Zolazepam-tiletamine-medetomidine versus butorphanol-azaperone-medetomidine for the immobilisation of captive leopards (*Panthera pardus*)

BY

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Submitted in partial fulfilment of the requirements for the degree of Master of Science (Veterinary Science) in the Department of Companion Animal Clinical Studies in the Faculty of Veterinary Science, University of Pretoria

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DECLARATION OF ORIGINALITY

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1. I understand what plagiarism is and am aware of the University's policy in this regard.
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Dr Roxanne Buck

LIST OF ABBREVIATIONS

%	percentage
˚C	degrees celsius
<	less than
=	equal to
>	greater than
±	plus or minus
BAM	butorphanol-azaperone-medetomidine
CNS	central nervous system
DAP	diastolic arterial pressure
DCA	damage causing animal
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ET Tube	endotracheal tube
f_R	respiratory rate
G	gauge (bore size of needles and catheters)
GABA	gamma amino butyric acid
GPS	global positioning system
IM	intramuscular
IPPV	intermittent positive pressure ventilation
IU	international units
IUCN	International Union for the Conservation of Nature
IV	intravenous
kg	kilogram
kPa	kilopascal
m	metre
m^2	square metre
MAP	mean arterial pressure
mg	milligram(s)
min	minutes
mmHg	millimeter(s) Mercury
mmol	millimole(s)
n	number of animals
NMDA	N-methyl-D-aspartate
p	estimation of statistical significance

P:F	ratio of partial pressure of arterial oxygen to fraction of inspired oxygen
PaO_2	partial pressure of oxygen
$P(A\text{-}a)O_2$	alveolar to arterial oxygen partial pressure gradient
PCO_2	partial pressure of carbon dioxide
PeCO2	partial pressure of expired carbon dioxide
$P(a\text{-}E')CO_2$	arterial to end-tidal carbon dioxide partial pressure gradient
pH	negative log of hydrogen ion concentration
SANBI	South African National Biodiversity Institute
SAP	systolic arterial pressure
SpO_2	peripheral oxyhaemoglobin saturation
SVR	systemic vascular resistance
T	difference in time to safe approach for group BAM vs group ZM
T_{15}	15 minutes after immobilisation
T_{45}	45 minutes after immobilisation
V/Q	ventilation/perfusion ratio
ZM	zolazepam-tiletamine-medetomidine

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SUMMARY

Zolazepam-tiletamine-medetomidine versus butorphanol-azaperone-medetomidine for immobilisation of captive leopards (*Panthera pardus*)

By

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Promoter: Dr Roxanne Buck

Co-promoter: Prof Adrian Tordiffe

Department: Companion Animal Clinical Studies

Degree: Master of Science (Veterinary Science)

Objective To compare the immobilisation time and cardiopulmonary effects of zolazepam-tiletamine-medetomidine (ZM) and butorphanol-azaperone-medetomidine (BAM) in captive leopards (*Panthera pardus*).

Study design Prospective, clinical study

Materials and Methods 17 adult, captive leopards were immobilised by remote injection of either a combination of zolazepam-tiletamine (1.5 mg kg^{-1}) and medetomidine (0.04 mg kg^{-1}) (ZM, n = 10)

or a combination of butorphanol (0.3 mg kg^{-1}), azaperone (0.12 mg kg^{-1}) and medetomidine (0.12 mg kg^{-1}) (BAM, n = 7). Time to safe approach, judged by absent responses to an ear flick and tail tug, was recorded as the immobilisation time. Following immobilisation, cardiopulmonary parameters were recorded and two arterial blood gas samples analysed. After 40 minutes, anaesthesia was reversed using atipamezole (0.2 mg kg^{-1}) for group ZM or atipamezole (0.6 mg kg^{-1}) and naltrexone (0.3 mg kg^{-1}) for group BAM. Recovery time was recorded as time from injection of reversal agent to head up. Data is reported as mean ± standard deviation and compared using a general linear mixed model ($p < 0.05$).

Results For ZM, doses administered were zolazepam-tiletamine 1.3 ± 0.6 mg kg^{-1} and medetomidine 0.04 ± 0.018 mg kg^{-1} while those for BAM were butorphanol 0.33 ± 0.05 mg kg^{-1}, azaperone 0.13 ± 0.02 mg kg^{-1} and medetomidine 0.13 ± 0.02 mg kg^{-1}. Immobilisation time was significantly faster for BAM (5.8 ± 1.1 minutes) than for ZM (11.8 ± 3.3 minutes, $p = 0.008$). Both treatments resulted in hypertension, with mean arterial blood pressure of 154 ± 46 mmHg with ZM and 137 ± 12 mmHg with BAM. BAM resulted in clinically significant hypoxaemia (arterial oxygen tension 52.8 ± 4.4 mmHg), while arterial oxygen tension was higher with ZM (72.6 ± 8.0 mmHg, $p = 0.027$). Arterial carbon dioxide tension was lower with ZM (26.4 ± 2.9 mmHg) than BAM (44.8 ± 3.9 mmHg, $p < 0.001$). Recovery time was no different between treatments ($p = 0.604$).

Conclusion Both combinations provided acceptable immobilisation for field use. Supplementation with oxygen is recommended, especially when using BAM.

Immobilisation of leopards

Leopards *(Panthera pardus*) are large carnivores belonging to the Felidae family. Due to human population expansion, a decrease in the availability of prey and an increase in human-wildlife conflict, the wild felids are among the mammals most vulnerable to extinction (Stein et al., 2020; Miller and Fowler, 2015). A direct result of the decline in the free-roaming population is an increased need for human intervention in both free-roaming and captive population management to ensure species survival.

Chemical immobilisation of wild animals is the use of sedative and anaesthetic drug, which are usually administered by remote injection, to achieve a level of sedation or chemical restraint that facilitates humane handling of the animal and ensures personnel safety. The ideal immobilising drug should be potent, to reduce the required drug volume; have a rapid onset of effect; have a wide safety margin and minimal side effects and be completely reversible by the administration of an antagonist (Kock & Burroughs, 2012). These qualities are essential when working with wild animals to reduce stress during induction, minimise the risk of the darted individual moving too far before becoming recumbent and ensuring it is able to defend itself against predators or competitors following reversal. Although no single drug currently embodies all of these properties, by combining different drugs in an immobilisation protocol, one can utilise their synergism to collectively meet these criteria (Chinnadurai et al., 2016).

Historically wild felids were immobilised with large volumes of a dissociative anaesthetic drugs, originally phencyclidine and then more recently, ketamine (as reviewed by Wenger et al., 2010). Immobilisation with these drugs alone was characterised by involuntary limb movements, maintenance of cranial reflexes, lack of reversibility and very rough and prolonged recoveries. In the

years that followed, sedatives and tranquillisers were added to the dissociative anaesthetic drugs to reduce the dose administered and to produce a calmer induction and partially reversible immobilisation (Semjonov et al., 2017; Fyumagwa et al., 2012).

Combinations of a cyclohexylamine dissociative anaesthetic drug with both benzodiazepine and alpha-2 adrenoceptor agonist sedatives are commonly used to provide a predictable and partially reversible immobilisation of wild felids (Kock & Burroughs, 2012). The most common combination of these drugs, zolazepam-tiletamine and medetomidine has been widely used for the immobilisation of many wild felids, including lions (*Panthera leo),* cheetahs (*Acinonyx jubatus*)and tigers (*Panthera tigris)* (Kock & Burroughs, 2012; Stegmann & Jago, 2006; Fahlman et al., 2005). Recently, a combination of an opioid, sedative and tranquilizer, butorphanol-azaperone-medetomidine (BAM) has been used in lions and cheetahs to produce a reliable and almost entirely reversible immobilisation, without any dissociative anaesthetic drugs (Semjonov et al., 2017; Semjonov et al., 2019). This combination may represent an alternative option for use in leopards.

To the authors' knowledge, a combination of ketamine and the alpha-2 adrenoceptor agonist xylazine, is the only immobilisation combination investigated in leopards (Deka et al., 2012; Belsare and Athreya, 2010). Although immobilisation was described as effective, the only clinical variables measured were heart rate, respiratory rate and temperature, which do not provide sufficient information on the cardiopulmonary effects of the combination, which is critical when considering a comparison between immobilisation protocols. Due to the sparsity of species-specific literature in leopards, most of the currently utilised immobilisation protocols for leopards are extrapolated from information garnered from research in other species and anecdotal experience, which is not ideal when inter-species differences can be so marked. Leopards have traditionally been difficult to study, in part due to their solitary nature, coupled with low population densities and distribution in remote

areas. In addition, being solitary predators, even captive populations often only comprise a small number of individuals.

Zolazepam-tiletamine and medetomidine

Tiletamine is a cyclohexylamine dissociative anaesthetic drug which is almost exclusively provided in proprietary formulations in combination with equal concentrations of the benzodiazepine zolazepam, as either Zoletil® or Telazol®. As with ketamine and with comparable pharmacodynamic effects, tiletamine acts on a number of receptors and the anaesthetic effects are generally ascribed to antagonism at N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors which results in inhibition of cellular excitatory potential due to the movement of cations (Ca^{2+}, Na^{+} & K^{+}) across the cell membrane (Lin, et al., 1993; Fahlman et al. 2005; Mion and Villevieille, 2013). Immobilisation with tiletamine is characterised by muscular hypertonicity, wide open eyes, an apneustic breathing pattern and maintenance of the majority of the cranial reflexes (Fahlman et al., 2005; Kock & Burroughs, 2012). Specific cardiopulmonary effects include a direct negative inotropic effect which is countered by sympathetic stimulation resulting in an increased heart rate and cardiac output as well as increased systemic and pulmonary vascular pressures (Berry, 2015). Cyclohexylamines do not reduce the respiratory response to hypoxia, and ventilation is generally well maintained, although apneustic breathing patterns are commonly observed (Ilkiw et al., 1996; Kaka et al., 2016).

There is no antagonist available for tiletamine and elimination is dependent on liver metabolism followed by urinary excretion. As such, care should be taken when administering very high or repeated doses as recovery can become exponentially prolonged (Pawson and Forsyth, 2008).

