Eesti Maaülikool
Estonian University of Life Sciences

EVALUATION OF A FIXED-DOSE COMBINATION OF BUTORPHANOL-AZAPERONE-MEDETOMIDINE (BAM) FOR CHEMICAL IMMOBILISATION OF AFRICAN LION, BLESBOK, AND CHEETAH

BUTORFANOOLI-ASAPEROONI-MEDETOMIDIINI (BAM) FIKSEERITUD ANNUSTE KOMBINATSIOONI KASUTAMINE AAFRIKA LÕVI, BLESBOKI JA GEPARDI KEEMILISEKS IMMOBILISEERIMISEKS

ALEKSANDR SEMJONOV

A Thesis
applying for the degree of Doctor of Philosophy in Veterinary Science

Väitekiri
filosoofiadoktori kraadi taotlemiseks loomaarstiteaduse erialal

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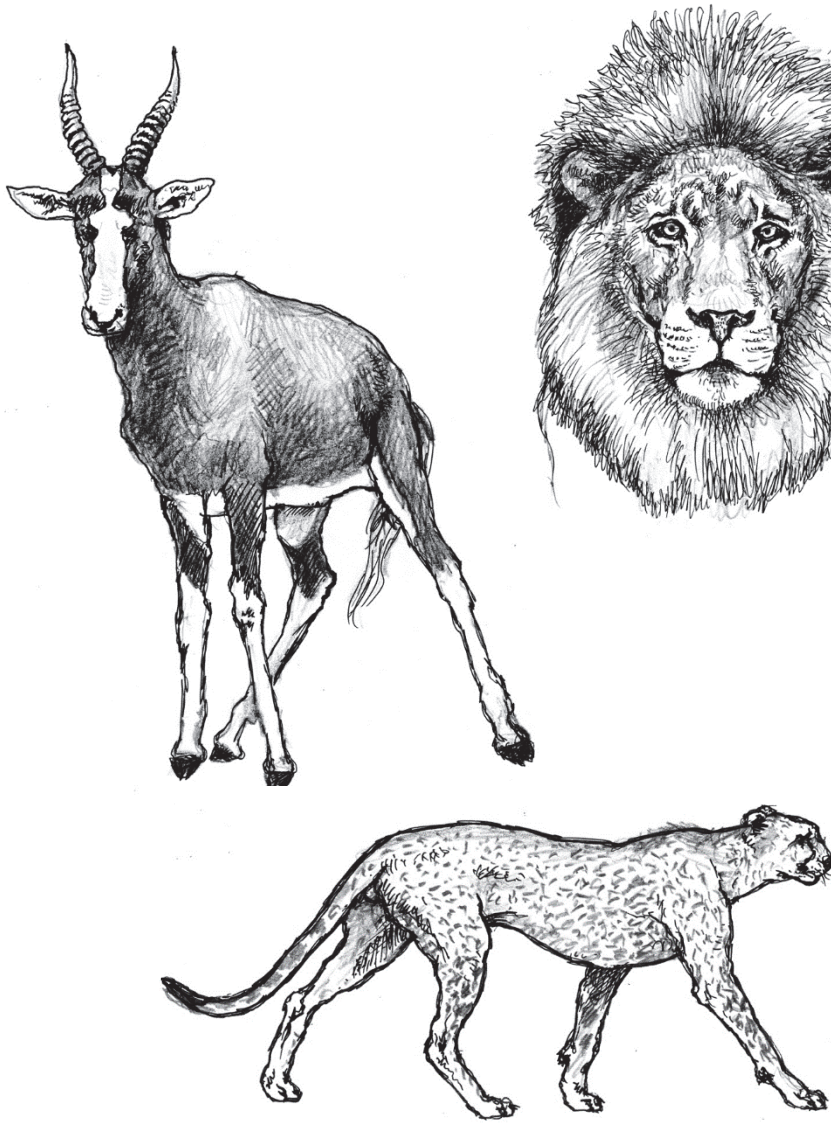
If I have ever seen magic, it has been in Africa

John Hemingway



CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (I-III in the text).

I **Semjonov A.**, Andrianov V., Raath J. P., Orro T., Venter D., Laubscher L., Pfitzer S. (2017). Evaluation of BAM (butorphanol-azaperone-medetomidine) in captive African lion (*Panthera leo*) immobilization. Vet Anaesth Analg. 44:883-889. doi: 10.1016/j.vaa.2017.02.001.

II **Semjonov A.,** Andrianov V., Raath J. P., Orro T., Laubscher L., Pfitzer S., Tiirats T. (2018). Evaluation of butorphanol-azaperone-medetomidine (BAM) in captive blesbok (*Damaliscus pygargus phillipsi*) immobilization. Vet Anaesth Analg. 45:496-501. doi: 10.1016/j.vaa.2017.03.011.

III **Semjonov A.,** Raath J. P., Laubscher L., Orro T., Pfitzer S., Tiirats T., Rogers P. S., Andrianov V. (2019). Evaluation of butorphanol-azaperone-medetomidine in captive cheetah (*Acinonyx jubatus*) immobilization. Vet Anaesth Analg. 46:90-95. doi: 10.1016/j.vaa.2018.09.038.

The contribution of authors to the research paper

Research paper	Original idea, study design	Data collection, sample analysis	Data analysis	Manuscript writing
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ABBREVIATIONS

5-HT – 5-Hydroxytryptamine
AUC – area under the curve
BAM – combination of butorphanol-azaperone-medetomidine
BMM – combination of butorphanol-midazolam-medetomidine
DBP – diastolic blood pressure
$ETCO_2$ – end-tidal carbon dioxide
GPS – global positioning system
GWLPT – Global White Lion Protection Trust
HR – heart rate
Inc – incorporation
IUCN – International Union for Conservation of Nature
KOR – κ-opioid receptor
Ltd – limited company
MAP – mean arterial pressure
MOR – μ-opioid receptor
NIBP – non-invasive blood pressure
$PaCO_2$ – partial pressure of carbon dioxide in arterial blood
PaO_2 – partial pressure of oxygen in arterial blood
Pty – proprietary company
RR – respiratory rate
SANS – South African National Standards
SBP – systolic blood pressure
SpO_2 – peripheral capillary blood haemoglobin oxygen saturation
USA – United States of America

1. INTRODUCTION

Chemical immobilisation and capture of wild animals are one of the most challenging fields of veterinary anaesthesia (Arnemo *et al.,* 2009). Surprisingly, there is only a small amount of clinical information that can be directly adopted from domestic species for this purpose. For example, the weight of the animal and its physiological status can only be determined once it is already immobilised; thus, only estimated body weight can be used for calculating the dose of an immobilisation agent. Diversity of animal species and variation in their physiology and behaviour are extremely wide. Not all wild animal species have scientifically evaluated immobilisation protocols, and it is often necessary to use combinations and doses from closely related species. During any kind of chemical immobilisation of wild animals, human safety must be the priority (Löe and Röskaft, 2004). Careful planning and preparedness are essential in any case of immobilisation of wild animals (Caulkett and Shuty, 2014). A veterinarian must be familiar with the natural history, behaviour, and physiology of the target species, and all equipment must be checked before use. Animal-related risks include injuries, allergic reactions, and zoonotic infections (Hill *et al*., 1998; Adejinmi and Ayinmode*,* 2008). Many wild animals are particularly dangerous, and it is essential that the animal reaches deep level of sedation or anaesthesia before any human gets close and that it remains in this level for the entire procedure (Arnemo *et al.,* 2014). Not only wild animals but also the potent drugs used for chemical immobilisation and drug delivery systems can be potentially dangerous for human health and life (Cording *et al.,* 1999; Chung *et al.,* 2000; Cattet *et al.,* 2006; Haymerle *et al.,* 2010). Unique risk assessment and emergency response protocol should be prepared for every situation prior to the operation. An experienced leader should be assigned, and every person participating in the chemical immobilisation of wild animal should be informed about the plan and know their role in the procedure.

Anaesthetic protocols, that are traditionally used for wild carnivores include dissociative anaesthetics with sedatives or benzodiazepines (Miller *et al*., 2003; Fahlman *et al.,* 2005; Zeiler *et al.,* 2013; Romagnoli *et al*., 2018). The disadvantages of these combinations are lack of antidotes for dissociative anaesthetics or high volume of drugs, which makes them impractical for administration (Herbst *et al*., 1985; Tomizawa *et al*., 1997;

Fahlman *et al.,* 2005). The most popular drugs for immobilisation of African antelopes are potent opioids (Grootenhuis *et al.*, 1976; Citino*,* 2003; Burroughs *et al.*, 2012). These drugs are highly controlled in terms of handling and storage. Besides this, potent opioids can cause respiratory depression and other severe complications in animals (Mich *et al.,* 2008).

In this thesis, a fixed-dose combination of butorphanol, azaperone and medetomidine (BAM) is considered as an alternative reversible protocol to previously used immobilisation protocols in African lion, blesbok and cheetah. The BAM in different concentrations was successfully used first in the USA for immobilisation of black bears (Wolfe *et al.*, 2008) and white-tailed deer (Miller *et al.*, 2009; Siegal-Willott *et al.*, 2009). The combination has shown good results, providing safe and reversible immobilisation (Wolfe *et al.*, 2008; Miller *et al.*, 2009). While there are several research works available regarding using BAM on American wild animal species, to our knowledge, this is the first time that this anaesthetic protocol has been used as a fixed-dose combination for the chemical immobilisation of African species, specifically African wild felids. African lions, blesbok, and cheetahs were chosen for the study owing to their availability in South Africa, the need for new safe and reversible protocol for these species, and the practical interest to the immobilisation of the selected species. Lion and cheetah are both vulnerable species according the International Union for Conservation of Nature (IUCN) Red List (Durant *et al.*, 2015; Bauer *et al.,* 2016). Chemical immobilisation of these species is an important part of conservation programmes. Blesbok is an important hunting species in the agricultural industry in South Africa. Translocation and disease testing of animals as a part of herd management are the main reason for chemical immobilisation of blesbok. The using of BAM in fixed-dose combination can simplify the immobilisation procedure and improve the safety and quality of chemical restraint.

The main aim of this thesis was to evaluate the novel anaesthetic protocol of BAM in a fixed-dose combination for chemical immobilisation of African lion, blesbok and cheetah.

2. REVIEW OF THE LITERATURE

2.1. Natural history of selected species

2.1.1. Lion

Lion (*Panthera leo*) belongs to the family Felidae of order Carnivora. This is one of the most majestic big cats and the second largest felid in the world. The species can be divided into two geographical subpopulations – African lion (*Panthera leo leo*) and Asiatic lion (*Panthera leo persica*) (Bauer *et al.,* 2016). Lions utilise a very broad range of habitats. African lion can be found in grasslands, savannas, forests, and even deserts. They are absent only in tropical rainforests and the deep Sahara Desert (Nowell and Jackson, 1996). The only habitat of Asiatic lion is the dry deciduous forest in The Gir National Park and Wildlife Sanctuary in West India (Bauer *et al.,* 2016).

The IUCN Red List classifies lion as vulnerable, and its population is in a decreasing trend (Bauer *et al.,* 2016). The main threats to lions are indiscriminate killing (mainly for protection of human life and livestock), loss of habitat and prey, and trophy hunting (Bauer *et al.,* 2016, Everatt *et al*., 2019). In addition, low genetic diversity and small population size have positively impacted the decline of the Asiatic lion population (Banerjee and Jhala, 2012). Inbreeding and several diseases (canine distemper virus, bovine tuberculosis) have also been a threat to lion subpopulations (Munson *et al*., 2008, Trinkel *et al*., 2011). The IUCN Red List estimates the lion population to be between 23,000 and 39,000 individuals worldwide (Bauer *et al.,* 2016).

There are some physical variations between subspecies and geographic regions, including coat colour, size, and mane characteristics. Lions of South Africa are generally larger than those of the eastern part of the range (Haas *et al*., 2005). Some of the lions included in the present study were white lions. White lion is a rare phenotype of African lion with coat colour variation and either yellow, blue, or green eyes. The white coat colour is not due to albinism, but rather leucism resulting from a double recessive allele (Turner *et al.,* 2015). They only present in the wild in the Timbavati Private Nature Reserve and southern Kruger National Park in South Africa (Turner *et al.,* 2015). Owing to their rarity, since the 1970s, many white lions were removed from the wild and sent to captive

breeding projects and to zoos around the world. According to the Global White Lion Protection Trust (GWLPT), at present, there are hundreds of white lions kept in captivity around the world and just 11 mature individuals in their natural habitat (GWLPT, 2018). Although white cubs were born repeatedly in the last years from normal-coloured lions carrying the white lion gene, the number of adult animals does not increase. This suggests that the white colour of the coat has a negative impact on hunting success and survival in the wild, although this hypothesis has not been proved (Turner *et al.,* 2015).

2.1.2. Blesbok

Blesbok or blesbuck (*Damaliscus pygargus phillipsi*) is a medium-sized antelope from the genus *Damaliscus*, within which there are two subspecies (blesbok and bontebok) that are distinguishable from one another only by differences in colour and in their geographical distribution (Burroughs *et al.*, 2012; Furstenburg, 2016). Blesbok is endemic to southern Africa, with distribution restricted to an area south of Zambezi River (Furstenburg, 2011, Dalton *et al.*, 2019). It can be primarily found on open grasslands, extending to altitudes up to 2000 m above sea level (East and Estes, 1999). Blesbok prefer grasslands with short grass (David and Lloyd, 2013). The distribution of blesbok is highly dependent on the availability of drinking water, as these animals must drink daily or at least every second day (Furstenburg, 2011). Blesbok are social gregarious antelopes that live in groups of up to 120 members (Dalton *et al.*, 2019).

Blesbok is classified as least concern in the IUCN Red List, and its population has been increasing. The estimated number of mature individuals is 55,000 on protected areas and wildlife ranches. Blesbok is widely used in commercial trophy hunting and in the meat industry (Dalton *et al.,* 2019). In South Africa, different colour variants are becoming progressively more valuable for game breeders, with animals being sold for record prices. As such, blesbok has become a financially important commodity that is being increasingly sold and translocated across South Africa.

2.1.3. Cheetah

Cheetah (*Acinonyx jubatus*) belongs to family Felidae of the order Carnivora, and is the fastest land animal. The maximum documented speed is 103 km/h (Sharp, 1997). In Africa, cheetah can be found in a very wide range of habitat from dry forest to grasslands and deserts (Durant *et al.*, 2015). There are reports of cheetah sighting at altitudes of 4000 m above sea level on the second-highest mountain in Africa – Mount Kenya (Young and Evans, 1993). According to Durant *et al.* (2010), cheetah has lower habitat selectivity than other carnivores. At present, Asiatic or Persian cheetah (*Acinonyx jubatus venaticus*) can only be found in the eastern-central region of Iran, where it mainly inhabits the desert areas (Farhadinia *et al.*, 2012).

The wild populations of cheetah are classified as vulnerable by the IUCN Red List, with population numbers continuously decreasing owing to habitat loss and persecution by livestock farmers. In Africa, cheetahs persist in only 10% of their historical distribution area (Durant *et al.*, 2015). Durant *et al.* (2017) estimated approximately 7,100 cheetah individuals in Africa and Iran in total. The captive cheetah population is an important component in conservation planning. Cheetahs have been recorded in zoos since 1829 in Europe, and the captive population continues to increase (Marker *et al.*, 2018).

2.2. Chemical immobilisation of wild animals

2.2.1. Carnivores: lion and cheetah

Both lion and cheetah belong to the family of big cats and, therefore, are particularly dangerous animals. They are often immobilised for routine procedures, such as clinical examination, sample collection, contraception, collaring, or medical treatment. Relocations for breeding projects also often require immobilisation. All drugs that are used for the immobilisation of large carnivores must be not only safe for the animals but also able to induce a deep plane of anaesthesia and guarantee the safety of people working with the animal (Fahlman *et al.,* 2005; Stegmann and Jago, 2006).

Traditionally, combinations of dissociative anaesthetics (ketamine and tiletamine) and sedatives (α_2-agonists) are used for the immobilisation

of wild carnivores (Miller *et al.*, 2003; Fahlman *et al.,* 2005; Zeiler *et al.,* 2013; Romagnoli *et al.*, 2018). The combination of tiletamine-zolazepam and medetomidine (Fahlman *et al.,* 2005) is considered to be the most widely used for the immobilisation of both wild and captive lions. The advantages of this combination include a low drug volume, rapid induction of anaesthesia, and deep plane of anaesthesia. The disadvantages of this combination are the long half-life of zolazepam and severe ataxia after medetomidine reversing, which makes quick recovery in an emergency situation impossible (Fahlman *et al.,* 2005). The same advantages and disadvantages were reported by other authors (Lewandowski *et al.,* 2002; Walzer and Huber, 2002; Stegmann and Jago, 2006) in terms of cheetah immobilisation. The combination of ketamine and medetomidine or ketamine and xylazine can be an alternative for short-time immobilisation. The advantage of this protocol is the short duration and fast-reversing ability of α_2-agonist (Kreeger and Arnemo, 2018). The disadvantage of this protocol is high volume of drugs, which makes it impractical for administration (Herbst *et al.*, 1985; Tomizawa *et al.*, 1997; Fahlman *et al.,* 2005). An important reported disadvantage of ketamine-medetomidine combination in lion immobilisation is sudden recovery (Quandt, 1992; Fahlman *et al.*, 2005; Kreeger and Arnemo, 2018). Stegmann and Jago (2006) compared three combinations for cheetah immobilisation: ketamine and medetomidine, ketamine and midazolam, and tiletamine/zolazepam and medetomidine. The authors did not observe any differences in induction time or blood pressure between the groups, but they noted lower heart rate and seizures in all cheetahs immobilised with ketamine-medetomidine.

The newer ketamine-free combination of butorphanol, midazolam, and medetomidine (BMM) has been successfully used in wild African lions, providing 45 min of safe immobilisation (Wenger *et al.*, 2010). The same combination was investigated earlier by Lafortune *et al.* (2005) for captive cheetah. The authors reported smooth and fast induction, adequate plane of immobilisation, and quick recovery after the use of atipamezole, flumazenil, and naltrexone for reversing. The same study noted the benefit of using ketamine-free protocol for quick reversing and for animals with kidney dysfunction (Table 1).

Table 1. Commonly used anaesthetic combinations for chemical immobilisation of African lion, blesbok and cheetah.

Species	Combination	References
African lion	Ketamine, xylazine	Herbst *et al.*, 1985
(Panthera leo leo)	Ketamine, medetomidine	Tomizawa *et al.*, 1997
		Kreeger and Arnemo, 2018
	Tiletamine/zolazepam, medetomidine	Fahlman *et al.,* 2005
	Butorphanol, midazolam, medetomidine	Wenger *et al.*, 2010
Blesbok	Etorphine	Williams and Riedesel, 1987
(*Damaliscus pygargus phillipsi*)		Burroughs, 1993
		Burroughs *et al.*, 2012
	Etorphine, azaperone	Gaudio *et al.*, 2020
	Thiafentanil	Burroughs *et al.*, 2012
	Thiafentanil, azaperone	Burroughs *et al.*, 2012
		Kreeger and Arnemo, 2018
Cheetah	Ketamine, xylazine	Janssens *et al.*, 1994
(*Acinonyx jubatus*)	Ketamine, medetomidine	Stegmann and Jago, 2006
	Tiletamine/zolazepam	Walzer and Huber, 2002
		Stegmann and Jago, 2006
	Tiletamine/zolazepam, medetomidine	Stegmann and Jago, 2006
	Butorphanol, midazolam, medetomidine	Lafortune *et al.*, 2005

2.2.2. Blesbok

Blesbok is very agile and fast and can easily be stressed. They may be difficult to immobilise by darting from a helicopter, as over-exertion can be a problem. Long induction can have devastating consequences, such as capture myopathy and death (Bartsch *et al.,* 1977).

