder animals in zoological institutions.[2] Captive management of nondomestic equids requires safe and reliable immobilization drug protocols for veterinary interventions, such as hoof trimming, that generally cannot be accomplished safely with behavioral restraint alone.[3]

The ultrapotent opioid (UPO) etorphine is the drug most commonly used to immobilize nondomestic equids because of its potency, rapid onset of action, reliability, reversibility, and the historic lack of availability of efficacious alternative protocols.[4] However, adverse effects of UPOs include respiratory depression, hypertension, muscle tremors, renarcotization, and the risk of accidental human exposure. Etorphine is usually administered in combination with a tranquilizer or sedative to reduce the etorphine dose required to achieve a working level of immobilization and to mitigate UPO-associated adverse effects.[4] Drug administration is usually by remote IM injection in nondomestic equids. A typical immobilization protocol for both wild free-ranging and captive Przewalski's horses combines etorphine with butorphanol and detomidine in a single dart.[3] At the Toronto Zoo, a two-step strategy consisting of premedication with detomidine IM followed by etorphine-acepromazine IM, all by remote dart injection, has historically been the immobilization protocol of choice for Przewalski's horses.

Some countries have experienced periodic shortages in etorphine supply, prompting zoo veterinarians to seek alternatives for nondomestic equid immobilization.[5] The UPOs carfentanil and thiafentanil have been trialed with variable success.[5,6] Several non-UPO combinations have

been reported in nondomestic equids managed in zoological institutions. Standing sedation is relatively easily achieved with non-UPO combinations such as butorphanol-detomidine, but, as in domestic horses, additional drugs (normally ketamine) are required to induce recumbency. Combinations of α_2-adrenergic agonists (such as medetomidine, xylazine, and romifidine) and ketamine or tiletamine-zolazepam have been used to induce recumbent immobilization in captive nondomestic equids.[7,8] Medetomidine-ketamine (MK) combinations reported in the zoological medicine literature have variable efficacy in Przewalski's horses with some individuals showing significant ataxia but failing to become recumbent, whilst others underwent calm induction, excellent muscle relaxation, and uneventful recovery.[7,9] In boma-confined wild zebra (*Equus zebra*), ketamine-butorphanol-medetomidine (KBM) was as effective as etorphine-azaperone, but adverse effects of KBM included ataxic and prolonged inductions, hypertension, and hypoxemia.[10]

The non-UPO combination butorphanol-azaperone-medetomidine (BAM) effectively immobilizes a wide range of nondomestic ungulate species, although reported adverse effects include prolonged inductions and hypoxemia.[11,12] Ketamine is added to BAM for the immobilization of nondomestic equids.[13] The aim of this study was to characterize and compare the quality of induction, maintenance, and recovery, and the cardiorespiratory effects of two drug protocols, ketamine-butorphanol-azaperone-medetomidine (KBAM) and detomidine-etorphine-acepromazine (DEA), administered by remote IM injection via dart, for the immobilization of captive Przewalski's horses. It was hypothesized that KBAM would result in greater muscle relaxation and higher PaO_2 than DEA.

Materials and methods

Research methodology was approved by the Toronto Zoo Animal Care & Research Committee (reference number 2018-09-19) and the Royal (Dick) School of Veterinary Studies Veterinary Ethical Review Committee (reference number 81.89).

Animals

Ten captive born Przewalski's horses were immobilized in April and October of 2019 for routine veterinary care at the Toronto Zoo. Seven mares, two geldings, and one stallion (estimated average body weight of 300 kg) were included in the study; all horses were clinically healthy.

Study design

This was a randomized, prospective, crossover clinical trial. Each horse received treatments KBAM and DEA in random order. The order in which horses received the two treatments was allocated using an online randomization plan generator, in which the 10 subjects were randomized into one block with balanced permutations.[14] Initial treatment allocation was concealed from the investigators during the randomization process, but no further blinding was performed during the animal phase because of the human safety concerns associated with exposure to etorphine and concentrated medetomidine. Animals received the first treatment in April 2019, and the alternative treatment in October 2019. The six-month washout period coincided with planned routine immobilizations for scheduled veterinary care.