Zolazepam is a benzodiazepine sedative drug that acts by modulating the inhibitory effects of gamma (γ) amino butyric acid (GABA) in the central nervous system. It does so by enhancing the affinity of

GABA for the presynaptic $GABA_A$ receptor which results in hyper-polarisation of the cell through a chloride ion influx, rendering the cell less excitable, thereby inhibiting the pathways responsible for arousal and awareness (Lin et al, 1993; Kock & Burroughs, 2012). This also results in muscle relaxant and anxiolytic effects which work to decrease the risk of convulsions and negate the muscular hypertonicity seen with the cyclohexylamines (Kock & Burroughs, 2012). Although there is a theoretical benefit of reversibility through the use of the benzodiazepine antagonist, flumazenil, its cost and short duration of action have been suggested to negate its usefulness in wildlife immobilisation (Kock & Burroughs, 2012; DeClementi, 2017).

Medetomidine is an alpha-2 adrenoceptor agonist with a higher affinity and specificity for alpha-2 adrenoceptors than the older sedative drugs, clonidine and xylazine. Medetomidine is widely documented as producing reliable sedation, analgesia, muscle relaxation, anxiolysis and significant anaesthetic sparing effects in a number of species (Sinclair, 2003; Buck et al., 2017; Semjonov et al., 2019). Due to a number of alpha-2 adrenoceptor subtypes and varied densities amongst individuals, however, there is marked species variation in response to medetomidine administration (Celly et al., 1998; Flaherty, 2013; Giovannitti et al. 2015). Due to effects on both peripheral adrenoceptors, a clinically significant effect of the alpha-2 adrenoceptor agonists is peripheral vasoconstriction (Sinclair, 2003). This results in an increase in systemic vascular resistance (SVR) which in turn causes an initial increase in blood pressure (Sinclair, 2003). Generally, this is followed by a baroreceptor reflex-mediated bradycardia that decreases cardiac output, and blood pressure returns to normal or slightly below normal values (Flaherty, 2013). Although ventilation is generally well maintained when alpha-2 adrenoceptors are used alone, respiratory effects are species specific and often relate to ventilation/perfusion mismatches, hypoventilation and pulmonary hypertension resulting in various levels of hypoxemia (Sarkar, 2017). Potentially less clinically significant effects are hyper-salivation and emesis (Sinclair, 2003). Due to medetomidine's sympatholytic effects, various forms of

sympathetic stimulation can result in delayed induction times after darting and spontaneous arousal during the immobilisation. This is an important factor to take into consideration when immobilising highly stressed animals (Kock & Burroughs, 2012).

Antagonism of the sedative effects of the alpha-2 adrenoceptor agonists can be achieved by administration of atipamezole or yohimbine. Atipamezole is preferred as it exhibits a greater alpha-2 to alpha-1 adrenoceptor selectivity, a higher alpha-2 adrenoceptor affinity and a half-life approximately twice that of medetomidine, thereby reducing the risk of re-sedation compared to when yohimbine is used (Sinclair, 2003; Kock & Burroughs, 2012).

The addition of medetomidine, with its potent anaesthetic sparing effects, has allowed for a reduction in the dose of zolazepam-tiletamine required for immobilisation of up to 80%, thereby decreasing the recovery time, improving the quality of recovery and markedly reducing the cost of immobilisation (Fahlman et al. 2005).

Zolazepam-tiletamine and medetomidine when used in combination for immobilisation of wild felids, is described as providing a relatively rapid and reliable induction, predictable working time with little risk of spontaneous arousal and a calm recovery as well as having a wide safety margin (Fahlman et al., 2005; Stegmann & Jago, 2006; Buck et al., 2017). However, with subsequent top-up administration, recovery can be extremely prolonged and particularly in a free-ranging environment it may be necessary to closely monitor the recovery over several hours before the animal is able to fend for itself (Pawson and Forsyth, 2008). Although commonly used for the immobilisation of leopards in clinical practice, there are currently no publications documenting its efficacy or the physiological effects in African leopards.

Butorphanol-Azaperone-Medetomidine (BAM)

Butorphanol is a synthetic opioid drug, described as having mixed agonist-antagonist effects, characterised by antagonistic effects at the mu-opioid receptor and agnostic effects at the kappa-opioid receptor (Miller et al., 2013). Due to its high affinity but low efficacy at the mu-opioid receptor it acts as a functional antagonist and only has poor and short-acting analgesic properties whereas its efficacy at the kappa-opioid receptor results in the sedative effects seen (Trescot et al., 2008). It is commonly used in combination with other tranquillisers and sedatives to provide an effective immobilisation protocol as on its own it only produces mild sedative effects (Bortolami & Love, 2015). Butorphanol has relatively few side effects and it can be completely and effectively reversed with naltrexone.

Azaperone is a butyrophenone-derivative drug that exhibits anti-dopaminergic and alpha-1 adrenoceptor antagonism (Kock & Burroughs, 2012). The short-acting neuroleptic sedative action of azaperone provides a reduction in stress during capture, handling and transport (Semjonov et al., 2017). Extrapyramidal signs can be noted with higher doses. Based on the concentration per millilitre in BAM, these would be extremely unlikely but should be noted as there is no antagonist (Kock & Burroughs, 2012). Through action at the alpha-1 adrenoceptor, azaperone causes vasodilation and a decrease in arterial blood pressure (Buss et al., 2016). Clinically there may be a mild increase in respiratory rate and it exhibits an anti-emetic effect, especially in combinations containing opioids (Pawson and Forsyth, 2008).

The potent sedative and anaesthetic sparing effects of the alpha-2 adrenoceptor agonist, medetomidine, appear to have positive synergism with butorphanol and azaperone (Ochi et al., 2014, Miller et al., 2009). Additionally, reversibility with atipamezole is advantageous.

Recently a proprietary formulation of butorphanol-azaperone-medetomidine (Bamanil®) has been produced by Wildlife Pharmaceuticals South Africa and is used under a section 21 permit as a research drug. Each millilitre of Bamanil® contains 30 mg butorphanol, 12 mg medetomidine and 12 mg azaperone and it can be reversed with atipamezole at a ratio of 3 to 5 mg of atipamezole per 1 mg of medetomidine and naltrexone at a ratio of 1 mg naltrexone per mg of butorphanol. As an alternative to or in combination with atipamezole, yohimbine can be used at a dose of 0.25 mg kg^{-1} in carnivores (Kock & Burroughs, 2012). It has been used successfully for immobilisation of lions and cheetahs (Semjonov et al., 2017; Semjonov et al., 2019). Proposed advantages of immobilisation with a combination of BAM include a small drug volume to administer and a calm and predictable induction with a long duration of action. Importantly, butorphanol and medetomidine can be antagonised with naltrexone and atipamezole, respectively, resulting in a combination that provides largely reversible sedation (Semjonov et al., 2017). Additionally, inclusion of the peripheral vasodilator azaperone with the medetomidine assists with blood pressure modulation (Semjonov et al., 2017).

Presently, little is known about the cardiorespiratory effects of either of these two drug combinations in leopards. The current use and recommended doses are extrapolated from published data in other wild felids, especially lions and cheetahs. However, drug effects and effective dosages should not be extrapolated across species, as allometric scaling has been demonstrated to not always be effective across wild animals (Carregaro et al., 2016). Additionally, differing temperaments of different species can result in vastly different efficacies of different drug combinations. A study comparing the effects of these drugs in leopards would therefore be useful to guide clinical practice.

INTRODUCTION

Leopards, along with the majority of Africa's free-ranging carnivores, face an ever-changing, human-dominated landscape with unprecedented levels of habitat destruction and unsustainable loss of biodiversity that has ultimately resulted in overall population decline. Due to their elusive nature,

leopards are a difficult species to count, and estimates of population size are highly variable, although the trend is general population decline and currently, leopards are listed as vulnerable to extinction on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Stein et al., 2020).

Despite their status as a vulnerable species, leopards are still regarded as pests. Two thirds of suitable leopard habitat is outside of protected areas, often times in heavily populated, human-dominated areas (Stein et al., 2020). The consequence is that conflict between leopards and humans is essentially unavoidable and to the inevitable detriment of the species.

The current wildlife trends indicate a shift towards highly managed ecosystems with a significant amount of human intervention. However, working with wild animals poses significant threat to personnel and animal safety without effective restraint (Chinnadurai et al., 2016). As wildlife habitats further contract and growing human pressures result in hard boundaries, the risk of increased human wildlife conflict is inevitably greater and with greater conflict comes a greater need for effective mitigation measures which often require safe and effective chemical restraint. Thus, identifying effective and safe immobilisation protocols across a wide range of species is vital to ensure safety and survival of both the wildlife and the people trying to save them.

AIMS AND OBJECTIVES

The primary aim of this study was to compare the induction time, cardiopulmonary effects and recovery time of using a combination of either zolazepam-tiletamine-medetomidine (ZM) or butorphanol-azaperone-medetomidine (BAM) for immobilisation of captive leopards.

The comparison focused on:

1. Ability to obtain rapid achievement of an adequate depth of sedation/anaesthesia to allow intubation and minor procedures (such as intravenous catheterisation and blood collection)
2. Ability to maintain stable and optimal cardiopulmonary function as indicated by systolic arterial blood pressure (SAP) and arterial partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), respectively.
3. Ability to provide a recovery that is rapid, calm and complete, thereby allowing immediate release.

HYPOTHESES

Immobilisation time

H0: There will be no difference in the immobilisation time between a combination of ZM and a combination of BAM in captive leopards.

H1: There will be a difference in the immobilisation time between a combination of ZM and a combination of BAM in captive leopards.

Cardiopulmonary effects

H0: There will be no difference in measured and calculated cardiopulmonary parameters (SAP, PaO_2, $PaCO_2$) between an immobilisation combination of ZM and a combination of BAM in captive leopards.

H1: There will be a difference in measured and calculated cardiopulmonary parameters (SAP, PaO_2, $PaCO_2$) between an immobilisation combination of ZM and a combination of BAM in captive leopards.