Potent opioids are the most popular drugs used for the chemical immobilisation of African antelopes (Grootenhuis *et al.*, 1976; Citino*,* 2003; Burroughs *et al.*, 2012). A dose of 2–3 mg of etorphine for adult blesbok rams and ewes is recommended, with the effects of etorphine being reversed by diprenorphine administration at a total dose of 6–9 mg per animal (2-3 times the dose of etorphine) (Williams and Riedesel, 1987; Burroughs, 1993; Burroughs *et al.*, 2012). The same dose of thiafentanil (reversed by 40–60 mg of naltrexone) can also be used, and some users claim that a mixture of etorphine and thiafentanil provides a better knockdown than etorphine alone (Burroughs *et al.*, 2012; Kreeger

and Arnemo, 2018). A number of sedatives and tranquilisers can also be included in the immobilising mixture, or can be used alone for tranquilisation. Azaperone has been used together with both etorphine and thiafentanil (Kreeger and Arnemo, 2018; Gaudio *et al.*, 2020). However, etorphine-azaperone combination caused greater ventilatory impairment than etorphine alone (Gaudio *et al.*, 2020). Alternatively, 5 mg of xylazine or 3–5 mg of detomidine has also been used in conjunction with thiafentanil, noting that this α_2-agonist needs to be reversed first with yohimbine or atipamezole before the opioid can be reversed (Burroughs *et al.*, 2012) (Table 1).

One of the biggest problems with the use of potent opioids in chemical immobilisation mixtures is that these substances need to be highly controlled in terms of their handling, storage, and record-keeping. Beyond these practical considerations, potent opioids have also been reported to be associated with hyperthermia, respiratory depression, poor muscle relaxation, and capture myopathy (Mich *et al.,* 2008).

2.3. Clinical pharmacology of selected drugs

Butorphanol-azaperone-medetomidine (BAM) is a fixed-dose combination of three active components: butorphanol (30 mg/ml), azaperone (12 mg/ml), and medetomidine (12 mg/ml). The effect of the combination is reversible. In carnivores and ungulates, atipamezole is used to reverse medetomidine at five times the actual dose of medetomidine in mg, and naltrexone hydrochloride is used to reverse butorphanol at one time the actual dose of butorphanol in mg. Azaperone is a short-acting tranquilliser that has no antidote available. Nevertheless, owing to the low dose of azaperone in the combination and its negligible effect on recovery, it does not affect recovery from anaesthesia (Wolfe *et al.*, 2008; Miller *et al.*, 2009).

2.3.1. Butorphanol

Butorphanol tartrate is a synthetic κ-opioid receptor (KOR) agonist that can act either as a partial agonist or antagonist at the μ-opioid receptor (MOR) (KuKanich and Papich, 2018). It has been used in a wide variety of both domestic and wild animal species (Radcliffe *et al.,* 2000; Larsen *et al.,* 2002; Spelman, 2004; Leann *et al.*, 2010; Schnellbacher, 2010; Laricchiuta *et al.*, 2012; Bush *et al.*, 2014; Horowitz *et al.,* 2014).

Butorphanol is commonly used as an adjunct anaesthetic agent in small animals, horses, and wild animals. Owing to its action on the KOR, butorphanol has strong sedative effect that occurs at lower dosages than does its analgesic effect (KuKanich and Papich, 2018). Butorphanol administered at clinical doses has minimal cardiovascular effects. The main adverse effects can be species-specific and may include dysphoria, mydriasis (cats), and decreased motility of the gastrointestinal tract. Butorphanol has lower respiratory depressant effects than MOR-agonists (Commiskey *et al.*, 2005; KuKanich and Papich, 2018). However, in some species, such as humans, primates, and pigeons, butorphanol exhibits grater activity on the MOR and thus induces significant respiratory depression (Zucker *et al.*, 1987; Butelman *et al.*, 1995; Commiskey *et al.*, 2005). The sedative effect of butorphanol has been used for standing sedation of elephants, kiang, Somali wild ass, southern white rhinoceros, western bongo, wisent, yak, water buffalo, Bactrian camel, and greater one-horned rhinoceros (Neiffer *et al.,* 2005; Bapodra *et al.*, 2014; Bouts *et al.*, 2017). The antagonistic activity of butorphanol on the MOR has been used in white rhinoceroses immobilised with etorphine to improve the cardiopulmonary adverse effects of the potent opioid (Wenger *et al.*, 2007; Boardman *et al.*, 2014; Haw *et al.*, 2014; Langhout *et al.,* 2016).

2.3.2. Azaperone

Azaperone is a butyrophenone derivative that has tranquilising effect (Lees and Serano, 1976; Posner, 2018). Azaperone mainly acts as an antagonist of D_2-dopamine receptors in mesolimbic-mesocortical pathways in the brain. In addition, its antagonistic effect on D_1, serotonin (5-HT), α_1, and histamine receptors has been reported (Potter and Hollister, 2001). Antagonism on dopamine receptors is associated with the side effects of azaperone and other butyrophenones. The most commonly observed adverse effects are extrapyramidal signs, such as dyskinesis, muscle tremors, and restlessness (Potter and Hollister, 2001). The effect of azaperone as an antagonist of α_1-adrenoreceptors produces some level of vasodilation and mild decrease in blood pressure, with a slight increase in heart rate (Clarke, 1969; Lees and Serrano, 1976, Serrano and Lees, 1976). In veterinary medicine, azaperone is mainly used in the field of swine medicine, where it has been used for calming animals and controlling aggression when mixing different groups, during transportation, and for obstetrics (Blackshaw, 1981; Ruediger and

Schulze, 2012; Posner, 2018). Lately, azaperone has been used in many wildlife species. The first indication for using azaperone in wildlife was the tranquilisation to calm wild animals during transport or the reduction of stress after capture and in the period of habituation. For these purposes, azaperone was successfully used in white-tailed deer (Read and McCorkell, 2002), roe deer (Mentaberre *et al.*, 2010a), Iberian ibexes (Mentaberre *et al.*, 2010b), and southern chamois (Mentaberre *et al.*, 2010c; Mentaberre *et al.*, 2011). Vasodilation produced by azaperone reduces hypertension associated with etorphine immobilisation in white rhinoceroses (Buss *et al.*, 2016) and African elephants (Still *et al.*, 1996). Azaperone alone was used for standing sedation in both African and Asian elephants (Schmitt *et al.*, 1996; Ramsay, 2000). At present, more and more investigators add azaperone to combinations with sedatives for their synergistic effect (Greth *et al.,* 1993; Bapodra *et al.*, 2014; Szabo *et al.*, 2015; Carregaro *et al.,* 2019; Gaudio *et al.*, 2020).

2.3.3. Medetomidine

Medetomidine is an α_2-adrenergic receptor agonist. Its α_2:α_1 ratio is 1620:1, which indicates that medetomidine is highly selective for α_2-receptors (Posner, 2018). Medetomidine has three main actions: sedation, analgesia, and muscle relaxation. It produces strong sedation and chemical restraint in most animal species – both domestic and wild (Cullen, 1996; Bryant *et al.*, 1998; Bush *et al.*, 2001; Larsen *et al.*, 2002; Miller *et al.*, 2003). The sedative effect of medetomidine is produced by presynaptic binding of α_2-receptors supraspinally and decreasing synaptic release of norepinephrine, which is the primary neuro-transmitter of the sympathetic nervous system (Scheinin and Schwinn, 1992; Cormack *et al.*, 2005; Posner, 2018). Its sedative effect can be species-specific. Ruminants are particularly sensitive to α_2-agonists owing to the presence of α_{2D}-receptors in the central nervous system, which are also associated with arousal (Scheinin and Schwinn, 1992). The analgesic effect of medetomidine occurs in the dorsal horn of the spinal cord via inhibition the release of norepinephrine and substance P (Hellyer *et al.*, 2007). Medetomidine provides good muscle relaxation and immobilisation owing to its effect on interneurons in the spinal cord (Sinclair, 2003). The most important side effects of α_2-agonists are related to the cardiovascular system (Yamashita *et al.*, 2000; Murell and Hellebrekers, 2005). They produce biphasic responses. After reducing the outflow of norepinephrine to the synaptic cleft, sympathetic tone

decreases and parasympathetic tone increases. As a result, negative inotropic effect on the heart and short-acting peripheral vasodilation occur. The effect on peripheral α_2-adrenoreceptors activates the endothelium of blood vessels and induce profound vasoconstriction that is stronger than the primary vasodilation produced by the lack of norepinephrine. To compensate for increased arterial blood pressure, baroreceptor-mediated reflex bradycardia occurs. The animal thus becomes hypertensive and bradycardic. After this, owing to weaker stimulation of peripheral adrenoreceptors, the systemic vascular resistance and blood pressure decrease. However, owing to the parasympathetic effect, heart rate remains low. In this phase, the animal can be normo- or hypotensive and bradycardic (Sinclair, 2003; Murrell and Hellebrekers, 2005; Posner, 2018). Medetomidine induces centrally controlled decreases in respiratory rate and minute volume. The effect can be stronger if medetomidine is combined with other respiratory depressants, such as a MOR agonist (Sinclair, 2003; Posner, 2018).

2.3.4. Hyaluronidase

Hyaluronidase is an enzyme that degrades hyaluronic acid by hydrolysing disaccharides (King *et al.,* 2018). Hyaluronic acid is present in connective tissue interstices throughout the body (Watson, 1993). Hyaluronidase converts hyaluronic acid into watery fluid and accelerates the absorption of injected drugs through tissues to blood vessels (Lewis-Smith, 1986). Hyaluronidase has been used in both human and veterinary local anaesthesia to protect cells from local anaesthetic-associated death and to improve the spread of a local anaesthetic through tissues (Watson, 1993; Onur *et al.,* 2013). Lately, hyaluronidase has been used to improve chemical immobilisation by reducing induction time in white rhinoceros (Kock, 1992), African elephant (Kock *et al.,* 1993; Osofsky, 1997; Mpanduji *et al.,* 2012), polar bear (Cattet and Obbard, 2010), blue wildebeest (Dittberner *et al.,* 2015), and wild cattle (Spadola *et al.,* 2019).

2.3.5 Butorphanol-azaperone-medetomidine

BAM has been evaluated in many species of wild animals. The first studies were conducted in the USA in both captive and wild black bears (Wolfe *et al.*, 2008) and white-tailed deer (Miller *et al.*, 2009; Siegal-Willott *et al.*, 2009). In all three studies, the authors reported that BAM provided reliable, safe, and reversible immobilisation. The anaesthetic induction

was rapid without excitement, and all animals were relaxed and did not react to painful stimuli as injections and blood collections. Free-ranging deer had significantly longer induction times than captive deer (Miller *et al.*, 2009; Siegal-Willott *et al.*, 2009). Physiological parameters were stable with minimal or no side effects. Recovery was smooth, but rapid compared with that of other combinations. Following these studies, the evaluation of BAM was continued in other species. Lapid and Shilo-Benjamini (2015) compared BMM with BAM in Nubian ibex, and found that BMM has more predictable effect for short-term immobilisation, although reversal time was significantly shorter with BAM. Watson *et al* (2016) described BAM as a safe protocol for immobilisation of Bennett's wallabies. The combination was safely used for minor procedures in American beavers (Roug *et al.*, 2018). The same combination was evaluated in captive caribou in Alaska (Hansen and Beckmen*,* 2018). Harms *et al.* (2018) have used BAM for both captive and free-ranging bison for helicopter-based immobilisation. The authors found this combination to be an alternative to potent opioids, as the immobilisation was stable with minimal side effects and short recovery. The latest studies showed that BAM can be recommended for rhesus macaques (Malinowski *et al.*, 2019) and wild pigs (Ellis *et al.*, 2019). However, some researchers noted hypoventilation and hypoxia in white-tailed deer and Rocky Mountain elk (Mich *et al.*, 2008; Wolfe *et al.*, 2014), and they recommend providing supplemental oxygen during the procedure.

In 2011, all rights to BAM fixed-dose combination outside North and South America were obtained by Wildlife Pharmaceuticals South Africa (Pty) Ltd. This was the start of research programme on African wildlife species. The original formulation of BAM used in the USA is 27.3 mg/ml of butorphanol, 9.1 mg/ml of azaperone, and 10.9 mg/ml of medetomidine (BAM^{TM}, Wildlife Pharmaceuticals, Inc., Windsor, Colorado, USA). The ratio of components was changed for the need of African species to 30 mg of butorphanol, 12 mg of azaperone, and 12 mg of medetomidine in 1 ml. The medetomidine dose was increased to provide safety for people working with immobilised carnivores and to decrease induction time. The dose of azaperone was increased to counteract the vascular effect of medetomidine and provide calm recovery.

3. AIMS OF THE STUDY

The main aim of the present study was to evaluate the novel reversible protocol of BAM in a fixed-dose combination for chemical immobilisation of African lion, blesbok, and cheetah.

The specific aims of this study were as follows:

1. to evaluate the effectiveness and safety of BAM fixed-dose combination for immobilisation of African lion, blesbok, and cheetah (**I, II, III**);

2. to evaluate the effect of hyaluronidase to the anaesthetic induction time of blesbok immobilised with BAM (**II**);

3. to compare the effects of two different combinations of antagonists – atipamezole and naltrexone and yohimbine and naltrexone – on recovery from anaesthesia in African lions immobilised with BAM (**I**).

4. MATERIALS AND METHODS

4.1. Study areas and animals

The trials of this thesis were performed in the Republic of South Africa. Three species of animals were included in the study: African lion, blesbok, and cheetah. All procedures were performed as a part of routine clinical work (**I, III**) or herd management (**II**).

Ethical considerationsns

The animals were immobilised for routine procedures, such as blood collection, disease testing, microchipping, GPS collaring, deworming, minor surgical procedure (wound treatment in one lion), application of contraceptive implants, and genetic material collection. The animals were handled in accordance with the Guidelines for the Accommodation and Care of Animals Used for Experimental and Other Scientific Purposes (European Commission, 2007), South African National Standard Zoo and Aquarium Practice (SANS, 2005), and the Animals Protection Act 71 of 1962 (South Africa, 1962).

African lion

A total of 20 immobilisation procedures were performed at Lechwe Lodge Private Game Farm in the Free State Province and at Moholoholo Wildlife Rehabilitation Centre in Limpopo Province in August 2014 (Group I: 13 lions) and September 2015 (Group II: 7 lions), respectively (**I**). All 13 lions in Lechwe Lodge Private Game Farm were white lions. The age of the animals in both groups varied from juveniles (2–3 months old) to adults. The body weight varied from 38 to 284 kg. The heaviest animal in this study was a captive geriatric obese white male lion. The elevation above sea level was 1399 m at Lechwe Lodge and approximately 520 m at Moholoholo Rehabilitation Centre. The ambient temperature ranged from 4.0 to 33.4 °C during the study.

Blesbok

Sixteen captive blesbok, housed together in enclosures at Ngongoni Private Game Farm at an altitude of 900 m above sea level in

Mpumalanga, were immobilised during September 2015 (**II**). The air temperature ranged from 18.2 to 32.2 °C.

Cheetah

Twelve captive cheetahs housed in open-air enclosures were immobilised at Hoedspruit Endangered Species Centre in the Republic of South Africa during January 2016 (**III**). The air temperature during the study ranged from 22.0 to 38.6 °C.

4.2. Medications, doses, and delivery methods

The BAM fixed-dose combination used in the present study was manufactured by Wildlife Pharmaceuticals South Africa (Pty) Ltd. Each millilitre of this solution contained the following active pharmaceutical ingredients: 30 mg of butorphanol, 12 mg of azaperone, and 12 mg of medetomidine.

Individual doses of butorphanol and medetomidine for lions (**I**) were calculated based on commonly accepted recommendations (Fahlman *et al.*, 2005; Wenger *et al.,* 2010) and the data collected during previous immobilisations of lions conducted by the authors. The body weight of the animals was estimated based on visual parameters. For blesbok (**II**), the individual doses of medetomidine were calculated based on previous reports in wild ruminants (Mich *et al.*, 2008; Miller *et al.*, 2009; Siegal-Willott *et al.*, 2009; Wolfe *et al.*, 2014). The individual doses of the combination for cheetah (**III**) were calculated based on a previous BAM study on African lions (Semjonov *et al.*, 2017).

Technical implementation of injections was carried out as follows.

African lion

A cartridge-fired projector (Model 389, Pneu-Dart, Williamsport, PA, USA) was used to deliver the medications to lions. Darts (Pneu-Dart Type 'C', Pneu-Dart, Williamsport, PA, USA) with a volume of 1–3 ml and length of 0.75–1.5 inches (19.05–38.1 mm) equipped with a 13–16-gauge needle with wire barbs were used. Remote darting was performed in enclosures from a vehicle or on foot from distances ranging from 3 to 21 m. Distance was measured using a Leupold RX-1000i (Leupold

and Stevens. Inc, NW, USA) rangefinder. The injections were administered into the femoral muscle.

Blesbok

A gas-powered dart gun (Pneu-Dart X-Caliber, Pneu-Dart, Williamsport, PA, USA) was used to deliver the drug combination. Darts with a volume of 2 ml and length of 19.05 mm equipped with a 14-gauge needle with wire barb (Wildlife Pharmaceuticals South Africa (Pty) Ltd, Mpumalanga, South Africa) were used. Remote darting was conducted in an enclosure of 6 × 8 m in size from an upper deck of the wall from distances ranging from 5 to 12 m. All injections were administered into the thigh muscle. For 7 out of 16 animals, 8000 unit of hyaluronidase (Hyaluronidase Type I-S from Bovine Teste; Sigma Aldrich, Missouri, USA) was added to the dart.

Cheetah

A pistol projector (Dan-Inject ApS, Borkop, Denmark) was used to deliver the drug combination. Darts (Dan-Inject ApS, Borkop, Denmark) with a volume of 1.5 ml with a 1.5 mm×30 mm plain needle were used (Dan-Inject ApS, Borkop, Denmark). Remote darting was performed from outside the enclosures at distances ranging from 3 to 5 m. All injections were administered into the thigh muscle.

All the immobilisation procedures were conducted between 6:00–13:00 and 15:00–17:00 h to avoid high noon ambient temperatures.

Reversal drugs

For lions, medetomidine was reversed with yohimbine hydrochloride (Yohimbine 6.25 mg/ml; V-Tech, Midrand, Gauteng, South Africa) at a dose rate of 0.2 mg/kg body weight in 13 animals (Group I), and with atipamezole (Antisedan 5 mg/ml; Orion Pharma, Espoo Finland) at five times the medetomidine dose in mg in seven cases (Group II). To reverse the effect of medetomidine, atipamezole at five times the medetomidine dose in mg was used in blesbok and cheetah.

Naltrexone hydrochloride (Trexonil 50 mg/ml; Wildlife Pharmaceuticals South Africa (Pty) Ltd) was used to reverse the effect of

butorphanol in all species at 1 mg to each mg of the actual butorphanol dose.