Sample size was calculated by power based on PaO_2 values obtained by arterial blood gas analysis. A clinically significant effect size of 15 mm Hg and SD of 10 mm Hg were derived

from prior clinical experiences of the authors. A power calculation was performed with power of 80% and a type I error of 5%, giving a sample size of eight horses.[15] An attrition rate of 20% was anticipated, so a final sample size of 10 horses was used.

Immobilization

Immediately before darting, each horse was separated from the herd and moved to a 4 x 8-meter outdoor pen accessed by sliding doors. Ambient temperatures ranged from 9°C to 16°C. Horses were randomly assigned to a treatment protocol as described above. For the DEA protocol, horses were premedicated with 40 mg detomidine IM followed 20 minutes later by 1.6-1.8 mL of a premixed compounded etorphine-acepromazine combination IM (etorphine HCl 2.45 mg/mL and acepromazine maleate 10 mg/mL, compounded from existing approved drug products with benzyl alcohol 0.5% preservative, Chiron Compounding Pharmacy Inc). For the KBAM protocol, horses received 200 mg ketamine combined with 4 mL of a premixed compounded butorphanol-azaperone-medetomidine combination IM (butorphanol tartrate 27.3 mg/mL, azaperone 9.1 mg/mL, medetomidine HCl 10.9 mg/mL, compounded from existing approved drug products, with inactive ingredients methylparaben NF powder, tartaric acid NF powder, citric acid USP anhydrous fine granular granules, sodium citrate USP anhydrous powder, and benzethonium chloride powder, Chiron Compounding Pharmacy Inc). All IM injections were administered via dart injection using a gas-powered pistol. Detomidine and KBAM were administered using 5 mL plastic darts and etorphine-acepromazine was administered using a 2 mL plastic dart, all with a 16-gauge, 38 mm, wire-barbed, side-ported, collared needle. Animals were darted from a distance of 3 to 5 meters into the hip or gluteal muscles. The time elapsed

from darting with KBAM or etorphine-acepromazine until the horse became recumbent was recorded ('induction time').

Once recumbent, heart rate (HR), respiratory rate (RR), and body temperature were monitored by palpation of peripheral pulse or stethoscope auscultation, visual count of thoracic wall excursions, and digital rectal thermometer, respectively. Physiological parameters were recorded at 5, 10, and 15 minutes after recumbency (T5, T10, and T15). Arterial blood samples of 2 mL were collected anaerobically at T5 and again at T15 by aseptically puncturing either the lateral metatarsal artery of the nondependent pelvic limb or the transverse facial artery with a 22-gauge needle attached to a heparinized syringe. Arterial blood was immediately analyzed for PaO_2, $PaCO_2$, SaO_2, pH, HCO_3^-, and base excess (BE) using a hand-held analyzer (i-STAT, Abbott Laboratories) at 37 °C. Supplemental oxygen was administered via a nasal cannula inserted into the ventral meatus of one nostril from a portable oxygen canister at a flow rate of 15 L/min, starting immediately after collection of the T5 arterial sample. During each immobilization horses underwent the following procedures: physical examination, venipuncture for blood collection, and prophylactic treatments as required (e.g., vaccinations) performed by veterinarians, and hoof trimming performed by certified farriers.

Following completion of the above procedures, antagonist drugs were administered IM by hand injection and horses were left to stand unassisted. For the DEA protocol, RX821002 was used to antagonize detomidine (0.1 mg RX821002 per mg detomidine) and naltrexone was used to antagonize etorphine (20 mg naltrexone per mg etorphine). RX821002 was compounded in-house from bulk substance (RX821002 hydrochloride powder reconstituted with sterile water to

2.5 mg/mL, Sigma Aldrich). For the KBAM protocol, medetomidine was reversed with atipamezole (5 mg per mg medetomidine) and etorphine was reversed with naltrexone (0.5 mg per mg butorphanol).