Recovery characteristics

H0: There will be no difference in recovery time (time from the administration of antagonist drug(s) to “head-up") between an immobilisation combination of ZM and a combination of BAM in captive leopards.

H1: There will be a difference in recovery time (time from the administration of antagonist drug(s) to “head-up") between an immobilisation combination of ZM and a combination of BAM in captive leopards.

BENEFITS ARISING FROM THE STUDY:

The study will help to characterise and compare zolazepam-tiletamine-medetomidine and butorphanol-azaperone-medetomidine immobilisation in leopards, with emphasis on immobilisation time, cardiovascular physiology and recovery time. Neither of the two combinations have been evaluated in leopards and the information gained in this study will go towards improving knowledge on the safe immobilisation leopards.

Experimental Design

The prospective, non-randomised clinical trial was approved by the Animal Ethics and Research Committee of the University of Pretoria (V051-17) (Addendum I).

Experimental Animals

Eighteen healthy adult leopards were included in the study. All leopards were housed in a semi-captive manner and data was collected during immobilisation required for routine health evaluations or to facilitate minor management procedures. Data was collected from leopards at three facilities: the AfriCat Foundation near Otjiwarongo, Namibia (n = 4), the Hoedspruit Endangered Species Centre in Limpopo, South Africa (n = 8) and Obetyane-o-Africa in Mpumalanga, South Africa (n = 6).

Immobilisation at the AfriCat Foundation and the Hoedspruit Endangered Species Centre facilitated pre-planned, annual health evaluations. Leopards at the AfriCat Foundation are housed individually in large, one-to-two-hectare, fenced camps with adjacent feeding compartments. AfriCat Foundation is at 1 460 m above sea level (barometric pressure during data collection 621 – 636 mmHg). Leopards at the Hoedspruit Endangered Species Centre (HESC) are housed either individually or in pairs, in fenced enclosures ranging from 800 m^2 to two hectares. HESC is 550 m above sea level (barometric pressure 759 – 762 mmHg). Leopards at Obetyane-o-Africa are housed in large, fenced one-to-two-hectare camps, either individually or in small family groups made up of related individuals (3 to 5 animals). Leopards were immobilised for collection of DNA database samples and relocation between camps. Obetyane-o-Africa is 1450 m above sea level (barometric pressure 671 – 683 mmHg). The facilities and sanctuaries that acted as sources for the study animals all complied with local environmental management permits and captive animal welfare standards.

Data Collection

Data collection took place opportunistically between June 2017 and July 2018.

Procedures

Each leopard was immobilised once for the purpose of the study, with one of two immobilisation combinations. The treatment groups were defined as Group ZM (zolazepam-tiletamine-medetomidine immobilisation combination) and Group BAM (butorphanol-azaperone-medetomidine immobilisation combination). Leopards were assigned to a treatment group based on geographic location. All leopards from the Hoedspruit Endangered Species Centre were allocated to the BAM Group (n = 8) and the leopards from AfriCat and Obetyane-o-Africa were allocated to the ZM Group (n = 10).

Data collection was sub-divided into three separate phases, namely, immobilisation, monitoring and recovery. An onsite data collection sheet accompanied each individual leopard (Addendum I). In addition to clinical parameters, signalment, and general data (date/time of the trial, animal identification, location, drug dosages and darting attempts) were recorded. If there were deviations from the protocol due to the administration of supplementary darts and drugs or having to apply rescue interventions, these were recorded.

Immobilisation

Animal managers were instructed to withhold food from all leopards for no less than 12 hours prior to immobilisation to reduce vomiting and the risk of aspiration pneumonia. Access to water was allowed until darting. Where the camp design allowed, and using meat as an incentive, the selected leopard was lured into an adjacent, smaller feeding enclosure to facilitate easier darting.

Immobilisation drugs were administered to all leopards by means of a dart, delivered using a 3 mL air-pressurised dart (Dan-Inject, Denmark) with a 2 x 30 mm collared needle (Dan-Inject, Denmark) fired from a gas-powered dart-projector (DanInject JM Special, Denmark) into the large muscle masses of either the shoulder or the hindquarters. Once effectively darted, all personnel were withdrawn to a distance where the leopard was no longer aware of their presence but could still be monitored.

Individual drug dosages were calculated based on a visual estimation of body mass coupled with historical data, when available. Leopards were weighed after immobilisation to calculate actual doses administered. The immobilisation combination included in the dart contained either:

a. Group ZM: 1.5 mg kg^{-1} zolazepam-tiletamine (Zoletil 100, Virbac, New Zealand) and 0.06 mg kg^{-1} medetomidine (Medetomidine 10 mg ml^{-1}; Kyron Prescriptions; South Africa)
b. Group BAM: butorphanol (0.3 mg kg^{-1}), azaperone (0.12 mg kg^{-1}), medetomidine (0.12 mg kg^{-1}) (Bamanil; butorphanol 30 mg ml^{-1}, azaperone 12 mg ml^{-1}, medetomidine 12 mg ml^{-1}; Wildlife Pharmaceuticals, South Africa) at a total volumetric dose of 0.1 ml per 10 kg.

The time of darting and the number of darting attempts were recorded.

Photo 1 Testing response to tactile stimulation to determine whether the leopard was adequately sedated and safe to approach.

Once the leopard was recumbent and apparently immobilised, it was approached. Initial evaluation of the depth of immobilisation comprised tactile stimulation with a long object and attempting to elicit a response by producing a loud noise. If the leopard did not respond to these stimuli, one person moved closer to the recumbent animal and an ear flick and tail tug test were used to assess responsiveness. A lack of response indicated that it was safe to work with the animal. This time was recorded as the "time to safe approach" and was considered the immobilisation time.

The leopard was then immediately blindfolded to reduce sensory stimulation and if needed, repositioned, and maintained in lateral recumbency. Tear gel (ISEE Eye Ointment, Virbac, South Africa) was applied to avoid drying out of the corneas. Initial physiological monitoring included respiratory rate, heart rate and rectal temperature. An intravenous catheter (20G Jelco, Smiths

Medical Ltd, South Africa) was aseptically placed and secured into either medial saphenous vein. The leopard was then lifted onto a stretcher and subsequently removed from the cage/camp and transported by vehicle to a set up procedure area for monitoring (up to 1 km distance).

Monitoring

Once at the procedure area, the leopard was intubated via an orotracheal approach with a cuffed silicone endotracheal (ET) tube (12 to 14 mm internal diameter) using an illuminated laryngoscope with a size 4 Miller blade. If anaesthetic depth was insufficient for intubation (indicated by increased jaw tone, swallowing, coughing or vocalisation), a bolus of ketamine (0.5 mg kg^{-1} IV; Ketamine Fresenius 100 mg ml^{-1}, Bodene trading as Intramed; South Africa) was administered. Additional boluses of ketamine were administered at 60 second intervals, until the appropriate anaesthetic depth was achieved. The dose of ketamine administered was recorded. The leopard remained intubated with the cuff inflated for the duration of the monitoring period to ensure a patent airway, protect against regurgitation and facilitate measurement of end-tidal carbon dioxide tension.

Photo 2 Testing depth of anaesthesia and jaw tone prior to intubation.

Balanced isotonic crystalloid fluids (Sodium Chloride Fresenuis 0.9%, Fresenius Kabi; South Africa) were administered at a rate of 10 ml kg^{-1} hr^{-1} via the medial saphenous vein for the duration of the immobilisation.

Cardiopulmonary parameters were continuously monitored using a multi-parameter monitor (Cardiocap 5; Datex-Ohmeda; Finland or Cardell 9500 HD Veterinary Monitor, Midmark Corporation, OH, USA) and recorded at 5-minute intervals, with the first measurement recorded at 10 minutes after the time to safe approach. Heart rate (HR) was measured from the electrocardiogram (ECG) with ECG electrodes in a base-apex configuration and correlated with the

rate calculated following thoracic auscultations with a stethoscope. End tidal carbon dioxide tension ($P_E'CO_2$) was measured using a side-stream gas sampler attached to the end of the ET tube (sampling rate 200 mL $minute^{-1}$). Respiratory rate was taken from the capnogram and correlated with visual appraisal.

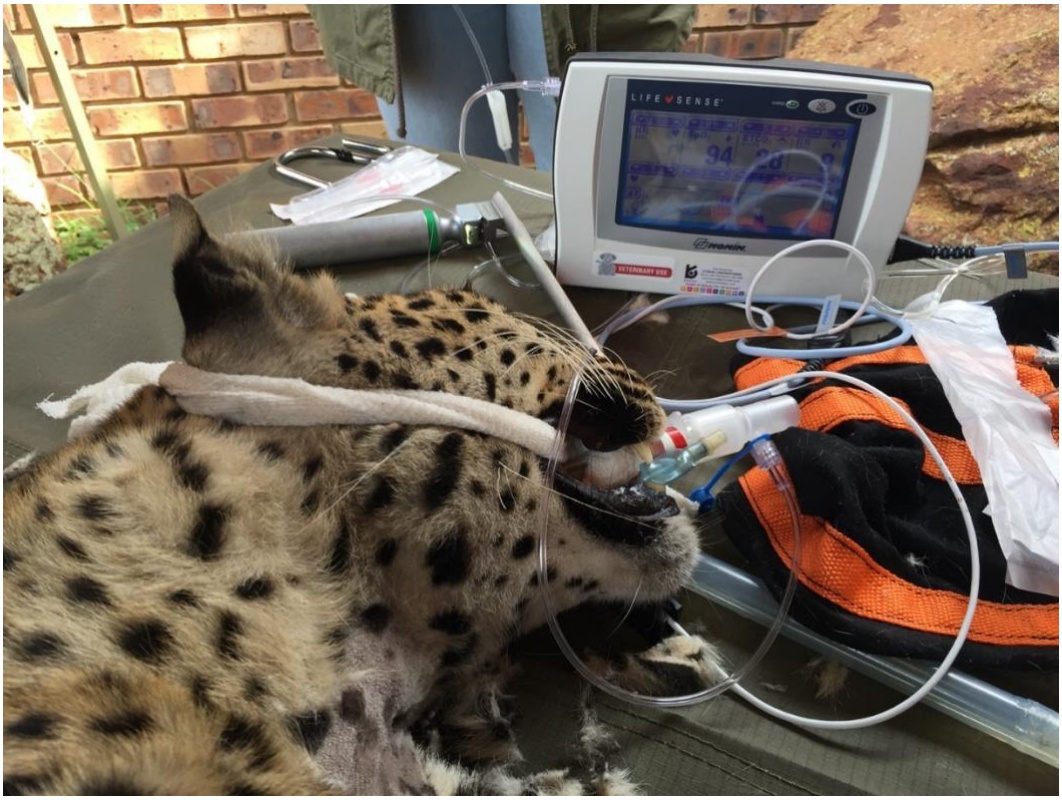


Photo 3 Intubated leopard showing placement of capnograph sampling port on the end of the endotracheal tube.