4.3. Immobilisation and manipulations of animals

4.3.1. Induction of anaesthesia

Two stages of anaesthetic induction were recorded: stage I – from the time of darting until the first signs of sedation; stage II – from the time of darting until sternal or lateral recumbency.

African lion

When the animal became recumbent, it was stimulated with a pole to determine if it was safe to handle. After the animal was blindfolded, it was placed on a stretcher, carried to the vehicle, and transported from the enclosure. Outside the enclosure, the lions were placed in a shaded area on a table in lateral recumbency. All animals were intubated using endotracheal tubes of 16–24 mm in diameter (Jorgensen Labs, Loveland, USA).

Blesbok

Once the animals reached recumbency, an additional 2 min was given before they were approached and blindfolded. If the blesbok went into lateral recumbency, it was placed in sternal recumbency immediately after approaching. Animals were placed on a stretcher, carried from the enclosure, and transported to a shaded area approximately 30 m from the enclosure. The animal was then settled on a table in sternal recumbency with the head fixed in an elevated position. All animals were intubated using endotracheal tubes of 10 mm in internal diameter (Jorgensen Labs, Loveland, CO, USA).

Cheetah

Once the animals reached lateral recumbency, an additional 5 min was given before they were approached and blindfolded. The animals were placed on a stretcher, carried from the enclosure, and transported by a vehicle to a building with controlled environment approximately 300 m from the camp, where monitoring could be performed. All animals were

intubated using endotracheal tubes (Jorgensen Labs, Loveland, CO, USA) of 9–11 mm in internal diameter.

4.3.2. Monitoring of anaesthesia

Registration of physiological parameters

In all species, heart rate, respiratory rate, body temperature, non-invasive blood pressure, and haemoglobin oxygen saturation were recorded every 5 min, beginning at 15–20 min after darting. A veterinary monitor (Capnovet Deluxe Multiparameter Monitor; Eickemeyer, Germany) was used for registration of physiological parameters. Auscultation with a stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M, USA) was performed every 5 min for the entire period of immobilisation. Respiratory rate was measured by observation of chest excursions. Capnography was performed for lions and blesbok (**I, II**). A pulse oximeter transducer was fixed on the tongue of the animal. A capnograph side-stream sampling tube was attached to the endotracheal tube. For simulation of the field procedure and to obtain respiratory parameters in animals breathing atmospheric air, animals were not supported with additional oxygen. A temperature probe was inserted into the rectum. A non-invasive blood pressure (NIBP) measuring cuff was placed on the antebrachium (**II, III**) or tail (**I**).

Blood sampling and analysis

To obtain reliable data of the content of blood gases, only arterial blood was investigated. Blood samples were collected for analysis of arterial blood gases, acid-base status, and blood biochemistry. Three to five samples were collected during immobilisation. A puncture was made anaerobically using a heparinised syringe with a 21-gauge needle. The femoral artery and median caudal artery were used for blood sampling in lions and cheetahs, and the auricular artery in blesbok. After blood collection, the puncture site was compressed using a finger for 2–5 min to avoid haematoma formation.

All blood sample analyses were conducted immediately using a portable analyser (i-STAT® STAT1 Portable Clinical Analyzer; Abaxis, Union City, USA) and cartridges (i-STAT cartridges CG4+ & CHEM8+; Abaxis). The variables measured included pH, partial pressure of arterial

oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chlorine, urea, creatinine, glucose, and ionised calcium levels. Actual base excess, actual bicarbonate, arterial haemoglobin, arterial blood oxygen saturation, and haemoglobin were calculated from the measured values. All parameters were temperature corrected.

4.3.2. Recovery

Time from darting until injection of reversal drugs was registered in all animals. The average duration of monitoring and of all manipulations was 70 min for lions, 50 min for blesbok, and 40 min for cheetah. After monitoring was finished, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd, Canada) to exactly determine their body weight. Animals were extubated when strongly marked palpebral reflex was observed. For recovery, lions and cheetahs were placed in lateral position, whereas blesboks were placed in sternal position under a shade on the ground. Antagonists were injected intramuscularly into the thigh muscles. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking and ear shaking; time to head lifting; time to standing; and time to fully coordinated motion (*i.e.*, full recovery). After injection of reversals, animals were left undisturbed.

4.4. Statistical analysis

For analysis of the effects of anaesthetic dosage on the HR, RR, SBP, DBP, MAP, SpO_2, $ETCO_2$, PaO_2, and $PaCO_2$ of the animals, the area under the curve (AUC) was calculated using a trapezoid method for every measurement during the immobilisation period. The mean AUCs were used as response variables in linear regression models. The actual anaesthetic dosage (calculated after the weighing of immobilised animals) and body weight were used as continuous explanatory variables. Sex was included in the models to control possible cofounding effects. For evaluation of the effect of RR on the PaO_2 and SpO_2 linear regression models using PaO_2 or SpO_2, mean AUCs as response variables and RR as an explanatory variable were used (**I, II, III**).

For analysis of the effect of BAM on anaesthetic induction (time from darting to recumbency) and recovery times, linear regression models were

used (**I, II, III**). The same model was used for the analysis of the effect of hyaluronidase and BAM to induction time and recovery time (**II**).

General linear mixed models (GLMMs) were used to analyse the overall time trend in lactate, arterial blood pH, and body temperature (**I, II, III**). Animals were included as random intercepts and polynomials of time (minutes) as fixed effects in increasing order. Isotropic spatial exponential covariance structure was used to model serial correlations of repeated measurements at the within-animal level in all models.

A nonparametric Man–n–Whitney test was used to compare recovery time between two groups of different antidotes (atipamezole and naltrexone and yohimbine hydrochloride and naltrexone) (**I**).

A backward elimination procedure was performed for all final models. The model's assumptions were verified by scatter and normality plots of standardised residuals. For statistical analysis, the STATA 14.0 software (Stata Corporation, TX, USA) was used. *P*-values of ≤ 0.05 were considered statistically significant.

5. RESULTS

5.1. Doses of BAM for African lion, blesbok and cheetah

The first aim of the present study was to prove the safety of BAM for the selected species of African wild animals and to find the correct dose. In the African lion study (**I**), the dose of BAM was estimated mainly according the dose of butorphanol and medetomidine, and gradually decreased towards the lowest safe recommended dose. The dose of BAM that provided full immobilisation after a single injection and deep plane of sedation was 0.006 ml/kg or 0.6 ml per 100 kg. With this dose rate, the doses of individual components were 0.18 mg/kg of butorphanol, 0.07 mg/kg of azaperone, and 0.07 mg/kg of medetomidine.

In the blesbok study (**II**), the dose used for lions did not provide full immobilisation, and this was increased to 0.017 ml/kg, where the doses of the individual components were as follows: butorphanol 0.34 mg/kg, azaperone 0.14, and medetomidine 0.14. This makes the dose of BAM for blesbok double the dose for lion. In the cheetah study (**III**), the same total dose of 0.5 ml was used for all adult animals. The average dose of BAM was 0.01 ml/kg, and the doses of the individual drugs were 0.29 mg/kg of butorphanol, 0.12 mg/kg of azaperone, and 0.12 mg/kg of medetomidine. All actual median doses and standard deviations for all three species are presented in Table 2.

Table 2. Medications and actual median doses ± standard deviation in mg/kg, median dose volume of fixed-dose combination of butorphanol-azaperone-medetomidine (BAM) in ml/kg, and total dose volume of BAM in ml in African lion, blesbok, and cheetah

Medication	African lion	Blesbok	Cheetah
Butorphanol	0.18±0.03	0.34±0.08	0.29±0.04
Azaperone	0.07±0.01	0.14±0.03	0.12±0.01
Medetomidine	0.07±0.01	0.14±0.03	0.12±0.01
BAM	0.0061±0.0009	0.017±0.003	0.010±0.001
Total dose volume	0.3–1.6	0.5–0.9	0.5

5.2. Induction of anaesthesia

In all cases, immobilisation occurred after a single injection of BAM, and no additional administration was needed. The first signs of sedation occurred within 1 to 5 min. For carnivores, the first signs included open mouth, ataxic gait, and lowering of the head; for blesbok, ears hanging down, wide stance of the front and back legs, and ataxia. The shortest average time for recumbency was shown in cheetah (4 min), followed by lions (7 min), and the longest induction occurred in blesbok (9.6 min). To decrease induction time in the blesbok study, hyaluronidase was added to the dart for seven blesbok, which decreased the time to the first signs of sedation from 3.8 min to 2.6 min and recumbency time from 9.6 min to 5.1 min. Table 3 shows mean times ± standard deviation of first signs and recumbency for all three species.

Table 3. Mean ± standard deviation of time (in minutes) after darting to the first signs of sedation and to recumbency for African lion, blesbok, and cheetah immobilised with a fixed-dose combination of butorphanol-azaperone-medetomidine (BAM) and blesbok, where BAM was combined with hyaluronidase

Parameter	African lion	Blesbok	Cheetah
Time to first sign (BAM)	3±1	3.8±0.9	2±1
Time to first sign (BAM and hyaluronidase)	-	2.9±0.7	-
Time to recumbency (BAM)	7±2	9.6±3.2	4±1
Time to recumbency (BAM and hyaluronidase)	-	5.1±0.8	-

The inductions were calm with no cases of severe excitement phase or vomiting observed. Immobilisations were stable, muscle relaxation was good, and no sudden arousals occurred. In nine cheetah and four lions, spontaneous twitches of the limbs were observed during the first 20–30 min of immobilisation. None of these movements were associated with too light level of sedation. Weak head and limb movements as a reaction to the changing of body position and weighing were observed in 14 lions after 1 h of procedure, which can indicate a decrease in anaesthetic depth. None of the animals reacted to intubation, extubation, changing of body position, or any painful stimulation such as blood drawing. The total duration of immobilisation was approximately 1 h in all three species.

5.3. Evaluation of physiological parameters

Cardiovascular function

Heart rate was stable throughout immobilisation in all three species. Slight bradycardia (defined as <50 beats/min) was reported in lions and blesbok throughout the entire period, and in cheetah in the first 20 min of immobilisation. Two cheetahs showed low heart rate (32–40 beats/min) during the entire procedure. No arrhythmias were detected. Arterial blood pressure was high in all species, with cheetah showing the highest (overall mean arterial pressure, 167 mmHg). It slightly decreased during immobilisation, but remained high even after 55–60 min since the beginning of anaesthesia (Table 4).

Respiratory function

Respiration was similar in lions and cheetahs. The overall respiratory rate was 15–20 breaths/min and stable throughout immobilisation. No apnoea was observed in any of the species, but apneustic-type breathing with a pause between inhalation and exhalation was recorded in five cheetahs. Neither inhalation nor exhalation was abnormal in quality. In seven cheetahs, bluish colour of the tongue was detected, although respiration was deep and regular. Seven cases of rare and shallow respiration in the induction phase were observed in lions. Compared with carnivores, all blesbok showed elevated respiratory rate (30–46 breaths/min). Respiration was regular, but mostly shallow.

All animals breathed the atmospheric air. SpO_2 was relatively low in the beginning of immobilisation, indicating hypoxia. In all three species, this parameter increased over time. Capnography was performed in all three species, but $ETCO_2$ values in the cheetah study were questionable and thus excluded from analyses. In lions and blesbok, $ETCO_2$ values were in acceptable ranges, although individual variations occurred and two lions showed hypercapnia ($ETCO_2$ >50 mmHg) during the entire procedure (Table 4).

Table 4. Mean ± standard deviation and range (min-max) of the main measured physiological parameters during chemical restraint with a fixed-dose combination of butorphanol-azaperone-medetomidine (BAM) in African lion, blesbok, and cheetah

Parameter	African lion	Blesbok	Cheetah
Heart rate (beats/min)	40±8	45±6	50±9
	(24–62)	(36–55)	(32–70)
Respiratory rate (breaths/min)	15±4	38±4	20±3
	(6–30)	(30–46)	(8–28)
Systolic blood pressure (mmHg)	170±20	166±11	197±19
	(118–236)	(150–190)	(122–209)
Mean blood pressure (mmHg)	142±16	137±7	167±19
	(104–193)	(127–151)	(106–186)
Diastolic blood pressure (mmHg)	131±16	118±3	151±19
	(79–177)	(113–123)	(96–176)
SpO_2[1] (%)	88±6	93±2	93±2
	(72–100)	(89–96)	(80–100)
$ETCO_2$[2] (mmHg)	41±8	41±4	–
	(18–59)	(32–49)	
Body temperature (°C)	38.2±0.7	39.1±0.9	38.2±0.7
	(36.6–39.5)	(37.1–40.0)	(36.8–39.1)

[1]Peripheral capillary blood haemoglobin oxygen saturation; [2]end-tidal carbon dioxide.

Environmental and body temperature

Most of the animals were darted either in the early morning or late afternoon to avoid high noon ambient temperatures. The air temperature ranged from 12 to 30 °C. However, two procedures of lion immobilisation were performed in the early morning when environmental temperature was 4–7 °C and four cheetah immobilisations in conditions of up to 38 °C. In the blesbok study (**II**), immobilisation procedures did not start if the ambient temperature was above 25 °C.

Body temperature initially increased in 14 lions, but slightly decreased after 50 min of immobilisation. In blesbok and cheetah, body temperature increased slightly over time but remained within acceptable ranges. Hyperthermia with rectal temperatures of >39 °C were recorded in 2 lions, 7 blesbok, and 1 cheetah. Hypothermia (rectal temperature <37 °C) was recorded in 1 lion and 1 cheetah immobilised in the early morning during low ambient temperature (Table 4).

Mild hypoxemia (PaO_2 60–80 mmHg) was recorded in most animals of all three species throughout immobilisation. Severe hypoxaemia with PaO_2 ranging from 50–59 mmHg was recorded in two male cheetahs in the first 30 min of immobilisation. Lactate levels steadily declined in all three species ($p<0.001$), indicating normal tissue perfusion. Moreover, in blesbok immobilised with the addition of hyaluronidase to the dart, lactate level declined more rapidly (interaction term $p=0.01$). Blood pH level decreased in cheetah ($p=0.023$) and lion ($p<0.001$), and was stable in blesbok over time (Table 5).

Table 5. Mean ± standard deviation and range (min-max) of the measured arterial blood parameters during chemical restraint with a fixed-dose combination of butorphanol-azaperone-medetomidine (BAM) in African lion, blesbok, and cheetah

Variable	African lion	Blesbok	Cheetah
PaO_2[1] (mmHg)	80±4	72±3	68±9
	(70–89)	(68–78)	(50–86)
$PaCO_2$[2] (mmHg)	31.2±3.4	45±2.5	33±5
	(22.2–39.9)	(41–49)	(29–45)
pH	7.34±0.03	7.44±0.04	7.33±0.02
	(7.28–7.41)	(7.36–7.5)	(7.25–7.41)
Lactate[3] (mmol/l)	0.54±0.27	1.32±0.83	0.3±0.2
	(0.30–2.64)	(0.44–3.06)	(0.30–0.88)

[1]Arterial partial pressure of arterial oxygen; [2]partial pressure of arterial carbon dioxide.

5.4. Recovery

Recovery after the administration of antagonists was smooth without any case of dysphoria. In the lion study, two combinations of antagonists were used. The recovery time after administration of naltrexone and yohimbine combination was significantly longer (22±7 min) than that of naltrexone and atipamezole combination, where the mean recovery time was 9±1 min ($p<0.001$). Thirteen lions reversed with naltrexone-yohimbine combination were severely ataxic compared with seven animals reversed with naltrexone and atipamezole, which showed just slight signs of ataxia. Cheetah showed similar recovery time after naltrexone and atipamezole administration. The shortest recovery time was observed in blesbok (Table 6).

Table 6. Mean ± standard deviation of time (in minutes) after administration of antagonists to the first signs of sedation and to full recovery (standing) for African lion, blesbok, and cheetah immobilised with a fixed-dose combination of butorphanol-azaperone-medetomidine (BAM)

Parameter	African lion	Blesbok	Cheetah
Time to first sign (naltrexone and atipamezole)	4±2	3.6±0.9	4.5±1.7
Time to first sign (naltrexone and yohimbine)	16±9	–	–
Time to standing (naltrexone and atipamezole)	9±1	4.8±0.7	9.0±3.6
Time to standing (naltrexone and yohimbine)	22±7	–	–

6. DISCUSSION

The main aim of the present study was to evaluate immobilisation quality and physiological responses using a fixed-dose combination of BAM in three species of African animals – lion, blesbok, and cheetah, and to provide practical guidelines for safe chemical immobilisation of these species. The main expected complications in wild animal anaesthesia are excessively long induction, which can cause an animal to injure itself; sudden awakening, which will be dangerous for personnel working with the animal; and physiological reactions to drugs, including hypo-ventilation or apnoea, hyper- or hypothermia, severe cardiovascular changes, or death.

6.1. BAM and dose volume

The present study showed that BAM in a fixed-dose combination is an efficient protocol for all three species of animals. Generally, the total dose volume of BAM did not exceed 1.5 ml, which makes it suitable for long-distance darting. Only in one very obese captive lion with body weight of 284 kg were higher drug volume and bigger dart needed. The dose volume of BAM for lion generally was up to 1.5 ml, which is lower than that of tiletamine-zolazepam-medetomidine (Fahlman *et al*., 2005). The total dose volume of BAM for adult blesbok immobilisation was 0.5–0.9 ml, which is similar or slightly lower than that of etorphine and azaperone combination (Gaudio *et al.,* 2020). Compared with other wild animals, adult cheetahs have no large variation in body weight (Marker *et al.,* 2003); thus, we used the same dose of 0.5 ml for all adult cheetahs. This dose volume is much lower than the total volume of ketamine-medetomidine or ketamine-medetomidine-midazolam combination (Stagegaard *et al.,* 2017), tiletamine-zolazepam combination (Walzer and Huber, 2002) or tiletamine-zolazepam, ketamine, and xylazine combination (Lewandowski *et al.,* 2002).

6.2. Induction of anaesthesia

In all animals from the three studies, immobilisation occurred after a single injection of BAM, and there was no need for additional drug administration. All inductions were smooth and calm. The shortest induction time was observed in cheetah (4±1 min) (**III**). The low amount of intramuscular and subcutaneous fat tissue as well as the

excellent blood supply of muscles in this species can be associated with rapid induction. The induction time was similar to the times reported in cheetahs immobilised with tiletamine-zolazepam combinations (Deem *et al.,* 1998; Lewandowski *et al.,* 2002; Walzer and Huber, 2002) and significantly shorter than those in cheetahs immobilised with ketamine and medetomidine (9.2±3.4 min); ketamine, midazolam, and medetomidine (11.3±10 min); or tiletamine-zolazepam and medetomidine (16.8±18.1 min) (Stegmann and Jago, 2006). In lion, the induction time was similar to that with butorphanol, midazolam, medetomidine combination (Wenger *et al.,* 2010), slightly longer that with tiletamine-zolazepam and medetomidine combination (Fahlman *et al.,* 2005), but significantly shorter than with ketamine-xylazine mixture (Stander and Morkel, 1991). Nevertheless, to guarantee the safety of people, carnivores were not approached earlier than 15 min after darting. The induction time of BAM in blesbok was considered relatively long (9.6±3.2 min) (**II**). The addition of hyaluronidase to the dart mixture significantly decreased induction time, both in terms of time to the first sign of sedation and time to recumbency. This could be expected because the addition of hyaluronidase has been shown in a number of studies to increase drug absorption and reduce induction time (Bush *et al.,* 2004; Cattet and Obbard, 2010; Dittberner 2011; Dittberner *et al.,* 2015). The effect of hyaluronidase is achieved through enzymatic break-down of the interstitial barrier between muscle fibres and break-down of the intercellular matrix, which is responsible for tissue integrity. This allows drugs to reach the central compartment much faster. As a result, the rate of drug absorption is enhanced, thereby facilitating faster immobilisation (Watson, 1993; Schulenburg *et al.,* 2007; Dittberner, 2011). Generally, even with the addition of hyaluronidase, the induction time in blesbok immobilised with BAM was longer than that with etorphine (2.5±0.4 min) or etorphine-azaperone combination (3.2±1.9 min) (Gaudio *et al.*, 2020).