Video recordings were made of the induction, maintenance, and recovery phases, and scored at a later date by a blinded observer using the scoring system of Matthews et al. (1995).[7] Horses were kept under observation until fully recovered, at which point they were reintroduced to the herd.

Statistical analysis

Data were analyzed by use of statistical software (R version 3.6.2, R Foundation for Statistical Computing) and graphics were constructed with the R package ggplot2.[16,17] Data from horses that required additional drugs during the induction period (re-darting or a ketamine bolus) were excluded from further analysis. For continuous data, distribution was assessed for normality using visual inspection of histograms, Q-Q plots, and performing the Shapiro-Wilk test. Results are presented as mean ± SD (sample range) for continuous data, and as median (interquartile range) for discrete data. Results were interpreted at the 95% level of confidence ($p \leq 0.05$). Data were tabulated, summary statistics determined, and induction time compared between combinations using a paired two-sample *t*-test. Data were not available for recovery times from October 2019 because of human error; therefore, recovery times were analyzed from 10 horses (5 DEA, 5 KBAM) in April 2019 using an unpaired two-sample *t*-test. Subjective quality scores had a non-parametric distribution, so the Friedman test was used to compare scores between combinations (response: score; treatment: protocol; block: horse).

189 Descriptive statistics for quantitative continuous data (HR, RR, body temperature, SaO_2, $PaCO_2$,
190 PaO_2, pH, HCO_3^-, BE) were tabulated at each time point and checked to identify any missing
191 values and recording errors. Data were plotted to visually check for differences between and
192 within protocols over time. Linear mixed model analyses were implemented using the R package
193 'lme4'.[18] The explanatory variables 'time [from onset of recumbency]' and 'protocol' were
194 selected on the basis of biological plausibility and clinical relevance and included as fixed effects
195 in the full model.[19] 'Horse ID' was included as a random effect to control for the similarity
196 between repeated measures within an immobilization procedure. Separate multivariable models
197 were created for each response variable. The fit of all models was examined by evaluating the
198 residuals to ensure no violation of the model's assumptions. This modelling strategy controlled
199 for the repeated measurements within an immobilization procedure, and also allowed for a direct
200 effect of protocol on the value of the monitored parameters as well as an effect of protocol on
201 changes in monitored parameters over time.[20] The 'lme4' package does not provide *p*-values for
202 variables, so these were generated using the *z* distribution from the Wald *t*-values provided in the
203 model output using the ANOVA function in the 'car' package.[21] These *p*-values were used to
204 evaluate the significance of fixed effects in the models obtained.[22] The standard errors of fixed-
205 effects coefficients were used to construct approximate Wald confidence intervals.[23]
206
207 **Results**
208 Animals
209 Of the 10 horses enrolled in the study, nine received both treatments in the randomized crossover
210 study. One mare allocated to KBAM at the first trial was subsequently mated and was in early
211 pregnancy at the time of the second trial; based on the preliminary finding of more severe

hypoxemia in horses immobilized with DEA and concerns about fetal viability, KBAM was used instead of DEA at the second trial for this individual. Therefore, data from two KBAM immobilizations were analyzed for this mare. The remaining nine horses received both protocols in random order; all horses recovered uneventfully from each anesthetic event. Two pregnant mares were immobilized with KBAM at approximately 12 weeks of gestation and subsequently birthed live foals at term.

Immobilization

Induction

All horses were agitated prior to and immediately following the initial dart. At 20 minutes after premedication with detomidine in the DEA group, all animals reached a moderate level of standing sedation and responded minimally to the etorphine-acepromazine dart. The mean ± SD induction time was slower ($p = 0.04$) for horses treated with KBAM (11.6 ± 4.2 min; range, 7 to 21 min) versus DEA (6.8 ± 2.4 min; range, 4 to 11 min).