Photo 4 Immobilised leopard instrumented for data collection at Obetyane-o-Africa.

An intravenous catheter (22G Jelco, Smith's Medical Ltd; South Africa) was aseptically placed into the dorsal pedal artery to facilitate continuous direct blood pressure measurement and intermittent blood collection for arterial blood gas analysis. Invasive SAP, mean (MAP) and diastolic (DAP) blood pressure as well as pulse rate were measured using a multi-parameter monitor (CardioCap 5; Datex-Ohmeda, Finland) connected via non-compliant tubing to an electronic strain gauge transducer (DTX Plus Disposable Transducer; BD Medical, South Africa) zeroed to atmospheric air pressure at the level of the right atrium.

A pulse oximeter (NONIN PureSat 2500 A; NONIN Medical Inc, MN, USA) was used to monitor peripheral oxyhaemoglobin saturation and pulse rate by placing the infrared transmittance probe on the tongue. The pulse rate was correlated with the heart rate from the ECG and auscultated heart rate.

Rectal temperature was recorded using a digital thermometer (H198509 Checktemp 1; Hanna Instruments, South Africa) placed into the rectum. If temperatures deviated from the normal range of

37.0 to 38.5 ˚C, measures were taken to maintain normothermia. Any leopard showing signs of hyperthermia (a rectal temperature greater than 39.5 ºC) was cooled using cold water, ice packs and fanning. Where the temperature decreased below 37 °C leopards were covered with blankets in an attempt to raise the body temperature.

Anaesthetic depth was constantly monitored by evaluating the medial and lateral palpebral reflexes, jaw tone and ear twitch reflex. In the event of the anaesthetic depth being too light, a ketamine bolus (0.5 mg kg^{-1}) was administered intravenously. Any supplementary administration of ketamine was recorded.

Arterial blood samples were anaerobically collected from the dorsal pedal artery cannula, in heparinised 1 ml syringes (Heparin Sodium Fresenius 1000 IU ml^{-1}; Bodene, trading as Intramed, South Africa). The first sample was collected at 15 minutes after "time to safe approach" (T_{15}) and a second sample was collected 45 minutes after "time to safe approach" (T_{45}). Samples were corrected for body temperature and evaluated on-site using the portable i-STAT Blood Gas Analyser (POCT Ltd, USA). The i-STAT CG4+ (i-STAT; POCT Ltd, USA) measured the arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$) and blood pH and lactate.

The total monitoring time was at least 50 minutes to allow for health evaluations and collection of blood samples for genetic typing or movement of animals between camps.

Recovery

Once all the required samples were obtained and parameters recorded, all monitoring equipment was disconnected from the leopard. Thereafter, the leopard was transported to either a crate or a small enclosure adjacent to its main camp for recovery. The intravenous catheter as well as the blindfold

and earplugs were then removed. All non-essential personnel returned to the vehicles or areas of safety outside the enclosure and the leopard was extubated.

For Group ZM, the alpha-2 adrenoceptor antagonist, atipamezole (Alphanil 20 mg ml^{-1}, Wildlife Pharmaceuticals, South Africa) was administered at a 5:1 ratio to the medetomidine dose by intramuscular injection (0.2 mg kg^{-1}). For Group BAM, atipamezole at a 5:1 ratio to the medetomidine dose (0.6 mg kg^{-1}) as well as naltrexone (Trexinol 50 mg ml^{-1}, Wildlife Pharmaceuticals, South Africa) at a 1:1 ratio to the butorphanol dose (0.3 mg kg^{-1}) were administered simultaneously, in separate syringes, by intramuscular injection.

Recovery was monitored and 'head-up' time was recorded as recovery time. All animals were monitored continuously until standing and able to ambulate with minimal ataxia and then intermittently for the next 24 hours to ensure there were no incidences of re-sedation or other untoward effects.

Rescue Interventions

During the trial, leopard safety was maintained as the highest priority. The following potential concerns and rescue interventions were identified to create a protocol for emergency assistance when required.

Potential concerns:

1. Apnoea, defined as no appreciable breathing attempt for longer than 45 seconds (a shorter, more cautious time compared to the conventional 60 seconds based on clinical experience and welfare grounds).
2. Hypotension, defined as a mean arterial blood pressure of less than 60 mmHg for 120 seconds.

3. Respiratory compromise manifesting as a SpO_2 of less than 90% or PaO_2 of less than 60 mmHg
4. Cardiovascular collapse defined by asystole or pulseless electrical activity.
5. Severe hyperthermia manifesting as a temperature greater than 40.5 ˚C.
6. Inadequate sedation defined as a depth of immobilisation considered unsafe for personnel.

Rescue Interventions:

1. If not already intubated, endotracheal intubation followed by intermittent positive pressure ventilation (IPPV) with an adult ambu-bag attached to the ET tube.
2. Administer a balanced isotonic crystalloid fluid bolus at 30 mL kg^{-1}, intravenously, over 10 minutes until the MAP increases.
3. Begin IPPV at a rate of one breath every 6-8 seconds and provide supplemental oxygen.
4. Administer the reversal agent (atipamezole at 5 times the medetomidine dose, naltrexone at 1 times butorphanol dose) and begin cardiopulmonary resuscitation (Fletcher et al. 2012).
5. Immediately begin cooling measures by dousing the leopard with cold water and fanning it. Where possible, place ice packs in the axillary and inguinal areas. Continue cooling measures until normothermia is achieved.
6. Immediately administer an intravenous bolus of ketamine at a dose of 0.5 to 1.0 mg kg^{-1}, repeated at 60 second intervals until an adequate level of immobilisation is achieved

Data Analysis

Cardiopulmonary parameters and indices were interpreted based on data available for domestic cats and dogs where values for leopards were not established. Hypoxemia was defined as a PaO_2 less than 80 mmHg (10.7 kPa) and severe hypoxia as a PaO_2 less than 60 mmHg (Bach, 2008). Hypercapnia was defined as a $PaCO_2$ greater than 45 mmHg (6.0 kPa) (Johnson, 2017). Blood pressure was

interpreted based on SAP and risk of target organ damage, with normotension, prehypertension, hypertension and severe hypertension defined as SAP less than 140 mmHg, 140-159 mmHg, 160-179 mmHg and greater than 180 mm Hg, respectively (Acierno et al., 2018).

The ratio of PaO_2 to the inspired oxygen tension (21% for all animals) was calculated as the P:F ratio, with values above 250 mmHg expected in normal lungs (Wagner, 2015). The alveolar to arterial oxygen partial pressure gradient [$P(A\text{-}a)O_2$] was calculated as described by Wagner (2015), using the daily barometric pressure measured on day of data collection for each animal, water vapour tension of 47 mmHg and a respiratory quotient of 0.8. A normal gradient was considered less than 15 mmHg with a fractional inspired oxygen tension of 0.21. The arterial to end-tidal CO_2 partial pressure gradient [$P(a\text{-}E')CO_2$] was calculated as the difference between the $PaCO_2$ and the $PE'CO_2$, with the expected range in mammals considered 2 to 5 mmHg (Bhavani-Shankar et al., 1992).

Distribution of data was assessed for normality by evaluating descriptive statistics, plotting histograms and performing the Anderson-Darling test for normality and found to be parametric. Physiological, electrolyte and blood gas variables were compared between combinations using a *t*-test. Body masses, drug dosages and induction times were also compared between groups using a *t*-test. Additionally, the 95% confidence interval for difference between the means (CI) and Cohen's *d* (*d*) were calculated for each variable of comparison to assess the effect size. Time variables were compared using values in seconds and then converted to minutes for reporting. Changes over time for variables of interest (HR, SAP, MAP, DAP, respiratory rate, $P_E'CO_2$, and blood gas variables) were compared using a general linear mixed model (fixed factors: time and group; random factors: leopard; interactions: time and time x group). Significant findings underwent post-hoc pairwise comparisons with Tukey correction. Data were presented as mean ± standard deviation (SD). Data

were analysed using commercially available software (MiniTab 18.1; MiniTab Inc., USA) and results were interpreted with a 5% level of significance ($p < 0.05$).

RESULTS

A total of 18 adult leopards were immobilised for this trial. All of the study animals were deemed healthy prior to the commencement of the study and all animals successfully returned to their enclosures following immobilisation. All animals successfully completed the trial and all animals returned to their usual behaviour by the day following immobilisation, The weight of Group BAM leopards was 49.0 ± 9.4 kg and 37.9 ± 9.0 kg for Group ZM (p = 0.036).