6.3. Cardiovascular parameters

All physiological parameters in all three species recorded in the present study were stable throughout the procedures. In lions (**I**), a slight but stable bradycardia (defined as <50 beats per minute) was observed. The heart rate was slightly lower than that described in other studies using tiletamine-zolazepam-medetomidine (Fahlman *et al.,* 2005; Jacquier *et al.,* 2006), butorphanol-medetomidine-midazolam (Wenger *et al.*, 2010), or

ketamine-xylazine (Larsson *et al.*, 2008). The heart rate was very similar to that in black bears immobilised with BAM (Wolfe *et al.* 2008). Most probably, the bradycardia was as a result of the vasoconstrictive effect of medetomidine via peripheral α_2-adrenoreceptors, the increase in systemic vascular resistance, and baroreceptor-mediated reflex bradycardia (Sinclair, 2003; Posner, 2018). Slightly higher heart rates were recorded in cheetah immobilised with BAM (**III**). These were similar to the heart rates of cheetahs immobilised with ketamine and medetomidine (Stagegaard *et al.,* 2017), but lower than those with ketamine-midazolam-medetomidine (Stagegaard *et al.,* 2017), tiletamine-zolazepam and medetomidine (Deem *et al.*, 1998; Stegmann and Jago, 2006), or ketamine and midazolam (Stegmann and Jago, 2006). This is probably also due to hypertension, as was noted in all cheetahs with mean arterial pressure consistently exceeding 150 mmHg. Deem *et al.* (1998) reported similar results in cheetahs immobilised with tiletamine-zolazepam-medetomidine. The peripheral vasoconstriction caused by medetomidine in dogs and cats is transient and usually followed by hypotension (Sinclair, 2003). This effect may be species-specific, and the duration of hypertension has been shown to be longer with increasing doses of medetomidine (Kuusela *et al.,* 2000). Kuusela *et al.* (2000) has reported that in dogs, medetomidine at doses higher than 20 μg/kg can provide a longer duration of hypertension, which is caused by persistent interaction with peripheral α_2-adrenoreceptors. In the present study, the mean dose of medetomidine in cheetahs was 120 μg/kg. This fact can explain such a long duration of hypertension. To counteract this hypertension, azaperone is commonly included in drug mixtures, as it has been found to reduce hypertension through its α_2-adrenergic antagonistic effects (Clarke, 1969; Lees and Serrano, 1976; Hattingh *et al.*, 1994; Still *et al.,* 1996). In the present study, we could not observe this effect of azaperone. Therefore, the hypertension observed in the current study may not be entirely caused by the effects of medetomidine, but rather by other possible factors, such as high ambient temperature or stress. It has been assumed that cheetahs in captivity can suffer from chronic stress which may, as it does in humans, stimulate the sympathetic nervous system and induce hypertension (Terio *et al.,* 2004; Cassia *et al.,* 2015). Steggmann and Jago (2006) found that captive cheetahs immobilised with medetomidine and ketamine or ketamine and midazolam were also hypertensive. They suggested that medetomidine was not the only contributor to the high blood pressure. In seven cheetahs, bluish colour of the tongue was observed. Usually, this can be

a sign of circulatory or respiratory deficiencies. Miller *et al.* (2009) noted that the colour of oral mucous membranes should be monitored in addition to pulse-oximetry, especially when low SpO_2 values are observed during immobilisation with BAM. This colour can be explained as a result of the peripheral vasoconstrictive effects of medetomidine (Flacke *et al.*, 1993). This can also explain the relatively low oxygen saturation recorded in all cheetahs in the present study (SpO_2 <95%). Similar cardiovascular effect was observed in all blesbok (**III**), which were bradycardic (heart rate <55 bpm) and hypertensive. As in the cheetah study (**II**), the hypotensive effect of azaperone was not observed in blesbok immobilised with BAM. However, Gaudio *et al.* (2020) reported lower blood pressure in blesbok immobilised with a combination of etorphine and azaperone than in blesbok immobilised with etorphine alone.

6.4. Respiratory parameters

Respiration in all animals was normal and stable. The respiratory rate in lions (**I**) and cheetahs (**III**) was between 15 and 20 breaths per minute. Although no cases of apnoea were observed, some animals showed apneustic type of breathing. This respiratory pattern was characterised by inhalation followed by a pause in respiration and final exhalation, although neither inhalation nor exhalation was abnormal in terms of depth or duration. As medetomidine can supress respiration, especially in a combination with opioids, this effect might be explained by the synergistic interaction of these two drugs, although there is no published report that can confirm this statement. In both lions and cheetahs, PaO_2 values were within the range of 70–82 mmHg throughout immobilisation and indicated mild hypoxaemia (PaO_2 60–80 mmHg) (Read, 2003). This value was similar to that recorded in lion and cheetah immobilised with other drug combinations without additional oxygen supplementation (Fahlman *et al.*, 2005; Wenger *et al.*, 2010), and can be explained as the effect of hypoventilation due to drug side effects. In addition, all blesbok showed increased respiratory rates throughout the procedure. The respiratory pattern was regular, but shallow. This can be explained by the direct effect of butorphanol. Butorphanol has low intrinsic activity at the MOR and strong agonist activity at the KOR and sigma-receptors (Schnellbacher, 2010). Its activity as a sigma-receptor agonist results in the stimulation of respiratory drive (Bush *et al.*, 2014). All blesbok (**II**) had PaO_2 values slightly higher than 70 mmHg,

indicating mild hypoxaemia. In this case, it can be explained either with shallow respiration and ventilation of dead space or lower ventilation/ perfusion mismatch (V/Q). Mich *et al.* (2008) reported a similar pattern of respiration and hypoxaemia in white-tailed deer immobilised with a combination of butorphanol, azaperone, and medetomidine. The authors explained this as a result of negative changes in ventilation/ perfusion ratio and increased physiologic shunting due to both opioid and α_2-agonist administration. Wolfe *et al.* (2014) described similar results in Rocky Mountain elk immobilised with combination of butorphanol, azaperone, and medetomidine. They suggested that supplemental oxygen should always be kept at hand when using this combination.

6.5. Reverse and recovery

In blesbok (**II**) and cheetah (**III**), immobilisation was reversed with intramuscular injections of naltrexone and atipamezole. In blesbok, recovery times were good in all animals, with all animals being fully recovered to standing within less than 5 min after the administration of reversal agents. Time to the first sign of recovery was less than 3 min, and no significant differences were found in recovery times between animals immobilised with BAM only and those immobilised with BAM and hyaluronidase. Only mild signs of sedation (lowered head, lowered ears, or a wide-based stance) were observed within the first 5 min after standing, and these signs were transient with animals returning to normal behaviour thereafter. Complete and uneventful reversal is due to the efficacy of both naltrexone and atipamezole. Atipamezole is a highly specific and selective α_2-adrenoceptor antagonist devoid of significant interactions with other neurotransmitter receptors, and it has been shown to antagonise the sedative effects of α_2-adrenoceptor agonists, such as medetomidine (Karhuvaara *et al.,* 1991; Georoff *et al.,* 2010). Its half-life is twice that of medetomidine, and as a result, re-sedation is uncommon (Sinclair, 2003). Re-sedation has only been reported in non-domestic species via intravenous route of administration (Jalanka and Roeken, 1990; Ranheim *et al.,* 2003). Naltrexone is a highly specific opioid antagonist characterised by a long half-life in most species; thus, re-sedation is also unlikely with naltrexone (Swan, 1993). It is a competitive antagonist at the MOR, and its long half-life is attributable to the half-life of its active metabolite. Although no literature is available on the half-life of naltrexone in non-domestic species, various studies

have reported it to exceed 10 h (Lee *et al.,* 1988; Haigh, 1991). In lions (**I**), the recovery time was significantly shorter when using naltrexone and atipamezole than when using naltrexone and yohimbine. Both atipamezole and yohimbine were able to reverse the effect of medetomidine. Recovery with the naltrexone and atipamezole combination was smooth and calm. The mean recovery time was 9 min. The animals showed signs of slight ataxia once standing during the initial recovery. Recovery with the naltrexone and yohimbine combination was significantly longer (mean time of 22 min), but still faster than that with a tiletamine-zolazepam and medetomidine combination (mean time 33 min) (Fahlman *et al.* 2005). The 13 lions reversed with yohimbine were severely ataxic, which may be explained by the incomplete reversal of medetomidine by yohimbine, along with the effect of azaperone (Schwartz and Clark, 1998). It is important to remember that yohimbine is labelled only for intravenous administration, and the recovery of lions can be quicker if yohimbine is administered intravenously. Janssen *et al.* (2017) compared the effect of intramuscular administration of yohimbine and atipamezole for reversing xylazine in mice. The authors concluded that although the potency of yohimbine is similar to that of atipamezole, the onset of action of yohimbine was longer. The same conclusion was made by Ambrisko and Hikasa (2003) when they compared intramuscular administration of atipamezole and yohimbine to reverse the effect of medetomidine in dogs. Yohimbine has been successfully used to reverse the effect of xylazine in Asiatic lions, tigers, leopards (Seal *et al.*, 1987; Sontakke *et al.*, 2009), and wapiti (Renecker and Olsen *et al.*, 1986). Miller *et al.* (2004) compared yohimbine, atipamezole, and tolazoline to reverse the effect of xylazine in a combination with tiletamine-zolazepam in white-tailed deer. In that study, the mean recovery time after atipamezole administration was significantly shorter than that after yohimbine administration (89.7 and 112.0 min, respectively).

The newer peripheral α_2-antagonist vatinoxan (previously called MK-467 and L-659.066) has been described recently in numerous studies on both domestic and wild animals (Adam *et al.,* 2018; Jaeger *et al.,* 2019; Sainmaa *et al.,* 2019; Tapio *et al.,* 2019; Einwaller *et al.,* 2020). Vatinoxan does not cross or minimally crosses the blood-brain barrier, and therefore affects only the peripheral adverse effects of medetomidine. This was used to eliminate medetomidine-induced bradycardia,

hypertension, and hypoxia. Further research can be conducted to evaluate the combined use of BAM and vatinoxan in wild animal species.

7. CONCLUSIONS AND RECOMMENDATIONS

1. The BAM fixed-dose combination at the doses used in the three studies provided safe and reliable chemical immobilisation of African lion, blesbok, and cheetah. All physiological parameters were stable throughout the procedures. All animals were bradycardic and hypertensive. Although the respiration rate and depth were good, all animals showed mild hypoxaemia; therefore, additional oxygen supplementation may be necessary. None of the animals developed apnoea. No sudden arousals were observed. The chemical immobilisation provided by BAM was reversible with antagonists. Recovery was smooth and calm (**I, II, III**).

2. The hyaluronidase added to BAM significantly reduced anaesthetic induction time in blesbok. It can be advisable to use hyaluronidase together with BAM when chasing wild animals prior to darting (**II**).

3. The use of different antagonists to reverse the effect of BAM clearly affected the recovery time and quality of recovery after chemical immobilisation of African lion. The recovery time after intramuscular administration of naltrexone and atipamezole was significantly shorter than that after naltrexone and yohimbine administration. The degree of post-recovery ataxia was lower with naltrexone and atipamezole reversal (**I**).

4. We suggest to use a BAM fixed-dose combination (30-12-12) at doses of 0.6 ml/100 kg body weight for African lion and 0.1 ml/10 kg for smaller species of African wild carnivores (cheetah, leopard, and serval). For medium-sized antelopes, a dose of 0.15–0.2 ml/10 kg of BAM will be suitable. When chasing wild animals, the addition of 8000 IU of hyaluronidase to the dart is advised to accelerate induction time and prevent the development of capture myopathy. We recommend waiting for an additional 5 min after an animal reached recumbency before approaching. Physiological parameters should be carefully monitored throughout chemical immobilisation. Supplementation with additional oxygen is strongly advisable to prevent hypoxaemia in any case of chemical immobilisation. We recommend using naltrexone and atipamezole intramuscularly to reverse the anaesthetic effect of BAM (**I, II, III**).

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9. SUMMARY IN ESTONIAN

Butorfanooli-asaperooni-medetomidiini (BAM) fikseeritud annuste kombinatsiooni kasutamine Aafrika lõvi, blesboki ja gepardi keemiliseks immobiliseerimiseks

Sissejuhatus

Metsloomade keemiline immobiliseerimine ehk liikumatuks tegemine on veterinaaranesteesia üks keerulisemaid tahke (Arnemo jt, 2009). Koduloomade anestesioloogiaalasest teabest võib ainult väikese osa üle kanda metsloomadele. Näiteks saab looma kehamassi ja terviseseisundit kontrollida ainult siis, kui loom on juba anesteseeritud ehk tuimastatud. Metsloomaliikide mitmekesisus on harukordselt lai ja nende füsioloogilised iseärasused varieeruvad. Ulukitega töötav loomaarst peab hästi tundma erinevate loomaliikide bioloogiat, käitumist ja füsioloogiat. Ainult piiratud arvu loomaliikide puhul on olemas teaduslikult uuritud ja põhjendatud immobiliseerimisjuhendid. Ulukite anesteseerimise puhul vajab inimese ohutus teravdatud tähelepanu. Loomade põhjustatavad ohud hõlmavad traumasid, zoonootilisi infektsioone ja allergiaid (Hill jt, 1998; Adejinmi ja Ayinmode, 2008). Paljud liigid, eriti suured kiskjad, kuuluvad väga ohtlike loomade hulka. Selliste liikide immobiliseerimisel peab vältima liiga pinnapealset anesteesiat ja järsku ärkamist. Oht inimese elule ja tervisele ei tulene vaid metsloomadest endist, vaid ka anesteesias kasutatavatest ravimitest. Näiteks potentsed ehk tugevatoimelised ravimid võivad olla inimese jaoks eluohtlikud isegi väga väikestes kogustes. Iga metslooma anesteesias on tähtsal kohal riskide ennustamine, väljaselgitamine ja hindamine (Löe ja Röskaft, 2004).

Käesolev uuring viidi läbi Lõuna-Aafrika Vabariigis. Uuringu objektideks olid valitud Aafrika lõvi, blesbok ja gepard. Loomaliikide valik põhines immobiliseerimisvajadusel ning loomade kättesaadavusel Lõuna-Aafrika Vabariigis. Nii lõvi kui ka gepard on Rahvusvahelise Looduse ja Loodusvarade Kaitse Ühingu (IUCN) punase nimistu klassifikatsiooni järgi ohualtid liigid. Nende keemiline immobiliseerimine on mitme looduskaitseprojekti oluline osa. Blesbok on tähtis jahiliik Lõuna-Aafrika põllumajanduses. Karjatervise tagamisel on tähtis blesbokkide ümberasustamine ja kontroll haiguste suhtes ning seetõttu vajavad nad regulaarset immobiliseerimist.

Lõvi (*Panthera leo*) on üks maailma suuremaid ja karismaatilisemaid kiskjalisi. Eristada saab kahte alamasurkonda – Aafrika lõvi ja Aasia lõvi. Lõvid asustavad väga erinevaid elupaiku. Aafrika lõvisid võib kohata rohumaal, Aafrika rohtlas, metsas ja isegi kõrbes. Neid ei ole vaid troopilises vihmametsas ega sügaval Sahara kõrbes. Aasia lõvi ainus elupaik on kuiv heitlehine mets Giri rahvuspargis Lääne-Indias (Bauer jt, 2016).

IUCN-i punase nimistu järgi on lõvid ohualdis liik, kelle asurkonna suurus tõmbub pidevalt koomale. Peamised ohud on valimatu tapmine inimeste ja kariloomade kaitseks, elupaikade ning saakloomade arvukuse hävimine, trofeejaht, asurkonna vähene geneetiline mitmekesisus, nakkushaigused ja lähisugulasristumine (Bauer jt, 2016). Aafrika lõvide üks harvaesinev värvivariatsioon on valge lõvi. Valgel lõvil on kollased, sinised või rohelised silmad. Tegemist on leukismiga, mis erinevalt albinismist ei mõjuta silmade värvust. Looduses elavad valged lõvid ainult Timbavati eralooduskaitsealal ja Krügeri rahvuspargi lõunaosas Lõuna-Aafrika Vabariigis. Valged lõvikutsikad võivad sündida ka tavalistel lõvidel (Turner jt, 2015).

Blesbok (*Damaliscus pygargus phillipsi*) on keskmise suurusega antiloop, kes on endeemiline lõunapoolses Aafrikas. Neid võib leida avatud rohumaadel, mis ulatuvad mägedesse kuni 2000 m merepinnast. Blesbokkide levik sõltub joogivee kättesaadavusest, sest nad peavad jooma iga päev või äärmisel juhul üle päeva. Blesbok on sotsiaalne karjaloom ja karja suurus võib olla kuni 120 looma (Dalton jt, 2019).

IUCN-i punase nimistu järgi on blesbok klassifitseeritud ohuvälise liigina ja nende asurkond on suurenemas (Dalton jt, 2019). Blesbok on kaubanduslikus trofeejahis ja lihatööstuses tähtis liik. Viimasel ajal muutuvad blesboki erinevad värvivariatsioonid ulukikasvatajate jaoks üha väärtuslikumaks ja loomi müüakse rekordhindadega. Seetõttu on blesbokist saanud märkimisväärne majanduslik subjekt, keda müüakse ja transporditakse aktiivselt üle kogu Lõuna-Aafrika Vabariigi.

Gepard (*Acinonyx jubatus*) on tuntud kiireima maismaaimetajana. Selle looma maksimaalne dokumenteeritud kiirus on 103 km tunnis (Sharp, 1997). Gepardid asustavad erinevaid elupaiku kuivast metsast kuni rohumaa ja kõrbeni. Gepardeid on nähtud ka Aafrika kõrguselt teisel mäel – Mount Kenyal, kõrgusel 4000 m merepinnast (Young ja Evans,

1993). Gepardi väga haruldane alamliik Aasia gepard on tänapäeval säilinud ainult Iraani idapiirkonnas. IUCN-i punane nimistu klassifitseerib gepardit kui ohualdist liiki, kelle asurkonda ohustavad nii elupaikade hävimine kui ka loomakasvatajate tegevus. Tänapäeval asustab gepard ainult 10% oma ajaloolisest levilast (Durant jt, 2015).