The induction quality score did not differ between the protocols ($p = 0.16$). However, there were notable differences in induction behavior between horses treated with KBAM and DEA. During KBAM inductions, animals were moderately to severely ataxic with multiple episodes of stumbling and almost falling before becoming recumbent. Penile prolapse during induction was a consistent finding in the stallion and geldings darted with KBAM but not with DEA. During DEA induction, horses tended to stand still with their head down or head-pressing against the pen wall, then developed muscle tremors before falling heavily into lateral recumbency.

One animal in each group required supplemental induction drugs because they were sedated but remained standing following administration of the standard protocol; data from these immobilizations were excluded from subsequent analysis. In the DEA group, one horse received additional etorphine-acepromazine by IM dart injection, and in the KBAM group one horse was administered additional ketamine by IV injection. Both horses became recumbent following supplementation. Overall, data from 10 KBAM and eight DEA immobilizations were analyzed.

Maintenance

Most DEA horses showed severe muscle rigidity and tremors and spontaneous limb movements during the maintenance phase. In contrast, the KBAM maintenance phase was smooth and relaxed. Relaxation scores were significantly higher (i.e., poorer muscle relaxation) for DEA than KBAM ($p < 0.01$). One horse treated with DEA spontaneously stood up at T10; the horse was carefully approached, blindfolded, given an IV ketamine bolus, and became recumbent again with no further attempts to stand prior to administration of reversal agents.

Recovery

The mean ± SD recovery time was slower ($p < 0.01$) for horses treated with KBAM (19.6 ± 6.9 min; range, 2 to 30 min) compared to DEA (3.2 ± 0.8 min; range, 1 to 4 min). Recovery quality scores did not differ between DEA and KBAM ($p = 0.41$). All horses were unrestrained during recovery and stood at their first attempt; recoveries were quiet and uneventful. In the stallion and geldings immobilized with KBAM, penile prolapse persisted during the recovery period but resolved within 6 hours with no complications. No renarcotization was observed, and there was no study-associated morbidity or mortality.

Physiological variables

Heart rates were faster and more variable ($p < 0.01$) and respiratory rates were slower ($p < 0.01$) in horses treated with DEA compared to KBAM (Table 1). Apnea of 30 seconds or longer occurred in 6 of the 8 horses treated with DEA but was not observed when the horses were treated with KBAM. Rectal temperatures were significantly higher for horses treated with DEA ($p < 0.01$) (Table 1, Table 2) but remained within clinically acceptable limits. There was no evidence for any change in HR, RR, temperature, pH, $PaCO_2$, HCO_3^-, or BE as an effect of time (Table 2, Figure 1).

Arterial blood gases at T5 and T15 revealed hypoxemia that was moderate ($PaO_2 < 80$ mm Hg) to severe ($PaO_2 < 60$ mm Hg) in most horses (Figure 2). Horses immobilized with DEA were more severely hypoxemic than with KBAM ($p < 0.01$) (Table 2, Figure 2). Normocapnia and normal blood pH balance were recorded in all horses. Although horses treated with DEA had higher $PaCO_2$, HCO_3^-, and BE ($p < 0.01$) and lower pH values ($p = 0.01$) compared to KBAM horses (Table 2), these differences were within clinically acceptable limits.

Discussion

Przewalski's horses treated with DEA had significantly faster and less variable induction and recovery times than when treated with KBAM, and KBAM inductions were characterized by prolonged periods of incoordination in some individuals. This is consistent with previous work comparing etorphine-azaperone and KBM in zebras, in which KBM also resulted in a markedly slower and more ataxic induction than the etorphine combination.[10] In domestic equids, increasing duration of the induction and recovery periods is associated with heightened risk of injury caused by ataxic stumbling.[24] The length and character of KBAM inductions could potentially increase the risk of injury compared to DEA. One horse in each of the treatment groups failed to become recumbent following apparently correct dart placement. Possible causes for apparent drug failure include injection into fascial planes rather than deep IM injection, incomplete injection, incorrect drug compounding, errors in dart preparation, or the effects of sex and temperament.[6,25]

Poor muscle relaxation contributed to suboptimal immobilization quality for DEA compared to KBAM horses, as is observed in many species under the influence of UPOs.[26] Excessive muscle rigidity and spontaneous movements when the horse is recumbent presents a risk of injury to horse and handlers, increases rectal temperature, and makes monitoring the depth of immobilization challenging, as demonstrated by the spontaneous arousal of a DEA horse at T10.