Immobilisation

All animals in Groups ZM and Group BAM were successfully immobilised. For Group ZM, 1.31 ± 0.6 mg kg^{-1} zolazepam-tiletamine and 0.04 ± 0.018 mg kg^{-1} medetomidine were administered. For the first three animals in Group ZM, the actual dose administered was found to be much lower than the planned dose after weighing the animal due to underestimation of current body mass (1.2 mg kg^{-1} zolazapam-tiletamine and 0.03 mg kg^{-1} medetomidine). Body weight estimates were adjusted going forward to correct for the accidental underestimation. Actual doses administered are included in all drug calculations. For Group BAM, a total drug volume of 0.011 ± 0.002 ml kg^{-1} was administered, which equated to 0.33 ± 0.05 mg kg^{-1} butorphanol, 0.13 ± 0.02 mg kg^{-1} azaperone and 0.13 ± 0.02 mg kg^{-1} medetomidine. Time to safe approach was faster for Group BAM at 5.8 ± 1.1 minutes compared to 11.8 ± 3.3 minutes for Group ZM (p = 0.008, T = -4.24). In both groups, induction was calm and predictable. Three animals in Group ZM were inadequately anaesthetised prior to transport to the procedure area and were administered ketamine (1.0 mg kg^{-1}) immediately following intravenous catheterisation at the darting site. No ketamine was required in Group BAM.

Monitoring

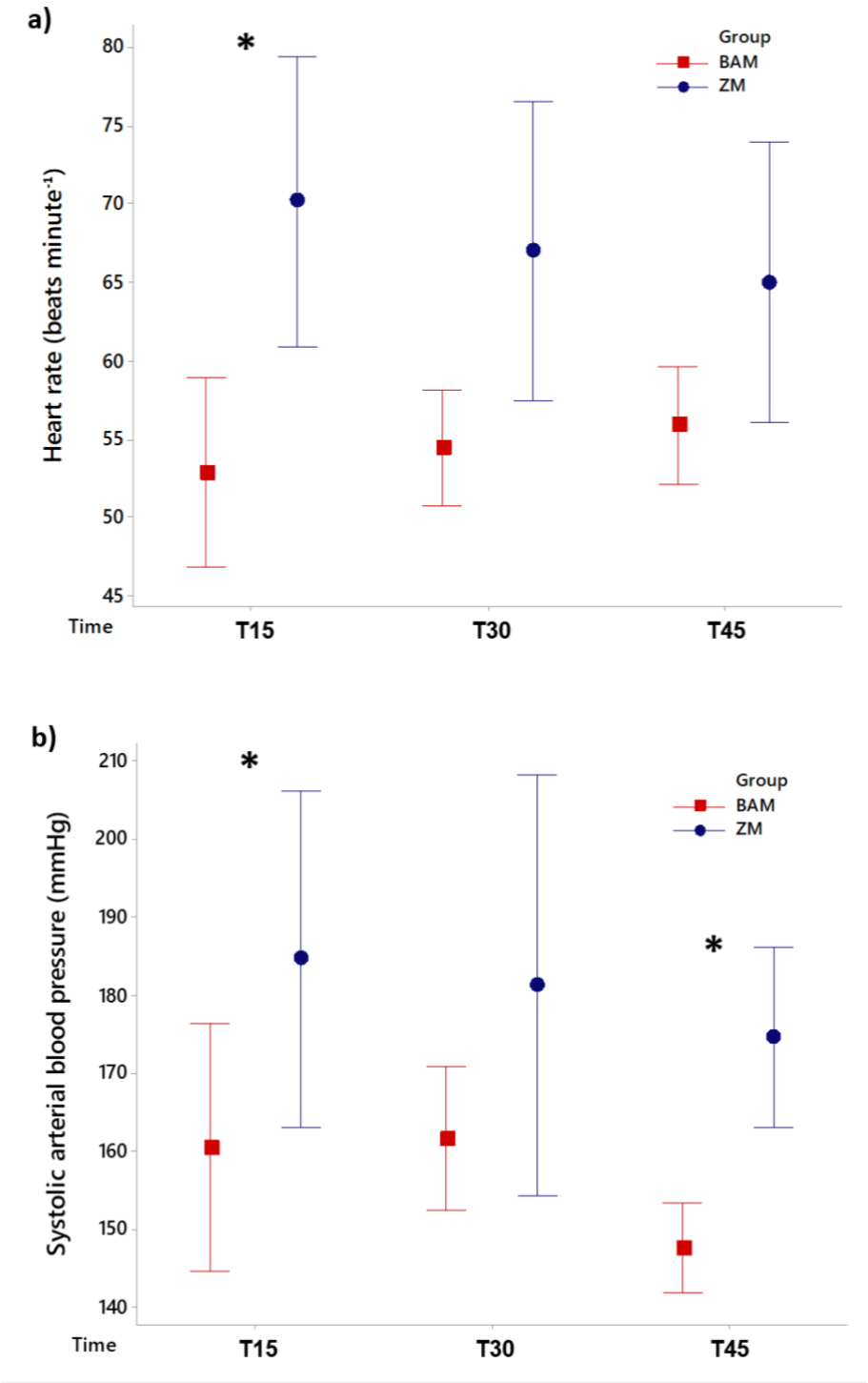
Two animals in Group ZM required a ketamine bolus (0.5 mg kg^{-1} each) prior to successful endotracheal intubation. The depth of immobilisation in all Group BAM animals was sufficient for

endotracheal intubation. The eyes of the leopards in Group ZM remained wide open with a slight medial palpebral reflex remaining present throughout the immobilisation. Conversely, the eyes of the Group BAM animals remained closed with complete absence of the medial and lateral palpebral reflexes. In both groups there was no response to stimulation (blood collection, application of monitoring equipment) or repositioning at any point during the immobilisation, with no need for ketamine during the monitoring period.

Physiological parameters were compared between groups at 15, 30 and 45 minutes (T_{15}, T_{30} and T_{45}, respectively) after 'time to safe approach', which indicated achievement of adequate immobilisation. At T_{15}, the heart rate of leopards in Group BAM was 53 ± 7 beats minute^{-1}, which was significantly lower than that for leopards in Group ZM of 70 ± 13 beats minute^{-1} (p = 0.019). Group BAM demonstrated a trend towards a slight increase in HR to 56 ± 4 beats minute^{-1} while Group ZM demonstrated a downward trend to 64 ± 14 beats minute^{-1} at T_{45}, although there was no significant differences in HR over time in either group, or between groups at the later timepoints (p = 0.915) (Figure 1a).

Leopards in both groups were hypertensive throughout the monitoring period (Figure 1b). Leopards in Group ZM were initially severely hypertensive, with SAP of 185 ± 37 mmHg, which was significantly higher than Group BAM, where SAP was 160 ± 13 mmHg (p = 0.049). There was a decreasing trend in SAP for both groups, although all animals remained hypertensive throughout.

Figure 1 Confidence interval plots (95%) of a) heart rate and b) systolic arterial blood pressure against time for eighteen leopards (*Panthera pardus*) immobilised with either zolazepam-tiletamine-medetomidine (ZM, n = 10) or butorphanol-azaperone-medetomidine (BAM, n = 8). Time is given after immobilisation with T1 representing 15 minutes after time to safe approach, T2 30 minutes after time to safe approach and T3 45 minutes after time to safe approach. * Indicates difference ($p < 0.05$) between groups at that timepoint.



The respiratory rate of leopards in Group BAM was 24 ± 5 breaths minute^{-1} at T_{15}, which was significantly higher than that of leopards in Group ZM, which was 14 ± 3 ($p < 0.001$) (Table 1). There was a gradual, non-significant, decrease in respiratory rate for Group BAM, while that for Group ZM remained constant, such that there were no differences between groups at T_{45} ($p = 0.330$).

Leopards in both groups were hypoxaemic throughout the study (Figure 2a), with Group BAM animals being severely hypoxaemic throughout. There was a small, non-significant, increase in PaO_2 over time for both groups, although leopards in Group BAM were still more hypoxaemic than those in Group ZM at T_{45} ($p < 0.001$). The P:F ratio was higher in Group ZM than Group BAM throughout maintenance (Table 1). The $P(A\text{-}a)O_2$ was above normal limits at both time points for Group BAM, whereas it remained within normal limits for Group ZM ($p < 0.001$).

Throughout the sampling period, the $P_E'CO_2$ was higher in Group BAM than Group ZM ($p = 0.002$), with no significant change in either group across the time periods ($p = 0.417$). For both groups, however, it remained within clinically acceptable limits. The $PaCO_2$ showed a similar trend, remaining higher for Group BAM (mild hypercapnia) than Group ZM throughout monitoring ($p < 0.001$), with no differences over time for either group (Figure 2b). The $P(a\text{-}E')CO_2$ was lower than expected in Group BAM when compared to reference values but showed an increase over the sample periods whereas reverse gradients were present in the Group ZM animals.

Arterial pH was within normal limits for both groups at all times, and no difference between groups at T_{15} ($p = 0.130$). Group BAM trended towards acidaemia whereas the blood pH for the ZM group increased over time, resulting in a significant difference between groups at T_{45} ($p = 0.003$) (Table 1). Blood lactate was within normal limits for both protocols, with no differences between groups or over time.