Nii lõvi kui ka gepard kuuluvad suurte kaslaste hulka ja on seetõttu eriti ohtlikud loomad. Sageli immobiliseeritakse neid selliste rutiinsete protseduuride jaoks nagu kliiniline läbivaatus, proovide võtmine, sündimuskontroll jne. Loomade ümberpaigutamine liigikaitselise tegevuse raames vajab samuti sagedast immobiliseerimist. Kõik kasutatavad ravimid peavad olema looma jaoks ohutud ja tagama samal ajal sügava anesteesia, garanteerides ka personali ohutuse (Fahlman jt, 2005; Stegmann ja Jago, 2006).

Kiskjate anesteesiaks kasutatakse traditsiooniliselt dissotsiatiivsete anesteetikumide (ketamiin ja tiletamiin) ja rahustite (α_2-adrenomimeetikumid) kombinatsiooni (Miller jt, 2003; Fahlman jt, 2005; Zeiler jt, 2013; Romagnoli jt, 2018). Tiletamiini-zolasepaami ja medetomidiini kombinatsioon on lõvide anesteseerimisel ilmselt kõige levinum (Fahlman jt, 2005). Selle eelis on väike ravimikogus, kiire anesteetiline induktsioon ja piisavalt sügav anesteesia. Puudus on aga tiletamiini ja zolasepaami väga pikk toimeaeg, mida ei ole võimalik antagonistide ehk vastandtoimeainetega peatada. Alternatiiviks võib olla ketamiini ja medetomidiini kombinatsioon, mida kasutatakse tavaliselt kiirete protseduuride jaoks. Selle segu eelis on lühike toimeaeg ja võimalus looma kiiresti äratada. Puudus on suur ravimikogus, mis muudab manustamise ebapraktiliseks, kuna lendsüstla maht on piiratud Herbst jt, 1985; Tomizawa jt, 1997; Fahlman jt, 2005). Ketamiini ja medetomidiini kombinatsiooni teine märkimisväärne puudus lõvide immobiliseerimisel on loomade järsk ärkamine (Quandt, 1992; Fahlman jt, 2005; Kreeger ja Arnemo, 2018).

Antiloopide immobiliseerimiseks kasutatakse Aafrikas kõige rohkem tugevatoimelisi opioide, nagu etorfiin ja tiafentanüül (Grootenhuis jt, 1976; Citino, 2003; Burroughs jt, 2012). Mõlemad ravimid tagavad kiire uinumise ja nende toimet saab kiiresti antagonistidega peatada. Kõige suuremad probleemid nende kasutamise puhul on ohtlikkus inimese tervisele ja elule ning kasutamise ja säilitamise range kontroll. Pealegi

tekitavad etorfiin ja tiafentanüül mitmeid ohtlikke kõrvaltoimeid, nagu hingamise pärssimine ja hüpertermia (Mich jt, 2008).

Butorfanool-asaperoon-medetomidiini kombinatsioon (BAM) on kolme aktiivse komponendi kombinatsioon fikseeritud annustes. Butorfanooli ja medetomidiini toimet saab vastavalt peatada naltreksooni ja atipamesooliga. Asaperoon on lühitoimeline trankvillisaator, millel puudub antagonist. Kombinatsioonis on asaperooni annus nii väike, et see ei avalda anesteesiast ärkamisele mingit toimet (Wolfe jt, 2008; Miller jt, 2009).

BAM-i on kasutatud mitme erineva loomaliigi immobiliseerimiseks. Esimesed uuringud, mis kirjeldasid BAM-iga esilekutsutud immobilisatsiooni usaldusväärse ja ohutuna, viidi läbi baribalil ja valgesabapampahirvel (Wolfe jt, 2008; Miller jt, 2009; Siegal-Willott jt, 2009). Loomade füsioloogilised näitajad olid protseduuri ajal stabiilsed ja kõrvaltoimeid ei märgatud. Edasised uuringud viidi läbi sellistel loomaliikidel nagu nuubia kaljukits, Bennetti känguru, Ameerika kobras, põhjapõder, Ameerika piison, reesusmakaak ja metssiga (Lapid ja Shilo-Benjamini, 2015; Watson jt, 2016; Roug jt, 2018; Hansen ja Beckmen, 2018; Harms jt, 2018; Malinowski jt, 2019; Ellis jt, 2019). Farmaatsiaettevõte Wildlife Pharmaceuticals South Africa (Pty) Ltd ostis 2011. aastal kõik õigused BAM-i kasutamiseks väljaspool Ameerikat.

BAM-i originaalkoostis Ameerikas oli järgmine: 27,3 mg/ml butorfanooli, 9,1 mg/ml asaperooni ja 10,9 mg/ml medetomidiini. Ravipreparaadi koostist muudeti Aafrika loomaliikide vajaduste kohaselt: 30 mg/ml butorfanooli, 12 mg/ml asaperooni ja 12 mg/ml medetomidiini. Medetomidiini annust suurendati kiskjaliste immobiliseerimisel inimeste ohutuse tagamiseks ja asaperooni annust suurendati medetomidiini kardiovaskulaarsete kõrvaltoimete vähendamiseks.

Töö eesmärgid

Käesoleva uuringu peaeesmärk oli BAM kombinatsiooni kasutamise hindamine Aafrika lõvide, blesbokkide ja gepardite keemiliseks immobiliseerimiseks.

Uuringu spetsiifilised eesmärgid:

1. hinnata BAM-i fikseeritud annuste kombinatsiooni toimet ja anesteesia ohutust kolme Aafrika loomaliigi (Aafrika lõvi, blesbok ja gepard) immobiliseerimisel (**I, II, III**);

2. hinnata hüaluronidaasi mõju anesteesia induktsiooni kiirusele blesbokkidel, keda immobiliseeriti BAM-i fikseeritud annuste kombinatsiooniga (**II**);

3. võrrelda kahe antagonistide kombinatsiooni – atipamesooli ja naltreksooni ning johimbiini ja naltreksooni – toimet Aafrika lõvi anesteesiast ärkamisele, kelle immobiliseerimiseks kasutati BAM-i fikseeritud annuste kombinatsiooni (**I**).

Materjal ja metoodika

Loomad

Kõik immobiliseerimisprotseduurid viidi läbi Lõuna-Aafrika Vabariigis. Uuringusse võeti kolm loomaliiki: Aafrika lõvi, blesbok ja gepard. Kõik protseduurid toimusid rutiinse kliinilise töö (**I, III**) või karja haldamise (**II**) raames. Loomad immobiliseeriti vereproovide võtmiseks, nakkushaiguste kontrollimiseks, mikrokiipide paigaldamiseks, parasiiditõrje tegemiseks või geneetilise materjali saamiseks. Üks lõvi immobiliseeriti haava kirurgiliseks raviks. Kokku immobiliseeriti 20 Aafrika lõvi, kellest 13 olid valged lõvid, 16 blesbokki ja 12 gepardit.

Ravimid, annused ja manustamisviisid

BAM-i kombinatsioon toodeti Wildlife Pharmaceuticals South Africa (Pty) Ltd tehases. Iga milliliiter sisaldas 30 mg butorfanooli, 12 mg asaperooni ja 12 mg medetomidiini. Individuaalsed annused lõvide jaoks arvutati välja varem avaldatud butorfanooli ja medetomidiini kasutamise uuringute andmete põhjal. Blesbokkide annuste arvutamise aluseks olid varasemad uuringud medetomidiini kasutamise kohta mäletsejalistel. Gepardite annuste arvutamisel võeti arvesse lõvide uuringu tulemusi.

Ravimit manustati distantsilt 3–21 m, kasutades kolme tüüpi anestesioloogilisi õhupüsse: Pneu-Dart Model 389 lõvidele, Pneu-Dart X-Caliber

blesbokkidele ning Dan-Injecti õhupüstol geparditele. Iga õhupüssi jaoks kasutati vastava süsteemi 1–3 ml mahuga lendsüstlaid. Protseduuri lõpus äratati loomad antagonistide abil üles. Butorfanooli vastandtoime-ainena kasutati naltreksooni. Medetomidiini toime lõpetamiseks kasutati atipamesooli (7 lõvi, kõik blesbokid ja gepardid) või johimbiini (13 lõvi). Atipamesooli manustati medetomidiini viiekordses annuses. Johimbiini annus oli 0,2 mg/kg. Kõik ravimid süstiti lihasesisesi.

Immobilisatsioon ja menetlused loomadega

Registreeriti kaks induktsioonistaadiumit: 1. staadium – aeg preparaadi manustamisest rahustamise esimeste tunnusteni; 2. staadium – aeg preparaadi manustamisest kuni looma lamama jäämiseni külili või rinnakul. Lõvide ja gepardite puhul anti inimese ohutuse tagamiseks viis lisaminutit, enne kui loomaarst lähenes.

Pärast reflekside kontrolli kaeti looma silmad kaitsemaskiga, paigaldati kanderaamile ja viidi aedikust välja. Lõvid ja gepardid olid anesteesia ajal külili. Blesbokkidele lähenes inimene kaks minutit pärast looma lamama jäämist. Loomad viidi aedikust välja ja tõsteti lauale rinnakule. Protseduuri ajaks intubeeriti kõik loomad.

Anesteesia jälgimine

Kõikide loomaliikide puhul järgiti standardset anesteesia jälgimise protokolli. Südame löögisagedust, kehatemperatuuri, vererõhku ja hemoglobiini hapnikuga küllastatust registreeriti iga 5 minuti järel, alustades 15–20 minutit pärast preparaadi manustamist. Anesteesia jälgimiseks kasutati veterinaarset monitori. Südame ja kopsude auskultatsiooniks ehk kuulatluseks kasutati stetoskoopi. Rinnakorvi liikumist jälgides mõõdeti hingamissagedust. Lõvidel ja blesbokkidel registreeriti väljahingatava õhu süsinikdioksiidisisaldus kapnograafia abil. Loomad hingasid atmosfääriõhku, lisahapnikku neile ei manustatud.

Veregaaside mõõtmine

Hingamiskvaliteedi hindamiseks mõõdeti arteriaalse vere gaaside sisaldust kõikidel loomadel. Protseduuri ajal võeti kolm kuni viis arteriaalse vere anaeroobset proovi, arterit punkteeriti hepariniseeritud

süstlaga. Lõvidel ja geparditel kasutati punkteerimiseks reie- või sabaarterit ning blesbokkidel kõrvaarterit. Proove analüüsiti kohe kaasaskantava veregaaside analüsaatoriga.

Ärkamine

Protseduuride keskmine kestus oli lõvidel 70 minutit, blesbokkidel 50 minutit ja geparditel 40 minutit. Anesteesia lõpuks olid kõik loomad kaalutud, ekstubeeritud ja viidud ärkamiseks tagasi aedikusse. Ärkamiseks asetati lõvid ja gepardid külili, blesbokid rinnakule. Kõik antidoodid manustati lihasesiseselt reiepiirkonda. Registreeriti järgmised ärkamisstaadiumid: aeg süstimisest esimeste ärkamistunnuste avaldumiseni, aeg pea tõstmiseni, aeg jalgadele tõusmiseni ja aeg jalgadel liikumiseni.

Statistiline analüüs

Ravimiannuse toime selgitamiseks südame löögisagedusele, hingamissagedusele, vererõhule, hemoglobiini hapnikuga küllastatusele ja veregaasidele arvutati kõveraalune pindala (*area under the curve*, AUC) iga immobiliseerimisperioodi jaoks. Keskmist AUC-d kasutati lineaarse regressiooni mudelites sõltuva muutujana. Statistiliseks analüüsiks kasutati programmi STATA 14.0.

Uurimistulemused

BAM-i annused lõvi, blesboki ja gepardi immobiliseerimiseks

Lõvide immobiliseerimiseks kasutatud BAM-i annus oli 0,006 ml/kg ehk 0,6 ml 100 kg kohta. Blesbokkide uuringus ei olnud lõvidel kasutatud ravimiannus antiloopide immobiliseerimiseks piisav ning BAM-i annust suurendati 0,017 ml/kg. Sellest järeldub, et blesboki immobiliseerimiseks läheb vaja üle kahe korra suuremat annust kui lõvide jaoks. Kõik täiskasvanud gepardid vaatamata soole said ühesuguse ravimiannuse – 0,5 ml looma kohta.

Anesteesia induktsioon

Kõik loomad jäid pärast esimest ravimiannuse manustamist sügavasse anesteesiasse. Preparaadi lisamanustamise vajadust ei tekkinud. Anesteesia esimesed tunnused ilmnesid 1–5 minutit pärast manustamist. Kiskjaliste puhul olid esimesed tunnused lahtine suu, ebakindel kõnnak ja pea alla laskmine; blesbokkide esimesed tunnused olid rippuvad kõrvad, jäsemete lai asetus ja ebakindel kõnnak. Kõige lühem keskmine aeg lamama jäämiseks oli geparditel (4 minutit), lõvidel kestis see 7 minutit ja kõige pikem induktsioon (9,6 minutit) oli blesbokkidel. Anesteesia induktsiooniaja kiirendamiseks blesbokkidel lisati seitsmele loomale algkombinatsiooni ka hüaluronidaasi. See kiirendas märkimisväärselt induktsiooni 9,6 minutilt 5,1 minutile. Hüaluronidaasi ensümaatiline aktiivsus lagundab lihastevahelises sidekoes hüaluroonhapet, kiirendades sellega preparaatide imendumist veresoontesse.

Anesteesia induktsioon toimus rahulikult, erutust ei tuvastatud. Immobilisatsioon oli stabiilne, lihaste lõõgastumine heal tasemel, järske ärkamisi ei olnud.

Füsioloogilised näitajad

Südame löögisagedus oli kõigil kolmel liigil kogu protseduuri vältel stabiilne. Lõvidel ja blesbokkidel registreeriti kerge bradükardia ehk südametegevuse aeglustumine (<50 lööki minutis) kogu protseduuri ajal, geparditel aga esimese 20 minuti jooksul. Rütmihäireid ei tuvastatud. Arteriaalne vererõhk oli kõrge kõigil loomaliikidel, samas kõige kõrgemad näitajad olid geparditel (keskmine rõhk 167 mm Hg). Protseduuri jooksul vererõhk langes, kuid jäi kõrgeks isegi 55–60 minuti pärast. Kõrget vererõhku ja reflektoorset bradükardiat võib seletada medetomidiini põhjustatud vasokonstriktsiooni ehk sooneahenemisega. Hingamine oli lõvidel ja geparditel sarnane. Üldine hingamissagedus oli 15–20 korda minutis ja jäi kogu protseduuri vältel stabiilseks. Hingamisseiskust ei täheldatud, kuid viiel gepardil esines sisse- ja väljahingamise vahel pause. Seitsmel gepardil täheldati suu limaskesta sinakat värvust, kuigi hingamine oli sel ajal sügav ja regulaarne. Seda saab samuti seletada medetomidiini toimega veresoontele. Võrreldes kiskjalistega oli blesbokkidel kõrge hingamissagedus – 30–46 korda minutis. Hingamine oli stabiilne ja regulaarne, kuid pigem pealispindne. Kõik loomad hingasid atmosfääriõhku. Hemoglobiini hapnikuga küllastatus oli protseduuri

alguses suhteliselt väike, mis viitab hapnikuvaegusele ehk hüpoksiale. Kõigil kolmel liigil suurenes see näitaja aja jooksul. Lõvide ja blesbokkide kapnograafianäitajad olid normi piires. Kõrge õhutemperatuuri vältimiseks immobiliseeriti praktiliselt kõik loomad varahommikul või õhtul. Õhutemperatuur jäi sel ajal vahemikku 12–30 ºC. Kaks lõvi immobiliseeriti hommikul väga vara, kui õhutemperatuur oli 4–7 ºC, ning neli gepardit õhutemperatuuril 38 ºC. Blesbokke ei immobiliseeritud, kui õhutemperatuur oli üle 25 ºC. Kehatemperatuur tõusis 14 lõvil, kuid hakkas langema 50 minutit pärast protseduuri algust. Blesbokkide ja gepardite kehatemperatuur tõusis kogu protseduuri vältel, kuid jäi normi piiridesse. Hüpertermia (>39 ºC) registreeriti kahel lõvil. Hüpotermia (<37 ºC) tekkis varahommikul immobiliseeritud lõvil ja gepardil.

Veregaasid

Enamikul loomadel täheldati kogu protseduuri vältel kerget hüpokseemiat ehk vere hapnikuvaegust (PaO_2 60–80 mm Hg). Protseduuri alguses registreeriti kahel gepardil tõsine hüpokseemia (50–59 mm Hg). Selle vältimiseks on soovitatav manustada anesteesia ajal lisahapnikku. Laktaadisisaldus vähenes ühtlaselt kõikidel loomadel. Hüaluronidaasi saanud blesbokkidel vähenes laktaadisisaldus kiiremini (p=0,01). Lõvidel ja geparditel alanes vere pH väärtus (vastavalt p<0,001 ja p=0,023) ning blesbokkidel see püsis stabiilsena.

Ärkamine

Pärast antagonistide manustamist ärkasid loomad rahulikult ilma düsfooriata. Lõvide uuringus kasutati kahte antagonistide kombinatsiooni. Ärkamine oli märkimisväärselt aeglasem johimbiini ja naltreksooni kasutamise korral (22±7 minutit) kui atipamesooli ja naltreksooni kasutamise korral (9±1 minutit) (p<0,001). Kolmteist lõvi, kes said johimbiini ja naltreksooni kombinatsiooni, olid ärkamisel väga uimased. Need loomad, kellele manustati atipamesooli ja naltreksooni, ärkasid täielikult, nähtava uimasuse tunnusteta. Kõige kiiremini ärkasid blesbokid (4,8±0,7 minutit).

Järeldused ja soovitused

1. Fikseeritud annuses BAM kombinatsiooni võib edukalt ja ohutult kasutada Aafrika lõvide, blesbokkide ja gepardite

immobiliseerimiseks. Anesteesia induktsioon toimus sujuvalt ja rahulikult. Protseduuride vältel olid kõik füsioloogilised näitajad stabiilsed. Kõikidel loomadel täheldati südametegevuse aeglustumist ja vererõhu tõusu. Kuigi hingamine oli stabiilne ja regulaarne, täheldati loomadel vere hapnikuvaegust. Seetõttu on lisahapniku manustamine iga protseduuri ajal põhjendatud ja soovitatav. Mitte ühelgi loomal ei registreeritud hingamisseiskust ega enneaegset järsku ärkamist. Ärkamine oli rahulik (**I, II, III**).

2. Hüaluronidaasi manustamine koos BAM-i kombinatsiooniga ühes lendsüstlas kiirendab anesteesia induktsiooni. Hüaluronidaasi kasutamine on soovitatav koos BAM-iga loomade aktiivse tagaajamise korral (**II**).

3. Antagonistide valik mõjutas ärkamise kiirust ja kvaliteeti. Atipamesooli ja naltreksooni manustamine kutsus esile kiirema ning täielikuma ärkamise uimasuse tunnusteta. Johimbiin ja naltreksoon äratasid looma aeglaselt ning loomad olid ärgates uimased (**I**).