Prolonged recoveries from KBAM immobilization may be attributable to incomplete reversal and the use of ketamine. In the DEA protocol, detomidine was reversed with RX821002 and etorphine with naltrexone, leaving only acepromazine to be metabolized, whereas for KBAM,

298 butorphanol was reversed with naltrexone and medetomidine with atipamezole, leaving both
299 ketamine and azaperone to be metabolized. In this study, RX821002 was used instead of
300 atipamezole to reverse detomidine because of its low cost, its α_2 to α_1 specificity ratio being
301 considered closer to that of detomidine, the small volume required, and the authors' positive
302 experience using this reversal in Przewalski's horses.[27] In equids, complete and rapid recovery
303 from immobilization is desirable to prevent disruption of social structure, misadventure, and
304 derangements in body temperature.[3] Prolonged recumbency during slow recoveries additionally
305 exacerbates hypoxemia caused by compression of the dependent lung, as well as increasing the
306 risk of neuropathy and myopathy in the dependent limbs.[28] Dividing antagonists equally between
307 the IV and IM routes results in rapid (< 5 min) recovery from KBAM in Przewalski's horses
308 (personal experiences of the authors, unpublished data). However, IV administration of α_2-
309 adrenoceptor antagonists can result in excitement and profound vasodilation with hypotension,
310 and thus the IM route is generally recommended except for emergency situations.[29] Compounded
311 butorphanol-azaperone-medetomidine and etorphine-acepromazine were used because approved
312 products were not available in Canada at the time of the study. Veterinarians should adhere to
313 compounding regulations and be aware that pharmacokinetic properties may differ between
314 compounded and approved products. The use of both etorphine and concentrated medetomidine
315 bears an inherent risk of human injury from accidental exposure.[30] Therefore, it is vital to pre-
316 emptively establish emergency protocols in case of accidental human exposure for both KBAM
317 and DEA.
318
319 Horses treated with DEA were severely hypoxemic (PaO_2 < 60 mm Hg) at all time points and
320 often apneic. Opioid-induced respiratory depression is the result of μ-receptor-mediated

depression of the central respiratory center.[31] Etorphine-associated skeletal muscle tremors increase cellular oxygen consumption and may therefore have further contributed to DEA-associated hypoxemia.[32] Despite not receiving a UPO, moderate hypoxemia (PaO_2 < 80 mm Hg) also occurred in horses treated with KBAM, as is consistently observed in nondomestic equids immobilized with medetomidine-ketamine combinations.[33] Similarly, PaO_2 commonly decreases from 100 mm Hg to 60 – 80 mm Hg within 5 minutes of anesthetic induction in domestic horses not administered oxygen.[34–36] This hypoxemia is mainly a result of ventilation-perfusion mismatch and, to a lesser extent, intrapulmonary vascular shunting because of atelectasis of the dependent lung fields that occurs during recumbency.[28,37] Ketamine, like etorphine, increases sympathetic tone and cellular oxygen consumption, which could have additionally contributed to the low PaO_2 in KBAM-treated horses in the present study.[38] Oxygen supplementation is therefore recommended with both KBAM and DEA protocols and, indeed, in all equid immobilizations.[37,39] In horses, oxygen insufflation at 50 L/min has been demonstrated to normalize PaO_2 if the nasal line was advanced into the pharynx or trachea.[40] In this study, nasal oxygen at 15 L/min only slightly increased PaO_2 over time. It could be that the nasal line was poorly positioned or that the flow rate was insufficient. However, if the predominant cause of hypoxemia was venous admixture, even at high flow rates, the fraction of inspired oxygen would have had very little impact on PaO_2. More research is required to establish optimal methods for respiratory support in immobilized nondomestic equids.