Figure 2 Confidence interval plots (95%) of a) arterial oxygen partial pressure (PaO_2) and b) arterial carbon dioxide partial pressure ($PaCO_2$) against time for eighteen leopards (*Panthera pardus*) immobilised with either zolazepam-tiletamine-medetomidine (ZM, n = 10) or butorphanol-azaperone-medetomidine (BAM, n = 8). Time is given after immobilisation with T15 representing 15 minutes after time to safe approach and T45 45 minutes after time to safe approach. * Indicates significant difference ($p < 0.05$) between groups at that timepoint

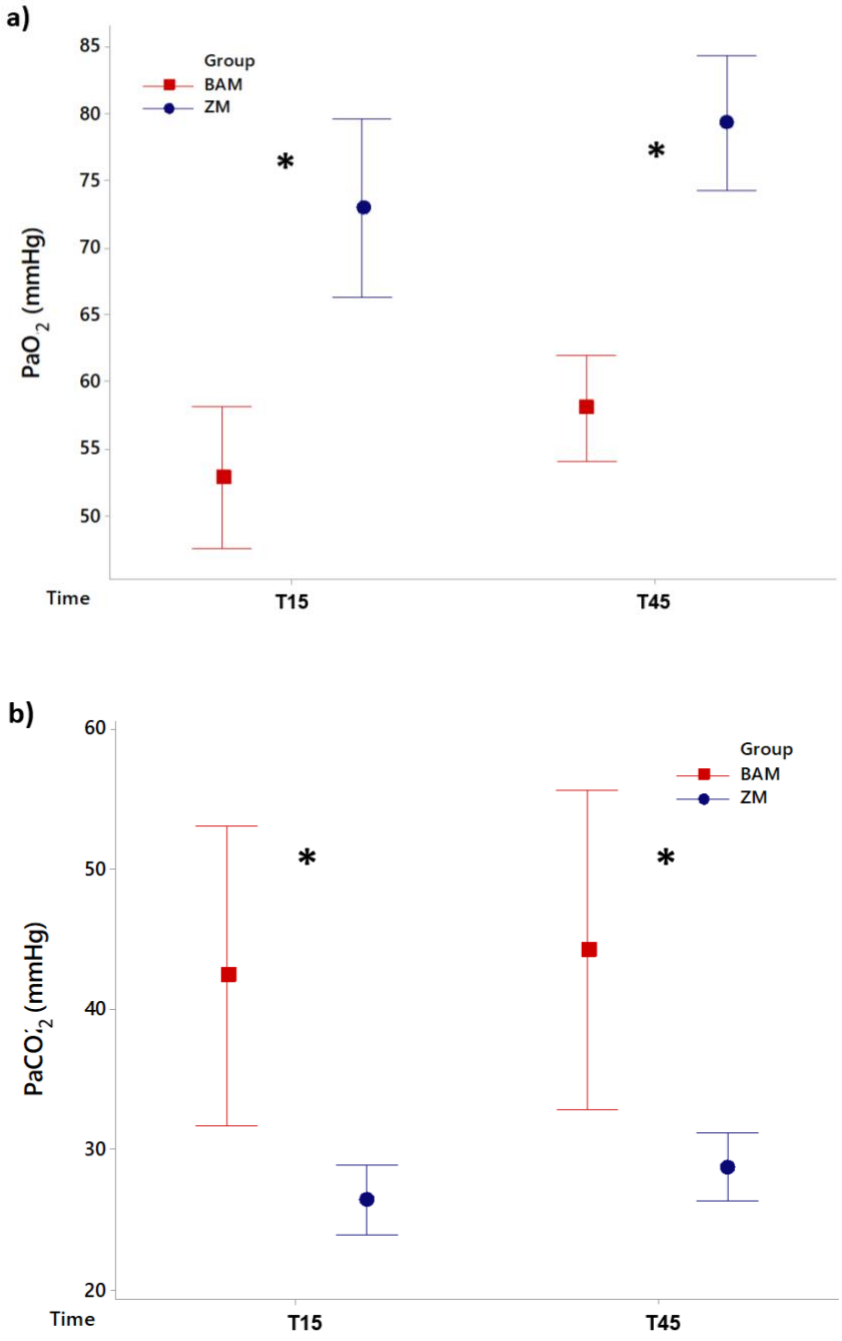


Table 1 Physiological and arterial blood gas parameters of 18 leopards (*Panthera pardus*) immobilised with the butorphanol-azaperone-medetomidine (n = 8) or zolazepam-tiletamine-medetomidine (n = 10). Parameters are reported as mean ±standard deviation at 15 minutes after immobilisation (T_{15}) and 45 minutes after immobilisation (T_{45}).

Parameter	Unit	Group	Time		Significance	
			T_{15}	T_{45}	Time	Group x Time
HR	beats minute^{-1}	BAM	53 ± 7	56 ± 4	p = 0.915	p = 0.514
		ZM	70 ± 13	64 ± 14		
f_R	breaths minute^{-1}	BAM	24 ± 5	18 ± 3	p = 0.102	p = 0.077
		ZM	14 ± 3	14 ± 5		
SpO_2	%	BAM	91 ± 4	94 ± 4	p = 0.598	p = 0.497
		ZM	95 ± 3	95 ± 5		
SAP	mmHg	BAM	160 ± 13	148 ± 6	p = 0.118	p = 0.804
		ZM	185 ± 37	175 ± 9		
DAP	mmHg	BAM	137 ± 12	121 ± 7	p = 0.006	p = 0.423
		ZM	141 ± 12	118 ± 11		
MAP	mmHg	BAM	145 ± 15	132 ± 5	p = 0.074	p = 0.770
		ZM	151 ± 8	136 ± 13		
Temp	˚C	BAM	38.7 ± 0.6	38.9 ± 0.8	p = 0.726	p = 0.288
		ZM	38.4 ± 0.8	37.8 ± 0.8		
pH		BAM	7.30 ± 0.01	7.27 ± 0.01	p = 0.965	p = 0.183
		ZM	7.34 ± 0.05	7.37 ± 0.02		
PaO_2	mmHg	BAM	52.8 ± 4.3	58.0 ± 3.2	p = 0.027	p = 0.814
		ZM	73.0 ± 8.0	79.3 ± 4.8		
	kPa	BAM	7.04 ± 0.57	7.73 ± 0.42		
		ZM	9.70 ± 1.10	10.57 ± 0.64		
$PaCO_2$	mmHg	BAM	42.4 ±8.6	44.2 ± 9.2	p = 0.417	p = 0.915
		ZM	26.3 ± 2.9	28.7 ± 2.3		
	kPa	BAM	5.65 ± 1.15	5.89 ± 1.23		
		ZM	3.51 ± 0.39	3.83 ± 0.31		
$PE'CO_2$	mmHg	BAM	39.8 ± 5.3	40.8 ± 5.1	p = 0.530	p = 0.863
		ZM	31.3 ± 3.6	33.0 ± 4.1		
	kPa	BAM	5.30 ± 0.71	5.44 ± 0.68		

		ZM	4.17 ± 0.48	4.40 ± 0.54		
P:F		BAM	251.4 ± 20.3	276.2 ± 15.1	$p = 0.027$	$p < 0.001$
		ZM	347.4 ± 38.0	377.6 ± 23.0		
$P(a\text{-}E')CO_2$	mmHg	BAM	1.6 ± 4.0	3.4 ± 3.2	$p = 0.906$	$p = 0.517$
		ZM	-1.57 ± 2.0	-4.16 ± 2.4		
$P(A\text{-}a)O_2$	mmHg	BAM	43.9 ± 13.0	36.5 ± 11.9	$p < 0.001$	$p = 0.651$
		ZM	20.4 ± 11.7	8.9 ± 4.6		
Lactate	mmol L^{-1}	BAM	1.12 ± 0.5	0.7 ± 0.2	$p = 0.113$	$p = 0.602$
		ZM	1.0 ± 0.5	0.8 ± 0.4		

HR heart rate, f_R respiratory rate, SpO_2 peripheral oxyhemoglobin saturation, SAP systolic arterial pressure, DAP diastolic arterial pressure, MAP mean arterial pressure, Temp rectal temperature, PaO_2 arterial partial pressure of oxygen, $PaCO_2$ arterial partial pressure of carbon dioxide, P:F ratio of partial pressure of arterial oxygen to fractional inspired oxygen, $PE'CO_2$ partial pressure of expired carbon dioxide, $P(a\text{-}E')CO_2$ partial pressure of arterial oxygen minus partial pressure of mean expired carbon dioxide, $P(A\text{-}a)O_2$ alveolar to arterial oxygen partial pressure gradient

Recovery

The recovery time for Group BAM was 12.8 ± 2.9 minutes, which was no different to the ZM group animals, where recovery times were 15.1 ± 9.6 minutes ($p = 0.604$; $t = 0.55$). Recovery for both groups was relatively calm. Leopards were observed for 24 hours following immobilisation, with no ill effects observed.

DISCUSSION

Combinations of either butorphanol-azaperone-medetomidine or zolazepam-tiletamine-medetomidine were effective in immobilising captive leopards in a predictable manner with acceptable cardiopulmonary function. However, both combinations resulted in hypertension and BAM resulted in clinically significant hypoxaemia. To the authors' knowledge this is the first study evaluating and comparing the cardiopulmonary effects as well as the practical efficacy of any of the commonly used immobilisation protocols in leopards.

Both combinations effected a relatively rapid and calm induction followed by an adequate sedation to allow for safe manipulation and carrying out of minor procedures without any indication of spontaneous arousal. The first three leopards immobilised in the study with the ZM combination had seemingly prolonged induction periods and required additional sedation. This was thought to be a result of a too low initial dose (1.2 mg kg^{-1} zolazapam-tiletamine and 0.03 mg kg^{-1} medetomidine), based on inaccurate body mass estimation. Once the dose was adjusted, the induction times were much shorter and the depth of immobilisation sufficient to allow for intubation, manipulation and sample collection. The revised doses were also closer to those described for other wild felids, including lions and cheetahs (2 to 3 mg kg^{-1} zolazepam-tiletamine and 0.05 mg kg^{-1} medetomidine; Kock & Burroughs, 2012).

As neither combination has been previously studied in leopards, the target doses investigated were extrapolated from published material in other wild felids. Zolazepam-tiletamine-medetomidine has been used successfully at 1.2 mg kg^{-1} zolazepam-tiletamine and 0.04 mg kg^{-1} medetomidine in cheetahs and 0.38 to 1.32 mg kg^{-1} zolazapam-tiletamine and 0.027 to 0.055 mg kg^{-1} medetomidine in lions (Buck et al., 2017; Fahlman et al., 2005). This is similar to the doses used in Group ZM in this study at 1.32 ± 0.31 mg kg^{-1} zolazepam-tiletamine and 0.044 mg kg^{-1} medetomidine. It has been

suggested that as a result of their fractious nature, a higher dose is required for the safe immobilisation of leopards, which may be supported by the need for additional sedation we saw when lower doses were used in Group ZM the early part of this study (Kock & Burroughs, 2012). Effective BAM dosages used in cheetah have previously been reported as 0.009 to 0.014 ml kg^{-1} (Semjonov et al., 2019) and 0.005 to 0.008 ml kg^{-1} in lions (Semjonov et al., 2017). The overall dose requirement is suspected to be lower in larger animals, such as lions than the smaller felid species, based on the assumption that larger animals have a lower metabolic rate (Kleiber, 1932 in Carregaro et al., 2016). A dose closer to that used effectively for cheetahs was investigated in this study, and ultimately 0.01 ± 0.009 ml kg^{-1}, provided calm, predictable and effective immobilisation.