4. Uuringute tulemuste põhjal võib järeldada, et BAM-i fikseeritud annuste kombinatsioon tagab pööratava anesteesia, mis on suhteliselt ohutu nii loomale kui ka temaga töötavale inimesele. Me soovitame kasutada BAM-i järgmistes kogustes: 0,6 ml/100 kg Aafrika lõvide jaoks ning 0,1 ml/10 kg väiksemate Aafrika ulukkaslaste jaoks (gepard, leopard ja serval). Keskmise suurusega antiloopide jaoks soovitame kasutada doosi 0,15–0,2 ml/10 kg. Antiloopide aktiivsel tagaajamisel soovitame anesteesia induktsiooniaja lühendamiseks lisada lendsüstlasse 8000 IU hüaluronidaasi. Inimese ohutuse tagamiseks soovitame enne loomale lähenemist oodata viis lisaminutit pärast looma lamama jäämist. Tüsistuste tekkimiste vältimiseks tuleb anesteesiat jälgida kogu protseduuri vältel ning hüpokseemia vältimiseks toetada hingamist lisahapnikuga. Loomade äratamiseks soovitame kasutada atipamesooli ja naltreksooni kombinatsiooni.

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I

ORIGINAL PUBLICATIONS

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RESEARCH PAPER

Evaluation of BAM (butorphanol–azaperone–medetomidine) in captive African lion (*Panthera leo*) immobilization

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Abstract

Objective The combination of butorphanol, azaperone and medetomidine (BAM) with subsequent antagonism by naltrexone–yohimbine or naltrexone–atipamezole was evaluated for reversible immobilization of captive African lions (*Panthea leo*).

Study design Prospective, clinical trial.

Animals Twenty lions, 11 males and nine females, weighing 38–284 kg were immobilized in South Africa.

Methods The BAM volume dose rate administered was 0.005–0.008 mL kg^{-1} (0.6 mL 100 kg^{-1}). Physiologic variables were recorded every 5 minutes. Four arterial blood samples were collected from all animals at 20, 30, 40 and 50 minutes after immobilization for analysis of blood-gases and acid-base status.

Results The actual doses administered were as follows: butorphanol, 0.18 ± 0.03 mg kg^{-1}; azaperone, 0.07 ± 0.01 mg kg^{-1}; and medetomidine, 0.07 ± 0.01 mg kg^{-1}. The inductions were calm and smooth, and induction time ranged from 4 to 10 minutes (7 ± 2 minutes). The amount of time needed to work with each lion was 70 minutes, and no additional drug doses were needed. Heart rate (40 ± 8 beats $minute^{-1}$) and respiratory frequency (15 ± 4 breaths $minute^{-1}$) were stable throughout immobilization. The mean arterial blood pressure of all animals was stable but elevated (142 ± 16 mmHg). The rectal temperature slightly increased over time but remained within acceptable range. The recovery time was significantly shorter when using naltrexone and atipamezole (9 ± 1 minutes) compared to using naltrexone and yohimbine (22 ± 7 minutes).

Conclusion and clinical relevance The BAM combination proved to be reliable for general veterinary anaesthesia in lions. During anaesthesia, minor veterinary procedures such a blood collection, intubation, vaccination and collaring could safely be performed with no additional dosing required.

Keywords azaperone, BAM, butorphanol, lion, medetomidine.

Introduction

African lions (*Panthera leo*) are often immobilized for routine procedures such as microchipping, collaring, disease prevention and medical treatment. These immobilizations require the use of drugs that are safe, not only for the animals but also for the people working with them (i.e., induce a deep plane of anaesthesia) since lions are notoriously aggressive and dangerous.

Traditionally, dissociative anaesthetics (ketamine and tiletamine) combined with relatively small concentrations of sedative and tranquilizing medications

(xylazine, medetomidine, detomidine) are applied for the anaesthesia of lions (Fahlman et al. 2005; Jacquier et al. 2006; Fyumagwa et al. 2012). Combinations of ketamine–xylazine and tiletamine–zolazepam–medetomidine are considered to be the most suitable combinations (Herbst et al. 1985; Fahlman et al. 2005; Jacquier et al. 2006). The application of all the above-mentioned combinations has both advantages and disadvantages (Herbst et al. 1985; Tomizawa et al. 1997; Fahlman et al. 2005; Jacquier et al. 2006; Fyumagwa et al. 2012; Kreeger & Arnemo 2012). More recently, a combination of butorphanol, medetomidine and midazolam (BMM) has been successfully used in free-ranging lions for a period of 45 minutes (Wenger et al. 2010).

BAM (the combination of butorphanol–azaperone–medetomidine), as described in this article, is a dry mixture, containing 300 mg of butorphanol tartrate, 120 mg of azaperone tartrate and 120 mg of medetomidine hydrochloride. The use of this combination has been reported in species such as white-tailed deer (*Odocoileus virginianus*) (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009), rocky mountain elk (*Cervus elaphus nelsoni*) (Wolfe et al. 2014), Nubian ibex (*Capra nubiana*) (Lapid & Shilo-Benjamini 2015), bighorn sheep (*Ovis canadensis*) (Smith et al. 2014) and black bear (*Ursus americanus*) (Wolfe et al. 2008). It has been reported to produce reversible anaesthesia without hyperthermia and good analgesia. In addition, it can be delivered with a low-volume dart (Lance 2008). One disadvantage noted in cervids is that this combination may result in significant hypoxia, and oxygen supplementation is recommended (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009).

To our knowledge, this is the first time that this combination has been used as a ready-made medication for the immobilization of a felid species, specifically lions. The aims of this study were to acquire comprehensive and reliable monitoring data as well as develop, explore and describe the use of BAM for the immobilization of captive African lions.

Materials and methods

Animals, medications and delivery methods

Twenty lions (11 males and nine females) were immobilized with BAM on the Lechwe Lodge private game farm in the Free State province (August 2014; 13 lions) and at Mohololo Wildlife Rehabilitation Center in the Limpopo province (September 2015; seven lions) in the Republic of South Africa. The animals were immobilized for clinical examination, Global Positioning System (GPS) collaring, deworming, contraceptive implantation and genetic material collection.

The BAM solution (BAM; Wildlife Pharmaceuticals South Africa (Pty) Ltd., South Africa) was prepared by dissolving one vial containing 300 mg of butorphanol tartrate, 120 mg of azaperone tartrate and 120 mg of medetomidine hydrochloride in 10 mL sterile water for injection (Pharma-Q water for injection; Pharma-Q, South Africa). Each millilitre of the solution contained 30 mg of butorphanol, 12 mg of azaperone and 12 mg of medetomidine. The individual doses for the combination were calculated based on commonly accepted recommendations for use of the above-mentioned active agents as well as the data acquired during previous immobilizations of lions conducted by the authors. The body weight of the animals was estimated based on visual parameters.

All the animals were immobilized between 6:00–13:00 and 15:00–17:00 hours in order to avoid high, midday environmental temperatures. The air temperature ranged from 4.0 °C to 33.4 °C. The elevation above mean sea level was 1399 m at Lechwe Lodge and about 520 m at the Mohololo Rehabilitation Center.

A cartridge fired projector (Pneu-Dart Model 389; Wildlife Pharmaceuticals (Pty) Ltd.) was used to deliver the anaesthetic. Darts (Pneu-Dart Type 'C') with a volume of 1–3 mL and a length of 19–38 mm, and 13–16 gauge needles with wire barbs were used (Wildlife Pharmaceuticals (Pty) Ltd.). Remote darting was done in enclosures from inside a vehicle or on foot from distances ranging from 3 to 21 m. Distance was measured using Leupold RX-1000i rangefinder (Leupold & Stevens, OR, USA). The injections were administered into the femoral muscles.

For reversing the effect of the medetomidine in 13 cases, a formulation of 6.25 mg mL^{-1} yohimbine hydrochloride at a dose rate of 0.2 mg kg^{-1} body weight was used. In seven cases, atipamezole (Antisedan, 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in milligrams was used. Naltrexone hydrochloride (Trexonil 50 mg mL^{-1}; Wildlife Pharmaceuticals (Pty) Ltd.) was used to reverse butorphanol at one times (mg to mg) the actual butorphanol dose. All injections were administered intramuscularly (IM).

Monitoring and manipulations of animals

Two stages of induction were timed: stage I—from the time of the darting until the first signs of sedation,

including open mouth, ataxic gait and lowering of the head; stage II—from the injection time until sternal or lateral recumbency. Once the animals reached lateral recumbency, they were blindfolded and transported from the enclosure to a controlled environment, no further than 100 m from the enclosure, where monitoring could be performed. All animals were intubated using endotracheal tubes (Jorgensen Labs, CO, USA; 16−24 mm in diameter). Every 5 minutes, beginning at 15−20 minutes after darting, monitoring of physiological parameters [heart rate (HR), respiratory frequency (f_R), oxygen saturation (SpO_2), end-tidal carbon dioxide ($P_E'CO_2$), noninvasive blood pressure and body temperature] was conducted using a veterinary monitor (Capnovet Deluxe Multiparameter Monitor; Eickemeyer, Germany). Auscultation using a stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M, MN, USA) was performed every 5 minutes for the entire period of immobilization. The level of muscle relaxation was assessed based on the general muscle tone and position of the lower jaw using a 3-point scale. Level 1 indicates the absence of muscle tone, level 2 indicates a light tone and level 3 indicates a strongly marked tone. Capillary refill time and palpebral reflex were additionally registered.

Arterial blood samples were collected from the femoral or median caudal artery at 20, 30, 40 and 50 minutes after darting. The samples were immediately analysed using a portable analyser (i-STAT1 Portable Clinical Analyzer; Abaxis, CA, USA) and cartridges (i-STAT cartridges CG4+ & CHEM8+; Abaxis). Variables measured included pH, partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chlorine, urea, creatinine, glucose and ionized calcium levels. Actual base excess, actual bicarbonate, arterial haemoglobin, oxygen saturation and haemoglobin were calculated from the measured values.

The animals were extubated 65−70 minutes after the beginning of immobilization provided a strongly marked palpebral reflex was observed. After extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co., Ltd., BC, Canada) and transported back to the enclosure by vehicle. In the enclosure, the lions were placed in the lateral position on the ground. Antagonists were then injected IM into the femoral muscle region. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking time to head lifting, time to standing and time to fully coordinated movement (i.e., full recovery).

Statistical analysis

For exploring the anaesthetic dosage effect on the lions' HR, f_R, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), SpO_2, $P_E'CO_2$, PaO_2 and $PaCO_2$, and the area under the curve (AUC) was calculated using a trapezoid method for every measurement for the immobilization period (60 minutes). Linear regression models were used with the mean AUC as the response variable. The exact anaesthetic dosage used (calculated after weighing of immobilized lions) was divided into three levels (low: 0.52−0.56 mL 100 kg^{-1}; $n = 6$, medium: 0.59−0.64 mL 100 kg^{-1}; $n = 8$; high: 0.66−0.83 mL 100 kg^{-1}; $n = 6$) as grouping variable and were included as an explanatory variable. Age was divided into two levels (subadults, $n = 9$; adults, $n = 11$) as the grouping variable and sex (males; $n = 11$ and females; $n = 9$), and their interaction with dose groups were added into every model. The model's assumptions were verified by scatter and normality plots of standardized residuals.

For comparing recovery time between different antidote groups (atipamezole; $n = 7$ and yohimbine hydrochloride; $n = 13$), a nonparametric Mann−Whitney test was used. For linear regression models and Mann−Whitney test, STATA 13.0 software (Stata Corporation, TX, USA) was used.

General linear mixed models (GLMMs) were used to explore the overall time trend in lactate, arterial blood pH and body temperature and differences in time trend between the dosage groups. Lions were included as random intercepts and polynomials of time (minutes), with interactions with the dosage group added as fixed effects in increasing order. The overall time trend differences between groups were tested using the F test. Isotropic spatial exponential covariance structure was used for modeling serial correlations of repeated measurements at the within-lion level in all models. Initially, sex and age group were included as fixed factors in all models. A backward elimination procedure was performed for the final models. The NLME package (Pinheiro J, Bates D, Debroy S, Sarkar D: Linear and nonlinear mixed effect models; R package version 3.1-73, Austria; 2006) with statistical software R 3.2.2 (R-soft. R Development Core Team R: A language and environment for statistical computing; R Foundation for Statistical Computing, Austria; 2006) was used for fitting these GLMM models.

All fitted model assumptions were verified by scatter and normality plots of standardized residuals. A p value ≤ 0.05 was considered statistically significant. Data are reported as mean ± standard deviation (range).

Results

Data from 11 male lions weighing 166 ± 78 (38–284) kg and from nine female animals weighing 116 ± 29 (72–162) kg were used in this study. In all 20 animals, immobilization occurred after a single injection of BAM. There was no need for additional injections to achieve immobilization. The following dose rates were used: BAM volume dose rate range was 0.005–0.008 mL kg^{-1} (0.006 ± 0.001 mL kg^{-1} or 0.6 mL 100 kg^{-1}). The total dose ranged from 0.3 to 1.6 mL. The actual doses were as follows: butorphanol, 0.18 ± 0.03 g kg^{-1}; azaperone, 0.07 ± 0.01 mg kg^{-1}; medetomidine, 0.07 ± 0.01 mg kg^{-1}. The first sign of induction occurred between 1 and 5 minutes after the injection (3 ± 1 minutes). The inductions were observed to be calm and smooth with no side effects. Vomiting was not observed in any of the lions. Five animals remained sleeping in sternal recumbency and never attained lateral recumbency but were immobilized; 15 lions went to lateral recumbency. The induction time ranged from 4 to 10 minutes (7 ± 2 minutes). There was no association between the variations in the BAM dose and the range of induction times recorded.

Immobilization was stable, and no sudden arousals were observed. Good muscle relaxation was evident in all cases. Low jaw muscle tone disappeared within 10–15 minutes after injection, except for one female lion whose low jaw muscle tone was still present 22 minutes after administration. Capillary refill times were less than 2 seconds, and mucous membrane colour was normal in all animals. Palpebral reflex disappeared at the 15th minute of the procedure and reappeared at the 45th–50th minute in the case of 12 animals, and at the 60th minute in the case of eight animals. In two animals, a weak palpebral reflex was registered during the entire period of immobilization. Four lions exhibited spontaneous limb twitches during the first 20 minutes of immobilization. None of the lions showed reaction to intubation, extubation or other painful procedures (e.g., blood collection). No apnoea was observed in any of the lions. One hour after the beginning of the procedure, during weighing, weak head and limb movements were observed ($n = 14$). The duration of immobilization of one lion without additional doses was a little more than 1 hour.

Table 1 presents the mean ± standard deviation and range of the main monitoring variables, and Fig. 1 presents the measured physiological

Table 1 Physiologic variables and arterial blood gases in captive African lions darted with BAM (butorphanol–azaperone–medetomidine). Average values are presented for the periods 10–20, 25–35, 40–50 and 55–60 minutes after darting and for the entire immobilization (overall). Results are presented as mean ± standard deviation and (range).

Variable	Unit	Timepoint				
		10–20	25–35	40–50	55–60	Overall
Heart rate	beats $minute^{-1}$	40 ± 8	42 ± 8	40 ± 7	42 ± 8	40 ± 8 (24–62)
Respiratory frequency	breaths $minute^{-1}$	15 ± 4	16 ± 4	16 ± 4	14 ± 3	15 ± 4 (6–30)
Rectal temperature	°C	38.0 ± 0.8	38.3 ± 0.7	38.3 ± 0.7	38.2 ± 0.8	38.2 ± 0.7 (36.6–39.5)
Systolic blood pressure	mmHg	175 ± 19	176 ± 23	170 ± 16	168 ± 14	170 ± 20 (118–236)
Diastolic blood pressure	mmHg	133 ± 11	130 ± 14	129 ± 12	129 ± 10	131 ± 16 (79–177)
Mean arterial pressure	mmHg	148 ± 14	143 ± 16	142 ± 11	137 ± 11	142 ± 16 (104–193)
SpO_2	%	86 ± 5	87 ± 5	89 ± 4	92 ± 4	88 ± 6 (72–100)
SaO_2	%	90 ± 4	92 ± 4	94 ± 4	94 ± 4	91 ± 5 (85–97)
PaO_2	mmHg	77 ± 4	76 ± 4	81 ± 5	82 ± 4	80 ± 4 (70–89)
	kPa	10.3 ± 0.5	10.1 ± 0.5	10.8 ± 0.7	10.9 ± 0.5	10.7 ± 0.5 (9.3–11.9)
$P_{E'}CO_2$	mmHg	39 ± 8	42 ± 7	41 ± 8	42 ± 6	41 ± 8 (18–59)
$PaCO_2$	mmHg	32.6 ± 5.7	30.1 ± 3.5	31.4 ± 3.5	32.1 ± 3.3	31.2 ± 3.4 (22.2–39.9)
	kPa	4.3 ± 0.8	4.0 ± 0.47	4.2 ± 0.5	4.3 ± 0.4	4.2 ± 0.5 (3.0–5.3)
pH		7.34 ± 0.03	7.33 ± 0.03	7.32 ± 0.03	7.32 ± 0.03	7.34 ± 0.03 (7.28–7.41)
Lactate	mmoL L^{-1}	0.65 ± 0.54	0.55 ± 0.32	0.52 ± 0.22	0.44 ± 0.16	0.54 ± 0.27 (0.30–2.64)

SpO_2, haemoglobin oxygen saturation measured by pulse oximetry; SaO_2, arterial haemoglobin saturation (calculated value, temperature corrected); PaO_2, partial pressure of arterial oxygen (measured value, temperature corrected); $P_{E'}CO_2$, end-tidal carbon dioxide measured by capnography; $PaCO_2$, partial pressure of arterial carbon dioxide (measured value, temperature corrected); pH corrected to rectal temperature.

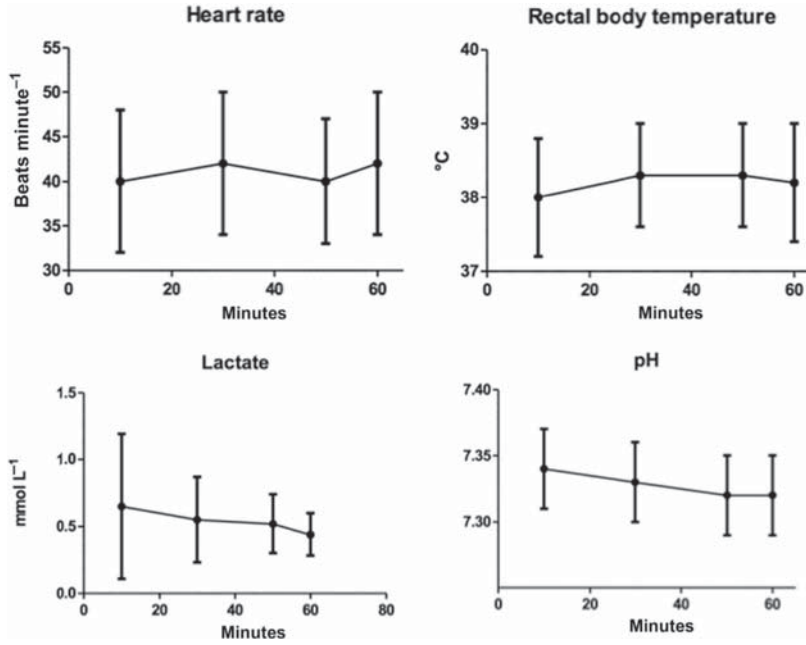

Figure 1 The dynamics of heart rate, rectal body temperature, arterial blood pH and lactate (mean ± standard deviation) during immobilization of African lions (10–60 minutes after darting) with a combination of butorphanol, azaperone, medetomidine (BAM).

parameters during chemical restraint. Mean blood pH level ($p < 0.001$) and lactate levels ($p < 0.001$) steadily declined in all animals (Fig. 1). The administered dose (low: 0.52–0.56 mL 100 kg^{-1}, $n = 6$; medium: 0.59–0.64 mL 100 kg^{-1}, $n = 8$; high: 0.66–0.83 mL 100 kg^{-1}, $n = 6$) did not influence any of the physiological parameters tested.