The PaO_2 values reported here are much lower than values reported for domestic horses that were recumbent, anesthetized, or both.[37] This finding may be the result of interspecies variation, an effect of the anesthetic agents, or sampling error. It is possible that there was collection of

venous or mixed sample because of difficulty palpating peripheral pulses in some of the horses, which would decrease oxygen content. Additionally, on colder days there was a time delay from sample collection to analysis because the analyzer needed to be warmed before the sample could be processed in the field; this could also decrease oxygen content of the sample.[41] Despite very low PaO_2 on a number of occasions, all horses recovered from each anesthesia uneventfully with no reported long-term consequences such as cognitive deficits. Thoroughbred horses have much lower PaO_2 during exercise than when at rest and autoregulation of cerebral and cerebellar blood flow is maintained in exercising horses despite this hypoxemia and hypercapnia.[37,42,43] It is not known to what extent the autoregulatory response of brain blood flow to hypoxemia is attenuated during anesthesia with KBAM and DEA.

In summary, KBAM may provide a useful alternative to etorphine-based combinations for captive Przewalski's horses. Horses treated with KBAM had subjectively better immobilization quality during the maintenance phase, compared with apnea and severe muscle tremors and rigidity in horses treated with DEA. However, KBAM induction and recovery periods were longer and less predictable compared to DEA, which could increase the risk of injury caused by ataxic stumbling and may therefore limit the usefulness of KBAM to situations in which the horse's environment can be controlled to prevent misadventure. Low PaO_2 (KBAM) to severe hypoxemia (DEA) occurred with both protocols, highlighting the critical importance of diligent monitoring and oxygen supplementation. Although the risk of UPOs to human health is well-understood and KBAM may therefore be viewed as a 'safer' option than etorphine, the potential danger of accidental human exposure to potent medetomidine should not be ignored when working with KBAM.

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Figure legends

Figure 1.

Graphs illustrating monitored parameters between 5 and 15 minutes post-recumbency within individual Przewalski's horses (*Equus przewalskii*) immobilized with two different drug protocols administered by IM dart. Ketamine-butorphanol-azaperone-medetomidine is indicated by the black dashed lines, and detomidine-etorphine-acepromazine is indicated by the grey solid lines. Analysis of linear mixed models found that protocol had a significant effect for all parameters ($p < 0.05$), but time under anesthesia only had a significant effect on the partial pressure of arterial oxygen (PaO_2, $p = 0.02$), indicated by *

Figure 2. Partial pressure of arterial oxygen (PaO_2) over time in Przewalski's horses (*Equus przewalskii*) immobilized with ketamine-butorphanol-azaperone-medetomidine (black) and detomidine-etorphine-acepromazine (grey). For each plot, the box represents the interquartile (25^{th} to 75^{th} percentile) range, the horizontal line in the box represents the mean, whiskers represent the range, and each dot represents an outlier result. The dashed lines represent the upper reference limit for moderate ($PaO_2 < 80$ mm Hg) or severe ($PaO_2 < 60$ mm Hg) hypoxemia. Analysis of linear mixed models found significant effects for both protocol ($p < 0.01$) and time ($p = 0.02$).

518 **Tables**

519 **Table 1.** Summary statistics for physiological parameters and arterial blood gas analysis over time in 10 captive Przewalski's horses

520 (*Equus przewalskii*) immobilized with ketamine-butorphanol-azaperone-medetomidine (KBAM) or detomidine-etorphine-

521 acepromazine (DEA). All values represent mean ± SD (range). NA = not available, no sample collected.