When chemically immobilising wild animals, and particularly free-roaming animals, it is beneficial to achieve as short an induction time as possible to limit the risk of the animal running too far before becoming recumbent which has both the benefit of reducing the risk of physical exertion, which may be associated with the development of capture myopathy, and the time required in searching for a darted animal if it has disappeared out of sight. Immobilisation time was quicker with BAM than with ZM. Although the immobilisation times were delayed in the first three immobilisations of the ZM group animals, this was ascribed to dosing for incorrect body weight estimates, and once this was adjusted with the remaining animals in the group, recumbency times were similar to those considered appropriate in cheetahs and lions, which have been reported as 8.0 to 15.0 minutes and 3.5 to 9.5 minutes, respectively (Buck et al., 2017; Fahlman et al., 2005). In cheetahs and lions immobilised with the BAM combination, immobilisation times of 4.0 to 6.0 minutes and 4.0 to 10.0 minutes were reported respectively, which is similar to Group BAM animals in this study (Semjonov et al., 2019; Semjonov et al., 2017). Although this study focused on captive animals, there may be benefit with the faster immobilisation time seen when using BAM for immobilisation, in free-roaming animals, where the quicker induction time both decreases the risk of losing sight of the darted animal as well

as the period of flight and thus exertion, which if delayed would be detrimental (Chinnadurai et al., 2016). However, further investigations of both drug combinations in free-roaming animals would be suggested prior to their use.

Immobilisation time was standardised for purposes of this study as the “time to safe approach" as it makes practical sense when working with potentially dangerous carnivores and maximises personnel safety. The limitation of this approach is that immobilisation times may appear longer when there was no opportunity to attempt to illicit a response through tactile stimulation and personnel safety was prioritised over testing immobilisation. Further limitations are encountered when comparing immobilisation times to other studies which have not defined what was considered the immobilisation time and further complicate comparison. However, the times in this study remain acceptable for both groups.

The resting values for systolic, mean and diastolic arterial blood pressure in leopards have not been determined, however, they are assumed to be similar to domestic dogs and cats. Severe hypertension, particularly with SAP above 180 mmHg, is thought to pose a high risk of target organ damage and thus generally undesirable (Acierno et al., 2018). Acute hypertension can result in haemorrhage and oedema throughout the body, with the brain and lungs being of particular concern, while sustained hypertension can result in long-term, irreversible organ damage, particularly to the heart, brain (encephalopathy), kidneys and retinas (ocular retinopathy, choroidopathy or retinal detachment) (Acierno et al., 2018). Animals in Group ZM were severely hypertensive initially (SAP 185 ± 37 mmHg) with mild improvement across the sampling period (SAP 175 ± 9 mmHg). However, the blood pressure was still higher than in Group BAM, where animals were initially hypertensive (160 ± 13 mmHg) before improving to prehypertensive levels (defined as SAP 140 to 160 mmHg) by the end of the sampling period. Arterial blood pressure is the product of SVR and cardiac output (CO),

which is itself the product of heart rate (HR) and stroke volume (SV). The SVR is primarily influenced by arteriolar radius and blood viscosity. Medetomidine was used in both immobilisation combinations and has been shown to cause significant, dose-dependent increases in SVR (Pypendop and Verstegen, 1998). The increase in SVR results from stimulation of peripheral alpha-2 adrenoceptors on the vascular smooth muscle, resulting in vasoconstriction, which is then seen clinically as an increase in blood pressure (Giovannitti et al., 2015; Pypendop and Verstegen, 1998).

When comparing the two protocols with regards to their medetomidine dose alone, one would expect higher blood pressure in Group BAM than Group ZM, given the higher dose of medetomidine in this combination. The lower blood pressure in Group BAM could be explained by a few factors. Firstly, the medetomidine-induced vasoconstriction has a ceiling-effect, where beyond a certain dose further increase in the medetomidine dose do not cause further vasoconstriction (Pypendop, and Verstegen, 1998). In domestic dogs, a reduction in the medetomidine dose by up to six times from the initial doses of 0.03 to 0.04 mg kg^{-1} did not have a significant influence on the cardiovascular effects (Pypendop, and Verstegen, 1998). Secondly, blood pressure is not dependent on SVR alone, and medetomidine also causes a reduction in cardiac output subsequent to the hypertension. The decreased CO results from the bradycardia, itself the result of a baroreceptor-mediated response to hypertension and decreased sympathetic tone (Pypendop & Verstegen 1998). Additionally, the effects of other drugs in the combination can also influence the blood pressure. Tiletamine, included in the ZM combination, has been shown to indirectly increase sympathetic activity through re-uptake inhibition of circulating catecholamines, most notably noradrenaline, causing an increase in heart rate and cardiac output which in turn could contribute to an increase in arterial blood pressure (Lin et al., 1993; Suleiman et al., 2012). Conversely, azaperone in the BAM combination, elicits peripheral alpha-1 adrenoceptor antagonist effects which may counteract the vasoconstrictive action of the medetomidine, resulting in a lower SVR and therefore relatively lower blood pressure in the Group

BAM animals (Mich et al., 2008; Buss et al., 2016). This combination of effects could explain the blood pressure differences between the two immobilisation protocols.

The lower heart rate exhibited by the Group BAM leopards can also most likely be attributed to the medetomidine whereby subsequent to the peripheral vasoconstriction and increase in blood pressure there is a baroreceptor reflex-mediated decrease in heart rate which is further compounded by the sympatholytic effect of medetomidine due to blockade of norepinephrine release (Lester et al., 2012). The converse was seen with the ZM group leopards, where their higher heart rate was likely a result of the sympathomimetic effects of the tiletamine counteracting the sympatholytic effects of the medetomidine. Interestingly, a reversal of the $P(a\text{-}E')CO_2$ gradient was seen in the Group ZM animals which may potentially indicate an increase in cardiac output (Karnik et al., 2017). Normally the $P(a\text{-}E')CO_2$ gradient is 5 mm Hg^{-1} or less and this is as a result of alveolar dead space which stems from gas mixing defects in normal lungs (Marshall, 2004).Reversal of the gradient can be seen with higher tidal volume, low respiratory rate and high cardiac output (Karnik et al., 2017).

There were clinically relevant differences in PaO_2 between the two groups. The Group BAM leopards were severely hypoxemic (PaO_2 < 60 mmHg) throughout the study, only showing a mild improvement towards the end of the sample period. In comparison, Group ZM leopards had higher PaO_2 at all timepoints, although they were also hypoxaemic at T_{15}. Hypoxemia typically results from one, or a combination, of the following causes: a low fraction of inspired oxygen, hypoventilation, ventilation/perfusion (V/Q) mismatching, intra-pulmonary shunting or impaired diffusion of oxygen from the alveoli into the blood (Sarkar et al., 2017).

Minute ventilation was not determined, and only respiratory rate, $P_{E'}CO_2$ and $PaCO_2$ were used as indicators of ventilation. Although the respiratory rates and $PE'CO_2$ were within the expected

reference intervals, there was a mild hypercapnia in the Group BAM animals. The hypercapnia could suggest mild hypoventilation which contributed to the hypoxaemia. Of interest, was that animals in Group ZM were not hypercapnic despite a lower respiratory rate (14 ± 3 and 14 ± 5 at T_{15} and T_{45} respectively). This suggests that either the tidal volume was insufficient in Group BAM or other mechanisms contributed to the decreased ventilatory efficiency. Unfortunately, tidal volume could not be measured in this study, and thus the cause cannot be definitively stated. However, this does highlight the potential pitfalls of using only respiratory rate to monitor ventilation. Alternatively, the normal $PaCO_2$ could potentially indicate a compensatory hyperventilation in response to the hypoxemia in Group ZM (Wagner, 2014), as it has been demonstrated that cyclohexylamine drugs, such as tiletamine, maintain the compensatory response to hypoxaemia better than other anaesthetic and sedative drugs (Lin et al., 1993).

The widening of the $P(A\text{-}a)O_2$ gradient, and the normal $P(a\text{-}E')CO_2$, is highly suggestive of oxygen diffusion impedance or right-to-left intrapulmonary shunting rather than ventilation/perfusion mismatching or hypoventilation as major causes of the hypoxaemia (Wagner 2015). Shunting and impaired diffusion of oxygen are often associated with immobilisation combinations containing butorphanol and medetomidine (Mich et al., 2008; Celly et al, 1997). Immobilising drugs within the opioid drug class are known to cause hypoxemia as a result of centrally mediated respiratory depression, decreased chest wall compliance, increased upper airway resistance and hypoventilation which are generally a result of mu-opioid receptor activity. Butorphanol as a mu-receptor antagonist and kappa-receptor agonist is less likely to cause the opioid induced respiratory effects but likely plays a role when in combination with medetomidine (Mich et al., 2008). Among other pharmacological effects contributing to hypoxemia, medetomidine has been shown to increase pulmonary artery pressure in dogs leading to pulmonary hypertension which limits the time available for oxygen diffusion as the transit time of blood is markedly decreased, the ultimate result of which

is right-to-left intrapulmonary shunting and oxygen diffusion deficits (Buck et al., 2017; Meyer et al, 2015; Rolfe et al, 2012). In addition, with a large variation of the alpha-2 receptor subtypes between species, it could explain the apparent greater sensitivity of leopards than what is measured in other species at comparable doses. This becomes ever more likely when you consider the alveolar-arterial O_2 gradient which was significantly increased with all the BAM group leopards, indicating a diffusion deficit as a result of the described mechanisms. Celly et al. (1997) described an increase in shunt fraction as a result of medetomidine administration, the pathophysiological mechanism was postulated to be as a result of an increase in pulmonary resistance due to pulmonary oedema which, although not described in carnivores, could potentially play a contributing role to the hypoxia.