There was a significant difference in recovery time after the administration of naltrexone and yohimbine [22 ± 7 (7–34) minutes; $n = 13$] *versus* naltrexone and atipamezole [9 ± 1 (8–1) minutes; $n = 7$] ($p < 0.001$).

Discussion

The present study indicates that BAM is an efficient immobilization protocol for lions. The results show that induction time is similar to the BMM combination (Wenger et al. 2010), slightly longer than the tiletamine–zolazepam–medetomidine combination (Fahlman et al. 2005), but considerably shorter than the ketamine–xylazine combination (Stander & Morkel 1991). The therapeutic dose rate of BAM solution is wide and assures a high degree of reliability when using BAM under field conditions. Based on the present study, the recommended dose of BAM for healthy lions is 0.6 mL 100 kg^{-1}. According to body weight, we recommend a total dose of 0.7–0.8 mL BAM for immobilization of an adult female or subadult male lion and a total dose of 1.0–1.2 mL for an adult male lion. The total volume of the drug is lower than the total volume of other combinations (Fahlman et al. 2005; Stander & Morkel 1991), which allows for the use of BAM with all types of remote delivery systems.

Only a few studies have reported on physiological parameters in detail in immobilized lions. The physiological parameters recorded during the present study were stable, and the cardiovascular parameters were within acceptable limits. A slight but stable bradycardia (defined as <50 beats minute^{-1}) was observed in all lions immobilized with BAM (Table 1). The HR was slightly lower than those reported in other studies using

tiletamine–zolazepam–medetomidine (Fahlman et al. 2005; Jacquier et al. 2006), BMM (Wenger et al. 2010) or ketamine and xylazine (Larsson et al. 2008). The HR was very similar to those in black bears immobilized with BAM (Wolf et al. 2008). The impact of dosage on HR and blood pressure does not seem to depend on the sex, body weight or the age of the animals. It can therefore be assumed that the bradycardia observed was because of the specific effect of medetomidine on peripheral α_2 adrenoreceptors resulting in an increase of systemic vascular resistant (Sinclair 2003). Rectal temperature initially increased in 14 lions, and this is similar to observations in other studies using alpha-2-adrenoreceptor agonists (Fahlman et al. 2005; Jaquier et al. 2006; Wenger et al. 2010). Hyperthermia may have been caused by high ambient temperatures (19–38 °C) and interference with normal thermoregulatory mechanisms by alpha-2-adrenoreceptor agonists or opioids (Wenger et al. 2010). Arterial blood variables revealed the presence of mild metabolic acidosis during chemical restraint. Values for PaO_2, arterial haemoglobin saturation (SaO_2), $PaCO_2$, pH and lactate remained within reference ranges reported for domestic cats (King 2004). The PaO_2 values were low and similar to values observed in lions immobilized with different drug combinations without additional oxygen supplementation (Fahlman et al. 2005; Wenger et al. 2010). The mean $PaCO_2$ in this study was 31.2 mmHg (4.16 kPa), which is within the normal range reported for domestic cats, indicating adequate ventilation (King 2004). It was also similar to $PaCO_2$ values observed in lions immobilized with a BMM combination (Wenger et al. 2010). The dose rate (low, medium or high) had no influence on heart rate, blood pressure or respiration parameters. This assures a high degree of reliability when practically applying the medication in field.

Using different reversal drugs clearly influences the recovery time and quality of recovery. The recovery time was significantly shorter when using naltrexone and atipamezole compared to when using naltrexone and yohimbine. Recovery with the naltrexone–atipamezole combination was smooth and occurred within 9 minutes. Animals showed signs of slight ataxia once standing during initial recovery. Recovery with the naltrexone–yohimbine combination was longer (22 ± 7 minutes) but still faster compared to the reported cases where a tiletamine–zolazepam–medetomidine combination was used in lions (mean time 33 minutes) (Fahlman et al. 2005). Thirteen lions reversed with yohimbine were severely ataxic, which may be explained by the incomplete reversal of the medetomidine by yohimbine.

In conclusion, the BAM combination at the doses used in this study proved to be a reliable immobilization protocol for lions. The advantages of BAM include a small drug volume for darting, calm and smooth induction, long duration of immobilization and ability to reverse the effects of immobilization drugs with naltrexone and atipamezole. Physiological parameters should be monitored throughout chemical restraint, and additional oxygen supplementation may be necessary.

Authors' contributions

Conception and design of the study, or acquisition of data, or interpretation of data: AS, VA, JPR, TO, DV, LL, SP; drafting of article or revising it critically: AS, VA, JPR, TO, DV, LL, SP; final approval of manuscript: AS, VA, JPR, TO, DV, LL, SP.

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Conflict of interest statement

Dr JP Raath is the owner of Wildlife Pharmaceuticals South Africa.

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II

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RESEARCH PAPER

Evaluation of butorphanol–azaperone–medetomidine (BAM) in captive blesbok immobilization (*Damaliscus pygargus phillipsi*)

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Abstract

Objective The fixed-dose combination of butorphanol, azaperone and medetomidine (BAM; 30, 12 and 12 mg mL^{-1}, respectively) with subsequent antagonism by naltrexone–atipamezole was evaluated for reversible immobilization of captive blesbok (*Damaliscus pygargus phillipsi*).

Study design Prospective, clinical trial.

Animals Sixteen blesbok (four males and twelve females), weighing 52.5–71.0 kg, were immobilized in South Africa.

Methods The total dose of BAM ranged from 0.5 to 0.7 mL for females and 0.7 to 0.9 mL for males. In seven animals chosen randomly, 8000 units of hyaluronidase was added to the dart. Physiologic variables were recorded every 5 minutes beginning at 10–20 minutes after darting. Arterial blood samples were collected three times at 20, 30 and 40 minutes after darting for analysis of blood acid-base status.

Results The mean administered doses of BAM were as follows: butorphanol (0.34 ± 0.08 mg kg^{-1}), azaperone (0.14 ± 0.03 mg kg^{-1}) and medetomidine (0.14 ± 0.03 mg kg^{-1}). The inductions were calm and smooth. The mean induction time was 9.6 ± 3.2 minutes with just BAM and 5.1 ± 0.8 minutes with BAM and hyaluronidase combination. Heart rate (45 ± 6 beats $minute^{-1}$) and respiratory frequency (38 ± 4 breaths $minute^{-1}$) were stable throughout immobilization. The mean arterial blood pressure for all animals was stable but elevated (137 ± 7 mmHg). Rectal temperature slightly increased over time but remained within an acceptable range. The recovery time after administering naltrexone and atipamezole was 4.8 ± 0.7 minutes.

Conclusion and clinical relevance The BAM combination proved to be reliable and effective in blesbok.

Keywords azaperone, BAM, blesbok, butorphanol, medetomidine.

Introduction

Blesbok (*Damaliscus pygargus phillipsi*) are gregarious medium-sized antelope that prefer the open grassland habitat of southern Africa. Ganhao et al. (1988) investigated the physiological responses of blesbok, eland (*Taurotragus oryx*) and red hartebeest (*Alcelaphus buselaphus*) to different capture methods, namely net capture, enclosure capture and chemical immobilization, and found that chemical immobilization elicited the lowest stress response. Chemical immobilization has become an essential part of research, in the treatment of sick or injured animals and during capture operations.

Etorphine is a widely used opioid for the chemical immobilization of blesbok (Williams & Riedesel 1987; Burroughs 1993; Kock & Burroughs 2012). Thiafentanil can also be used, and some users claim that a

mixture of etorphine and thiafentanil provides better induction than etorphine alone (Kock & Burroughs 2012). A number of sedatives and tranquilizers can also be included in the immobilizing mixture (Kock & Burroughs 2012).

One of the biggest problems with the use of powerful opioids in chemical immobilization mixtures is that they need to be highly controlled in terms of their handling, storage and record-keeping. Furthermore, these substances are not always easily accessible. Beyond these practical considerations, opioids such as thiafentanil have also been reported to be associated with hyperthermia, respiratory depression, poor muscle relaxation and capture myopathy (Mich et al. 2008).

The use of butorphanol, azaperone and medetomidine as a sedative combination provides a potentially useful alternative (Wolfe et al. 2008). Butorphanol is a synthetic opioid analgesic agent (partial agonist–antagonist), three to five times more potent than morphine. It can be combined with alpha$_2$-adrenergic agonists to produce profound sedation or light general anaesthesia (Neiffer et al. 2005). Azaperone is a short-acting neuroleptic sedative belonging to the class of butyrophenones that is often used in combination with opioids and alpha$_2$-agonists to reduce the stress from capture and handling (Kock & Burroughs 2012). Medetomidine is a potent alpha$_2$-agonist with sedative and analgesic properties that, in combination with butorphanol, provides smooth induction and good muscle relaxation. The combination of these three agents has been reported to provide safe and reversible immobilization in white-tailed deer (*Odocoileus virginianus*) (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009), rocky mountain elk (*Cervus elaphus nelsoni*) (Wolfe et al. 2014), Nubian ibex (*Capra nubiana*) (Lapid & Shilo-Benjamini 2015), black bears (*Ursus americanus*) (Wolfe et al. 2008) and African lions (Semjonov et al. 2017). Hyaluronidase is proteolytic enzyme. The effect of hyaluronidase is via enzymatic breakdown of the interstitial barrier between cells which in turn breaks down the intercellular matrix (responsible for tissue integrity) and allows drugs to reach the central compartment much faster. As a result, the rate of drug absorption is enhanced, thereby accelerating immobilization (Watson 1993; Schulenburg et al. 2007; Dittberner 2011).

The aims of this study were to evaluate the effectiveness and physiological responses of captive blesbok to BAM administered intramuscularly (IM) with and without hyaluronidase.

Material and methods

Sixteen blesbok (four males and twelve females) that required clinical examination, deworming, blood collection and genetic material collection were recruited for this study. They were housed together in enclosures on the Ngongoni private game farm at an altitude of 900 m above sea level in Mpumalanga, South Africa, and were immobilized in September 2015.

The butorphanol–azaperone–medetomideine fixed-dose combination (BAM), as used in this study, was produced by Wildlife Pharmaceuticals South Africa (Pty) Ltd. Each animal was darted with BAM. The individual dose was estimated based on animal size, and small, medium and large females were administered 0.5, 0.6 and 0.7 mL, and small, medium and large males 0.7, 0.8 and 0.9 mL, respectively. Each millilitre of the solution contained 30 mg butorphanol, 12 mg azaperone and 12 mg medetomidine. In seven randomly chosen animals of both sexes, 8000 units of hyaluronidase (Hyaluronidase Type I-S from Bovine Teste; Sigma-Aldrich, MO, USA) was added to the dart. All animals were darted between 5:00 and 12:00 or 15:00 and 17:00 hours to avoid the high, midday environmental temperatures.

A gas-powered dart gun Pneu-Dart X-Caliber (Pneu-Dart Inc., PA, USA) was used to deliver the drugs. Darts with a 2 mL capacity combined with a 19 mm long, 14 gauge needle with wire barb (Wildlife Pharmaceuticals (Pty) Ltd., South Africa) were used. Remote darting was performed in a 6 × 8 m enclosure from an upper deck of the wall at distances ranging from 5 to 12 m. All injections were administered into the femoral muscles.

To antagonize the effect of the medetomidine and butorphanol, atipamezole (Antisedan 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in milligrams and naltrexone hydrochloride (Trexonil 50 mg mL^{-1}; Wildlife Pharmaceuticals (Pty) Ltd., South Africa) at one time (mg to mg), the actual butorphanol dose was administered to reverse medetomidine and butorphanol, respectively. All injections were administered IM.

Monitoring and manipulations of animals

Two stages of induction were timed: stage I – from time of the darting until the first signs of sedation,

including ears hanging down, wide stance of the thoracic and pelvic limbs and ataxia; stage II — from the injection time until sternal recumbency. Once the animals reached recumbency, an additional 2 minutes were waited before animals were approached and blindfolded. If the animal went into lateral recumbency, it was placed in sternal recumbency immediately after approaching. Animals were placed on a stretcher, carried from the enclosure and transported to a shaded area around 100 m away. The blesbok was then placed on a table in sternal position with the head fixed in a lifted position. All animals' tracheas were intubated using endotracheal tubes 10 mm in diameter. Every 5 minutes, beginning at 10—20 minutes after darting, physiological parameters were measured. A veterinary monitor, the Capnovet Deluxe Multiparameter Monitor (Eickemeyer, Germany), was used to register heart rate (HR) and respiratory frequency (f_R), oxygen saturation (SpO_2), end-tidal carbon dioxide ($P_E'CO_2$), noninvasive arterial blood pressure [systolic (SBP), diastolic (DBP) and mean (MBP)] and body temperature. The pulse oximeter transducer was fixed on the tongue of the animal. The capnograph transducer was attached to the endotracheal tube. The temperature transducer was inserted into the rectum. The noninvasive blood pressure (NIBP) measuring cuff (Criticon Soft-Cuf nr 5, 8 × 15 cm; GE Healthcare, NY, USA) was placed on the thoracic limb. Auscultation with a stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M, MN, USA) was performed every 5 minutes for the entire duration of anaesthesia. The level of muscle relaxation was subjectively assessed based on the general muscle tone on a 3-point scale: level 1 — the absence of muscle tone; level 2 — a light tone; and level 3 — a marked tone. Capillary refill time was evaluated on the mucus membranes of the maxilla. Either the presence or absence of the palpebral reflex was additionally registered. Animals were observed for rumen tympani throughout the study period.

Three arterial blood samples were collected from each blesbok at 20, 30 and 40 minutes after darting using the auricular artery. The puncture was performed anaerobically using a heparinized syringe and a 21 gauge needle. Blood sample analysis was conducted immediately using a portable analyzer (i-STAT 1 Portable Clinical Analyzer; Abaxis, CA, USA) and cartridges (i-STAT cartridges CG4+, CHEM8+; Abaxis). Variables measured from arterial blood included pH, partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chloride, urea, creatinine, glucose and ionized calcium levels. Actual base excess, actual bicarbonate, oxygen saturation and haemoglobin were calculated automatically from the measured values by the portable analyzer.

The duration of immobilization was 50 minutes. The animals were extubated 40 minutes after the beginning of anaesthesia. After extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd., BC, Canada) to measure their body mass, and transported back to the enclosure. In the enclosure, the blesbok were placed in sternal position on the ground. Antagonists were then injected IM into the femoral muscle region. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking, time to head lifting and time to standing.

Statistical analysis

For the analysis of anaesthetic dosage effects and the effect of hyaluronidase addition on the blesboks' HR, f_R, SBP, DBP, MBP, SpO_2, $P_E'CO_2$, PaO_2 and $PaCO_2$, the area under the curve (AUC) was calculated using a trapezoid method for every measurement during the immobilization period (50 minutes). The mean AUCs were used as response variables in linear regression models. The exact anaesthetic dosage (calculated after weighting of immobilized blesbok) and body weight were used as continuous explanatory variables. Hyaluronidase (yes, $n = 7$; no, $n = 9$) and sex (male, $n = 4$; female, $n = 12$) were added into every model as two-level categorical variables.

For the analysis of the effect of hyaluronidase and BAM on induction (time to recumbency) and recovery, time linear regression models were used. Inverse transformation from induction time (to achieve normal distribution of model residuals) was used.

Linear mixed models were used to explore the overall time trend in lactate, arterial blood pH and body temperature and differences in time trend between the hyaluronidase administration groups. Blesbok were included as random intercepts and polynomials of time (minutes), with interactions with the hyaluronidase group added as fixed effects in increasing order. The overall time trend differences between groups were tested with an F test. Isotropic spatial exponential covariance structure was used to model serial correlations of repeated measurements at the within-animal level in all models.

A backward elimination procedure was performed for the all final models, and biologically meaningful interactions were tested. The model's assumptions were verified by scatter and normality plots of standardized residuals. For statistical analysis, STATA 14.0 software (Stata Corporation, TX, USA) was used. A p value ≤ 0.05 was considered statistically significant. Data are presented as median (range) and mean ± standard deviation.

Results

Data from four male animals weighing 66.5 (52.5–69.3) kg and from 12 female animals weighing 60.8 (52.0–71.0) kg were used in this study. All 16 blesbok injected with BAM were successfully immobilized. There was no need for additional injections to achieve immobilization. The following dose rates were used: 0.017 ± 0.003 mL kg^{-1} or 0.17 ± 0.03 mL 10 kg^{-1}. Total dose ranged from 0.5 to 0.9 mL. The actual doses were as follows: butorphanol (0.34 ± 0.08 mg kg^{-1}), azaperone (0.14 ± 0.03 mg kg^{-1}) and medetomidine (0.14 ± 0.03 mg kg^{-1}). The inductions were calm and smooth. All animals showed first signs of sedation, including drooping ears, eyelids and heads, within 3.4 ± 0.9 minutes after drug administration. The addition of hyaluronidase to the dart decreased the time to first sign of sedation from 3.8 ± 0.9 minutes (animals immobilized with only BAM) to 2.9 ± 0.6 minutes (animals immobilized with BAM and hyaluronidase) (p = 0.001). There was also a difference in induction time between animals immobilized with pure BAM (9.6 ± 3.2 minutes) and animals immobilized with BAM and hyaluronidase (5.1 ± 0.8 minutes) (p = 0.001) (Table 1). There was no correlation between the variations in the range of induction times recorded and the BAM dose (p = 0.285) or between the induction times and the body weight of animals (p = 0.917).

The quality of immobilization was considered good based on muscle relaxation, the absence of muscle twitching and the lack of significant response to intubation, painful stimuli (drawing blood) and handling. Capillary refill time in all animals did not exceed 2 seconds.

Table 2 presents the main monitoring variables including HR, f_R, SpO_2, $P_E'CO_2$, arterial acid-base balance and ventilation parameters and rectal body temperature during chemical restraint. There were no differences between the BAM and BAM with hyaluronidase groups. All parameters were considered acceptable for this species.

The rectal body temperatures of all animals increased slightly during immobilization but remained within an acceptable range (37–40 °C). No animals demonstrated ruminal distention or excessive salivation during immobilization.

All animals showed increased respiratory rates and mild hypoxaemia (PaO_2 <80 mmHg) (Fahlman 2014). Lactate levels steadily declined in all animals during immobilization time ($p < 0.001$). In animals immobilized with BAM and hyaluronidase, the lactate declined more steadily (interaction term $p = 0.01$).

All animals typically recovered within less than 5 minutes of administration of naltrexone and atipamezole. There was no difference in recovery time between animals receiving only BAM and animals receiving BAM with hyaluronidase (p = 0.811) (Table 1). Some signs of sedation were still observed in all animals within the first 5 minutes after standing.