522

Parameter	**Protocol**	**5 minutes**		**10 minutes**		**15 minutes**	
Heart rate (beats/min)	KBAM	37±6	(23 to 44)	40±7	(28 to 52)	39±5	(32 to 44)
	DEA	58±19	(32 to 84)	53±12	(40 to 78)	55±11	(40 to 68)
Respiratory rate (breaths/min)	KBAM	19±10	(6 to 36)	19±12	(6 to 40)	17±8	(8 to 30)
	DEA	8 ±7	(4 to 24)	6±3	(1 to 12)	5±2	(2 to 8)
Temp (°C)	KBAM	38.9±0.5	(38.2 to 39.7)	39.0±0.7	(38.1 to 39.9)	38.7±0.7	(37.6 to 39.7)
	DEA	39.2±0.4	(38.6 to 39.6)	39.2±0.4	(38.6 to 39.8)	39.2±0.5	(38.6 to 40.0)
Blood pH	KBAM	7.41±0.05	(7.34 to 7.48)	NA	NA	7.40±0.05	(7.34 to 7.49)
	DEA	7.35±0.06	(7.26 to 7.43)	NA	NA	7.37±0.06	(7.32 to 7.46)
PaO_2 (mm Hg)	KBAM	47.2±11.8	(26 to 62)	NA	NA	61±25	(35 to 114)
	DEA	27±9	(14 to 44)	NA	NA	35±13	(20 to 61)
SaO_2 (%)	KBAM	80±14	(47 to 93)	NA	NA	86±10	(67 to 98)
	DEA	45±22	(12 to 81)	NA	NA	59±20	(27 to 91)
$PaCO_2$ (mm Hg)	KBAM	41.9±4.4	(36.3 to 48.7)	NA	NA	44.9±6.6	(34.6 to 53.6)
	DEA	55.7±11.4	(38.6 to 78.9)	NA	NA	54.6±9.3	(40.8 to 68.3)
BE (mmol/L)	KBAM	2.1±2.3	(–2.0 to 5.0)	NA	NA	3.2±2.9	(–2.0 to 8.0)
	DEA	5.1±4.8	(–3.0 to 12)	NA	NA	6.3±4.4	(1.0 to 14)
HCO_3^- (mmol/L)	KBAM	26.69±2.0	(23.4 to 29.3)	NA	NA	27.9±2.7	(22.4 to 32.3)
	DEA	30.8±4.8	(23.6 to 36.7)	NA	NA	31.6±4.2	(26.3 to 38.2)

523

524 **Table 2.** Multivariable analysis using linear mixed-effects models for the effect of protocol (ketamine-butorphanol-azaperone-
525 medetomidine or detomidine-etorphine-acepromazine) and time on physiological parameters and arterial blood gas analysis in
526 immobilized Przewalski's horses (*Equus przewalskii*). The significance of fixed effects in each of the models was evaluated using
527 Wald's *p*-values and 95% CI around the estimates. * indicates a statistically significant effect ($p < 0.05$)

Parameter	**Effect of protocol**				**Effect of time**			
	Estimate	**Standard error**	**95% CI (Wald's)**	***p*-value (Wald's)**	**Estimate**	**Standard error**	**95% CI (Wald's)**	***p*-value (Wald's)**
Heart rate (beats/min)	–17.5	2.48	–22.33 to –12.62	< 0.01 *	–0.11	0.27	–0.64 to 0.42	0.68
Respiratory rate (breaths/min)	11.3	1.92	7.54 to 15.07	< 0.01 *	–0.27	0.22	–0.69 to 0.16	0.22
Temperature (°C)	–0.43	0.14	–0.70 to –0.16	< 0.01 *	–0.006	0.015	–0.03 to 0.02	0.71
Blood pH	0.04	0.02	0.008 to 0.074	0.01 *	0.0003	0.002	–0.0027 to 0.0034	0.84
PaO_2 (mm Hg)	24.4	4.94	14.74 to 34.09	< 0.01 *	1.09	0.47	0.17 to 2.00	0.02 *
$PaCO_2$ (mm Hg)	–11.8	2.61	–16.87 to –6.66	< 0.01 *	0.12	0.26	–0.38 to 0.63	0.63
BE (mmol/L)	–3.1	1.15	–5.32 to –0.79	0.01 *	0.11	0.11	–0.11 to 0.34	0.99
HCO_3^- (mmol/L)	–3.9	1.11	–6.10 to –1.75	< 0.01 *	0.10	0.11	–0.11 to 0.32	0.34

528