Hypoxemia has been described following the use of BAM for the immobilisation of white-tailed deer (*Odocoileus virginianus)* where the recommendation was made to institute nasal insufflation of oxygen (Mich et al., 2008). In that study, medetomidine-induced pulmonary changes, hypoventilation and specific butorphanol and medetomidine drug effects were purported to be the most likely factors contributing to the hypoxia (Mich et al., 2008). Interestingly, prior studies evaluating the cardiopulmonary effects of BAM in lions, cheetahs and blesboks (*Damaliscus pygargus phillipsi)* all demonstrated clinically acceptable PaO_2 levels at similar doses to those applied in this study (Semjonov et al., 2015; Semjonov et al., 2017).

When comparing the two protocols, what makes the hypoxemia in the BAM group even more noteworthy is that all Group BAM leopards were immobilised at an altitude of 550 m above sea level whereas the ZM group leopards were immobilised at altitudes no less than 950 m and as high as 1750 m above sea level. As altitude increases the barometric pressure decreases and with it the partial pressure of oxygen which poses a greater risk of hypoxia. With the calculated, expected PaO_2 at different altitudes, the difference between the two protocols is even more stark.

Prolonged hypoxemia and hypoxia which remains unchecked can have dire consequences, especially when compounded by a stressful or prolonged capture and induction which ultimately results in greater oxygen consumption on top of a decreased supply (Kock & Burroughs, 2012; Miller et al, 2013). The primary concern is cellular death in organs with high oxygen requirements, particularly the heart and brain, which can result in irreversible damage and decreased survivability. A further consequence is the shift from aerobic to anaerobic metabolism in an oxygen deprived state which results in the production of lactic acid though anaerobic glycolysis and a disturbance of the acid-base balance with a drop in pH (Goldhaber, 1997). The acidosis that follows can have a profound negative inotropic effect on cardiac function due to a desensitisation of the cardiac myofilaments to Ca^{2+} (Than et al., 1994). Without attempts to reverse a worsening hypoxic state and acidosis, death can result. In this trial, despite the hypoxaemia, however, the blood lactate levels for both groups were within normal limits for dogs (< 2 mmol L^{-1}) which may indicate adequate tissue oxygenation despite the hypoxemia. Thus, regardless of the cause of the hypoxaemia, the provision of supplemental oxygen would be recommended when immobilising leopards with both protocols and particularly with the BAM combination.

The ZM group leopards maintained a more effective level of ventilation which is common of combinations containing cyclohexylamine class drugs which result in a dissociative anaesthesia with minimal respiratory effects. A common manifestation of tiletamine or ketamine anaesthesia is an apneustic breathing pattern, which is characterised by a deep inspiration, followed by a pause at full inspiration and an expiration that appears to be insufficient and unmatched with the level of inspiration. An apneustic breathing pattern or an increased cardiac output and CO_2 production could explain the reverse end-tidal CO_2 gradients seen in the ZM group leopards (Karnik et al., 2017).

Recovery from immobilisation should be rapid and as complete as possible when working with wild animals, to allow animals to return to normal behaviour as soon as possible and to protect themselves. The period from the administration of the reversal drugs to 'head-up' time was considered the point of recovery to standardise the recovery time for animals reversed in a crate which may have inhibited their ability to stand and therefore affected evaluation of time to standing. The recovery time was similar in both groups. Although the azaperone in the BAM combination is not directly reversible, based on the level of arousal following reversal, its effects appear to be limited and therefore the reversal is generally expected to be rapid following the administration of naltrexone and atipamezole. Only the medetomidine in the ZM combination is feasibly reversible and therefore recoveries are dependent on redistribution or metabolism of tiletamine and zolazepam. The recovery times of the BAM group animals (12.8 ± 2.9 minutes) were slightly longer than those seen in BAM immobilised cheetah and lions, with recovery times of around 9 minutes in both species when immobilisation was reversed with naltrexone and atipamezole (Semjonov et al., 2019; Semjonov et al., 2017). In lions immobilised with ZM, recovery times were reported as 8 to 26 minutes (Fahlman et al., 2005), which is similar overall to the recovery times evaluated in the ZM group animals (15.1 ± 9.6 minutes). Lack of standardisation of 'recovery time' limits the ability to draw comparative conclusions between studies. However, based on the comparisons with other species, the recovery times of both protocols are acceptable for the immobilisation, particularly for captive leopards, where animals can be observed for complete recovery.

A possible source of study bias and the most significant limitation of this study was that the treatments could not be randomised as they were dictated by geographical location and attending veterinarian preference. This was an aspect of the study we had to adapt to though as a majority of the animals were immobilised as a part of the individual facility's annual management and health checks and we collected the data opportunistically.

Working with wildlife has the major limitation that pre-immobilisation cardiopulmonary parameters usually cannot be measured. Therefore, conclusions are often based on extrapolated data from similar species and domestic animals. This outlines an obvious limitation of this study, in that sample taking and recording could only take place once the leopard was safely immobilized. By that point the capture event and immobilising drugs would have already affected the individual's physiology. However, as the goal of the study was to describe and compare the effects of the immobilisation drugs on physiology, this could still be achieved without baseline values.

A further limitation was a small sample size, which may have resulted in type II error. However, leopards are notoriously dangerous to work with and it is difficult to source captive animals to include in the trial as they are infrequently immobilised. The number of animals in this study is similar to other studies on immobilisation of wild felids (Semjonov, 2017). Although inclusion of more animals could detect more definite differences between the two drug combinations, this trial provides a useful source of preliminary data for future studies.

CONCLUSION

Combinations of both butorphanol-azaperone-medetomidine and zolazepam-tiletamine-medetomidine for immobilisation provided field appropriate rapid induction times, clinically acceptable cardiorespiratory effects and smooth recoveries. Zolzepam-tiletamine-medetomidine, as seen in other wild felid immobilisation studies, resulted in a predictable and safe immobilisation in leopards at the recommended doses of 2-3 mg kg^{-1} zolazepam-tiletamine and 0.03 - 0.05 mg kg^{-1} medetomidine. The sustained, severe hypertension seen throughout the immobilisation with the ZM protocol should be taken into consideration and evaluated further for long term side effects. The butorphanol-azaperone-medetomidine combination resulted in a rapid, calm and reversible immobilisation in leopards at the recommended dose of 0.01 ml kg^{-1} of Bamanil®. The hypoxemia observed in Group BAM in this study was considered clinically significant and we recommend supplementation with oxygen when immobilising leopards with this combination. Either combination is appropriate for use for immobilisation of captive leopards.

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Contents:

Data collection form

Date: _____ Trial Number: _____

Leopard identification: _____

Actual time of darting: _____

Time from successful darting to safe approach: _____

Environmental Temperature: _____

Stress Score:

Before Darting		After Darting	
1	Calm; sitting/standing stationary without an attempt to escape; no vocalisation; * Relaxed and seemingly unaffected by our presence on approach	1	Calm; sitting/standing stationary without an attempt to escape; no vocalisation;
2	Mild agitation; Pacing; A few attempts to escape; Growling/Hissing occasionally; * Relaxed majority of the time but aware of our presence on approach	2	Mild agitation; Pacing; A few attempts to escape; Growling/Hissing occasionally;
3	Markedly distressed; Multiple attempts at escape; Constant vocalisation; * Very aware of our presence and moves off on approach	3	Markedly distressed; Multiple attempts at escape; Constant vocalisation;
4	Extreme distress; Vicious attempts at escape with loud vocalisation and serious risk of self-trauma; * Very skittish and runs off as soon as we approach to close	4	Extreme distress; Vicious attempts at escape with loud vocalisation and serious risk of self-trauma;

Immobilisation:

Zoletil (Zolazepam-Tiletamine)	Est: _____ mg/kg	ml
Domitor (Medetomidine)	Est: _____ mg/kg	ml
	Total Dart Volume	ml
	Darting Attempts	

OR

BAM	Estimated dose:	ml
	Total Dart Volume	ml
	Darting Attempts	

Intubation: **Intubation Time:_____**

Score (Circle)	Description
1	Orotracheal intubation easy; no ketamine needed
2	Ketamine bolus required for intubation
3	Multiple ketamine boluses required for successful intubation
4	Orotracheal intubation unsuccessful

Ketamine (If required): _____mg/kg _____ml

Monitoring:

Time Actual: _____	Heart Rate (bpm)	Resp Rate (bpm)	Temp (°C)	SpO2 %	Blood Pressure (Sys/Dia/Mean) (mmHg)	$EtCO_2$ (mmHg)	ABG (tick time):
0							
5							
10							
15							
20							
25							
30							
35							
40							
45							
50							
55							
60							

Lactate: (1) _____ Time: _____ (2) _____ Time: _____
Actual Body Weight: _____kg **Barometric Pressure: _____**

Estimated Age: _____ **Sex:** M/F

Recovery:

Atipamezole	mg/kg	ml
Naltrexone	mg/kg	ml
Other recovery drugs:		

Total Anaesthetic Time: _____

Extubation Time: _____

Head Up Time from atipamezole: _____

Recovery Score:

Early Recovery		**Late Recovery**	
1	Transition to alertness is calm; voluntary movements are coordinated and requires 1-2 attempts to stand	1	No ataxia Normal gait
2	May startle but generally calm; voluntary movements are semi-coordinated and requires 2-4 attempts to stand	2	Mild ataxia Gait abnormalities are minimal and return to normal quickly
3	Easily startled; voluntary movements are uncoordinated, resulting in paddling and multiple attempts at standing	3	Moderate ataxia Unsteady gait
4	Emergence delirium; uncoordinated thrashing and multiple attempts to stand	4	Severe ataxia for an extended period of time

Additional Notes & Comments:

Animal Ethics Certificate:

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Preliminary findings of the effects of capture technique on stress and immobilization quality and dose in Leopards (Panthera pardus)**
PROJECT NUMBER	**V051-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. R Buck**

STUDENT NUMBER (where applicable)	**U_04388402**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Leopards *(Panthera pardus)***	
NUMBER OF SAMPLES	**40**	
Approval period to use animals for research/testing purposes		**June 2017- June 2018**
SUPERVISOR	**Prof. G Zeiler**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 26 June 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

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