Discussion

The present study indicates that BAM (butorphanol–azaperone–medetomidine) is an efficient immobilization drug for blesbok. The addition of hyaluronidase to the dart mixture accelerated induction

Table 1 Mean ± standard deviation (SD) of induction and recovery times of blesbok, immobilized with BAM (butorphanol–azaperone–medetomidine) and BAM combined with hyaluronidase

	Group	
	BAM (n = 9)	**BAM and hyaluronidase (n = 7)**
Time to first sign of sedation (minutes)	3.8 ± 0.9	2.9 ± 0.7
Time to recumbency (minutes)	9.6 ± 3.2	5.1 ± 0.8
Time from injection of antidotes to first sign of recovery (minutes)	3.6 ± 0.9	3.5 ± 0.6
Time to standing (minutes)	4.8 ± 0.7	4.9 ± 0.5

Table 2 Physiologic variables and arterial blood gases in captive blesbok darted with BAM (butorphanol-azaperone-medetomidine). Mean values are presented for the periods of time 10–20, 20–30 and 30–40 minutes after darting and for the entire immobilization (overall). Results are presented as mean ± standard deviation and (range)

Variable		Timepoint (minutes)			
		10–20	20–30	30–40	Overall
HR	beats minute^{-1}	45 ± 7	44 ± 8	45 ± 7	45 ± 6 (36–55)
f_R	breaths minute^{-1}	37 ± 5	37 ± 5	39 ± 5	38 ± 4 (30–46)
T	°C	38.9 ± 0.8	39.1 ± 0.9	39.1 ± 1	39.1 ± 0.9 (37.1–40.0)
SAP	mmHg	169 ± 14	167 ± 12	161 ± 16	166 ± 11 (150–190)
DAP	mmHg	118 ± 7	118 ± 6	116 ± 7	118 ± 3 (113–123)
MAP	mmHg	140 ± 9	136 ± 10	135 ± 10	137 ± 7 (127–151)
SpO_2	%	90 ± 3	92 ± 2	93 ± 3	93 ± 2 (89–96)
PaO_2	mmHg	72 ± 3	76 ± 2	73 ± 3	72 ± 3 (68–78)
	kPa	9.6 ± 0.4	10.1 ± 0.3	9.7 ± 0.4	9.6 ± 0.4 (9.1–10.4)
$P_{E'}CO_2$	mmHg	41 ± 4	41 ± 3	41 ± 3	41 ± 4 (32–49)
$PaCO_2$	mmHg	45.0 ± 2.5	45.5 ± 1.9	46 ± 2.9	45 ± 2.5 (41–49)
	kPa	6.0 ± 0.3	6.1 ± 0.3	6.1 ± 0.4	6.0 ± 0.3 (5.5–6.5)
pH		7.42 ± 0.04	7.44 ± 0.03	7.45 ± 0.04	7.44 ± 0.04 (7.36–7.5)
Lactate	mmol L^{-1}	1.59 ± 0.96	1.32 ± 0.83	1.13 ± 0.73	1.32 ± 0.83 (0.44–3.06)

HR, heart rate; f_R, respiratory rate; T, Rectal temperature; SAP, systolic blood pressure; DAP, diastolic blood pressure; MAP, men blood pressure; SpO_2, haemoglobin oxygen saturation; PaO_2, partial pressure of oxygen; $P_{E'}CO_2$, end-tidal carbon dioxide; $PaCO_2$, partial pressure of carbon dioxide.

times, both in time to first sign of sedation and time to recumbency. This was expected since the addition of hyaluronidase has been shown to increase drug absorption and induction times (Bush et al. 2004; Cattet & Obbard 2010; Dittberner 2011; Dittberner et al. 2015).

All blesbok showed elevated respiratory rates and mild hypoxaemia throughout immobilization. This may be a direct effect of butorphanol. Mich et al. (2008) reported hypoxaemia in white-tailed deer (*Odocoileus virginianus*) immobilized with butorphanol, azaperone and medetomidine. The authors attributed this to increased venous admixture as a result of a lower ventilation/perfusion (V/Q) ratio and increased physiologic shunting because of both opioid and alpha$_2$-agonist administration. Wolfe et al. (2014) reported similar results in Rocky Mountain elk immobilized with butorphanol, azaperone and medetomidine and noted that hypoxaemia was most severe in animals that received high doses of the combination.

In all cases, animals were bradycardic (HR <55 beats minute^{-1}) as well as hypertensive. This is possibly resulting from medetomidine increasing initially the blood pressure as a result of peripheral vasoconstriction. The observed reflex bradycardia is therefore likely secondary to the medetomidine-induced hypertension. Although azaperone is reported to have a hypotensive effect because of a reduction in total peripheral resistance, no such effect was observed in these blesbok (Clarke 1969; Lees & Serrano 1976; Serrano & Lees 1976; Hattingh et al. 1994). This effect may be species-specific or perhaps was superseded by the hypertensive effect of medetomidine.

The main limitation of the present study was the small sample size of animals which decreases the statistical power of the study. All animals used in the study were in good condition and clinically healthy, and information about the effect of BAM on starving or injured animals is not available.

In conclusion, the BAM combination at the doses used in this study proved to be a reliable immobilization agent and an alternative for ultra-potent opioids for captive blesbok. Advantages of BAM include a small drug volume for darting, calm and smooth induction, long duration of immobilization and the ability to reverse the effects of immobilization with naltrexone and atipamezole. The addition of hyaluronidase to the dart mixture is advisable to decrease the induction times in cases of chasing wild animals. Physiological parameters should be monitored throughout chemical restraint, and oxygen supplementation may be necessary with this drug combination.

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Authors' contributions

All authors participated in the conception and design of the study, or acquisition of data, or interpretation of data as well as in drafting of the article or revising it critically. All authors approved the final version of the manuscript.

Conflict of interest statement

Dr JP Raath is the owner of Wildlife Pharmaceuticals South Africa (Pty).

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III

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SHORT COMMUNICATION

Evaluation of butorphanol-azaperone-medetomidine in captive cheetah (*Acinonyx jubatus*) immobilization

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Abstract

Objective The butorphanol-azaperone-medetomidine fixed-dose combination (BAM, respectively, 30-12-12 mg mL^{-1}) with subsequent antagonism by naltrexone-atipamezole was evaluated for reversible immobilization of captive cheetahs (*Acinonyx jubatus*).

Study design Prospective, clinical trial.

Animals Twelve cheetahs (six males and six females, weighing 37–57 kg) housed in enclosures, were immobilized at Hoedspruit Endangered Species Centre in the Republic of South Africa.

Methods BAM volume dose rate was 0.009–0.014 mL kg^{-1} (mean ± standard deviation 0.010 ± 0.001 mL kg^{-1}). Total dose in all animals was 0.5 mL. The actual doses were as follows: butorphanol (0.29 ± 0.04 mg kg^{-1}), azaperone (0.12 ± 0.01 mg kg^{-1}) and medetomidine (0.12 ± 0.01 mg kg^{-1}). Physiologic variables and quality of immobilization were recorded every 5 minutes beginning at 15–20 minutes after darting. Arterial blood samples were collected three times at 20, 30 and 40 minutes after darting from all animals for analysis of blood oxygenation and acid-base status.

Results The inductions were calm and smooth and mean induction time was 4.0 ± 1.1 minutes. Heart rate (50 ± 9 beats $minute^{-1}$) and respiratory frequency (20 ± 3 breaths $minute^{-1}$) were stable throughout immobilization. The recovery time after reversing with naltrexone and atipamezole was 9.1 ± 3.6 minutes.

Conclusions and clinical relevance BAM proved to be a reliable and cardiovascular stable drug combination for immobilization of cheetahs.

Keywords Azaperone, BAM, butorphanol, cheetah, immobilisation, medetomidine.

Introduction

Free ranging cheetah (*Acinonyx jubatus*) populations are listed as vulnerable on the International Union for Conservation of Nature red list with population numbers continuously declining in Africa (Durant et al. 2015). Chemical immobilization of cheetahs is often performed in relocation and breeding projects where animals require immobilization for routine management practices such as vaccinations and injury or disease treatment.

Although published reports on the use of immobilizing drugs in cheetahs is limited, some published studies have investigated a number of drugs and/or drug combinations. Recently, a number of combination drugs have been reported for immobilization in this species. Janssens et al. (1994) used a combination of ketamine and xylazine in a female cheetah.

Lewandowski et al. (2002) evaluated the use of tiletamine-zolazepam (TZ) in combination with ketamine and xylazine at various dosages. The combination provided rapid induction after a single intramuscular (IM) injection along with a safe, predictable working time, good muscle relaxation and analgesia. Stegmann & Jago (2006) compared the effects of either ketamine and medetomidine (KM), ketamine and midazolam (K/MID) or tiletamine, zolazepam and medetomidine (TZM). Although no differences were observed in induction time or blood pressure (BP) between the groups, the authors noted seizures in all animals immobilized with KM as well as a lower heart rate (HR).

The use of the TZ has been the popular choice for many veterinarians working with cheetahs in South Africa (Lewandowski et al. 2002; Walzer & Huber 2002; Stegmann & Jago 2006). However, without an antagonist, recovery usually takes a long time and while some authors report a smooth recovery, others have found that recovery is often rough with animals jerking their heads around and repeatedly hitting the floor for up to 30 minutes (Walzer & Huber 2002). Walzer & Huber (2002) found that the use of a partial antagonist such as flumazenil or sarmazenil shortened the recovery time and lead to a calmer and smoother recovery.

Most notably, reports on the majority of immobilization protocols mentioned that cheetahs could not be rapidly reversed, therefore animals were vulnerable to attack should they be released into the wild during recovery.

Lafortune et al. (2005) investigated the use of medetomidine, butorphanol and midazolam in captive cheetahs and found this combination to produce a smooth and fast induction, adequate immobilization for minor procedures and a quick recovery after reversal with atipamezole, flumazenil and naltrexone. The authors noted that this ketamine-free combination was particularly beneficial for animals with liver/kidney dysfunction. The aim of the current study was to investigate the use of a butorphanol-azaperone-medetomidine fixed-dose combination (BAM) for the safe and reversible immobilization of cheetahs. The study hypothesized that this combination could produce both rapid and smooth inductions as well as induce a good level of immobilization characterized by stable cardiopulmonary and respiratory parameters. In addition, the study aimed to show that reversal of this combination with naltrexone and atipamezole could produce rapid, uneventful recoveries that would be suitable for use in wild animals.

Materials and methods

Twelve cheetahs (six males and six females), housed in enclosures, were immobilized at Hoedspruit Endangered Species Centre in the Republic of South Africa during January 2016. The animals were immobilized for clinical examination, blood collection, microchipping, deworming and genetic material collection.

BAM, as used in this study, was produced by Wildlife Pharmaceuticals South Africa (Pty) Ltd., South Africa. Each mL of the solution contained the active pharmaceutical ingredients as 30 mg of butorphanol, 12 mg of azaperone and 12 mg of medetomidine. The individual doses for the combination were calculated based on a previous BAM study on African lions (Semjonov et al. 2017). The total dose in all animals was 0.5 mL. The body weight of the animals was estimated based on body condition. A pistol projector (Dan-Inject ApS, Denmark) was used to deliver the drug. Darts (Dan-Inject ApS) with a volume of 1.5 mL and a 1.5 × 30 mm plain needle were used (Dan-Inject ApS). Remote darting was performed from outside the enclosures at distances ranging from 3 to 5 m. All injections were administered into the muscles of the thigh. All the animals were darted between 6 am and 10 am, avoiding high mid-day environmental temperatures. The air temperature ranged from 22.0 to 38.6 °C.

Two stages of induction were timed: first from time of the darting until the first signs of sedation, including open mouth, ataxic gait and lowering of the head; second from the injection time until sternal or lateral recumbency. Once the animals reached lateral recumbency, an additional 5 minutes were given before animals were approached and blindfolded. Animals were placed on a stretcher, carried from the enclosure and transported by vehicle to a shaded area around 300 m from the enclosure, where monitoring could be performed. The tracheas of all animals were intubated using endotracheal tubes (Jorgensen Labs, CO, USA) 9–11 mm in diameter. Every 5 minutes, beginning at 15–20 minutes after darting, monitoring of physiological parameters [HR, respiratory frequency (f_R), oxygen saturation (SpO_2), noninvasive BP and rectal temperature] was conducted using a veterinary monitor (Capnovet Deluxe Multiparameter Monitor; Eickemeyer, Germany). Auscultation with a

stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M United States, MN, USA) was performed every 5 minutes for the entire period of immobilization. The level of muscle relaxation was assessed based on the general muscle tone and position of the lower jaw using a 3-point scale. Level 1 indicated the absence of muscle tone, level 2—a light tone and level 3—a strongly marked tone. Capillary refill time and palpebral reflex were additionally monitored.

Arterial blood samples were collected from the femoral or median caudal artery of the tail at 20, 30 and 40 minutes after darting. The samples were immediately analysed using a portable analyzer (i-STAT1 Portable Clinical Analyzer; Abaxis, CA, USA) and cartridges (i-STAT cartridges CG4+ & CHEM8+; Abaxis). Variables measured included pH, arterial partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chlorine, urea, creatinine, glucose and ionized calcium levels. Actual base excess, actual bicarbonate, arterial haemoglobin, arterial blood SpO_2 and haemoglobin were calculated from the measured values.

The average duration of monitoring and of all the manipulations was 40 minutes. The animals were extubated 45−50 minutes after the beginning of immobilization, provided a strongly marked palpebral reflex was observed. Following extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd., Canada) to determine their body weight and transported back to the enclosure by vehicle. In the enclosure the cheetahs were placed in the lateral position in the shade on the ground.

To reverse the effect of medetomidine, atipamezole (Antisedan 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in mg was used. Naltrexone hydrochloride [Trexonil 50 mg mL^{-1}, Wildlife Pharmaceuticals (Pty) Ltd.] was used to reverse butorphanol at a total dose of 15 mg in all animals. Antagonists were then injected IM into the muscles of the thigh. The following stages of recovery were recorded: time elapsed from injection until the first signs of recovery, including eye blinking; time to head lifting; time to standing; and time to fully coordinated movement (i.e. full recovery).

Statistical analysis

For the analysis of anaesthetic dosage effects on the cheetahs' HR, f_R, systolic and diastolic BP, mean arterial pressure (MAP), SpO_2, PaO_2 and $PaCO_2$, the area under the curve (AUC) was calculated using a trapezoid method for every measurement for the immobilization period (40 minutes). The mean AUCs were used as response variables in linear regression models. The exact anaesthetic dosage (calculated after weighing immobilized cheetahs) and bodyweight were used as continuous explanatory variables. Sex was included in models to control for a possible cofounding effect. For evaluation f_R effect on the PaO_2 and SpO_2 linear egression models using PaO_2 or SpO_2, mean AUCs as response variables and f_R as an explanatory variable were used.

For analysis of the effect of BAM on induction (time to recumbency) and recovery time, linear regression models were used.

Linear mixed models were used to explore the overall time trend in lactate, arterial blood pH and body temperature. Animals were included as random intercepts and polynomials of time (minutes) as fixed effects in increasing order. The isotropic spatial exponential covariance structure was used to model serial correlations of repeated measurements at the within-animal level in all models.

A backward elimination procedure was performed for the final models. The model's assumptions were verified by scatter and normality plots of standardized residuals. For statistical analysis, STATA 14.0 (Stata Corporation, TX, USA) software was used. All p values ≤ 0.05 were considered statistically significant. Data are reported as mean ± standard deviation (SD).

Results

The average duration of monitoring and of all manipulations was 40 minutes. Data from six male cheetahs weighing 54.1 ± 2.8 kg (range 50−57 kg) and from six female animals weighing 47.8 ± 5.6 kg (range 37−54 kg) were used in this study. In all 12 animals, immobilization occurred after a single injection of BAM. There was no need for additional injections to achieve immobilization. The following actual dose rates were used: BAM volume dose rate range was 0.009−0.014 mL kg^{-1} (0.010 ± 0.001 mL kg^{-1}). The total dose in all animals was 0.5 mL. The actual doses were as follows: butorphanol (0.29 ± 0.04 mg kg^{-1}), azaperone (0.12 ± 0.01 mg kg^{-1}) and medetomidine (0.12 ± 0.01 mg kg^{-1}). First signs were observed within 1−5 minutes (2 ± 1 minutes) after darting and all animals were recumbent between 3 and 7 minutes (4 ± 1 minutes) after darting.

There was no association between the BAM actual dose and the induction time ($p = 0.751$) recorded. The inductions were observed to be calm and smooth with no side effects. Vomiting was not observed in any of the cheetahs. Two animals remained in sternal recumbency and never attained lateral recumbency but were immobilized; 10 cheetahs went into lateral recumbency.

Immobilization was stable and no sudden arousals were observed. During the first 20–30 minutes of immobilization, spontaneous limb twitches were present in nine animals. Low jaw muscle tone disappeared within 10–15 minutes after injection and remained at level 1. Capillary refill times were less than 2 seconds in all animals. In seven animals, the colour of the tongue was bluish, while breathing was deep and stable at the same time. Palpebral reflex disappeared at the fifteenth minute of monitoring and did not reappear until recovery. None of the cheetahs showed any reaction to intubation, extubation, or other painful procedures (e.g. blood collection). No apnoea was observed in any of the cheetahs, but abnormal breathing patterns, characterized by inhalation followed by a pause in breathing and eventual exhalation, were recorded in five animals. The arterial blood gases were at an acceptable level throughout immobilization [PaO_2 69 ± 9 mmHg (9.2 ± 1.2 kPa) and $PaCO_2$ 33 ± 5 mmHg (4.4 ± 0.7 kPa)]. There was no association between f_R and PaO_2 ($p = 0.874$) or f_R and SpO_2 ($p = 0.0768$). The duration of immobilization of each cheetah without additional doses was little less than 1 hour. Recovery was smooth and calm. Time from injection of the antidotes to the first signs of recovery was 4.5 ± 1.7 minutes; time of full recovery was 9.0 ± 3.6 minutes. There was no correlation between recovery time and the actual BAM dose ($p = 0.854$). No mortalities occurred during the study.

Table 1 presents the mean ± SD and range of the main parameters measured during monitoring and the results of blood gases analyses. Blood pH levels ($p = 0.023$) and lactate levels ($p < 0.001$) steadily declined in all animals, which is indicative of normal tissue perfusion. MAP was elevated (167 ± 19 mmHg) and there was no association between MAP and the actual BAM dose ($p = 0.640$).

Discussion

Published reports on suitable immobilization protocols for cheetahs are very limited, although immobilization of this species is common, particularly in South Africa. Additionally, most studies report on the use of TZ or ketamine combinations. When animals are to be released into the wild, a fully reversible

Table 1 The physiological response of cheetahs to immobilization with butorphanol-azaperone-medetomidine fixed-dose combination (BAM) over a 40-minute period.

Variable		10–20 min[1]	20–30 min[1]	30–40 min[1]	Overall*[min-max]
Heart rate	Beats minute^{-1}	48 ± 10	50 ± 10	53 ± 9	50 ± 9 [32–70]
Respiratory frequency	Beats minute^{-1}	18 ± 3	19 ± 4	18 ± 3	20 ± 3 [8–28]
Rectal temperature	°C	38.1 ± 0.6	38.2 ± 0.7	38.3 ± 0.7	38.2 ± 0.7 [36.8–39.1]
Systolic blood pressure	mmHg	198 ± 22	197 ± 20	188 ± 18	197 ± 19 [122–209]
Diastolic blood pressure	mmHg	152 ± 20	151 ± 21	147 ± 16	151 ± 19 [96–176]
Mean arterial pressure	mmHg	172 ± 20	168 ± 21	163 ± 16	167 ± 19 [106–186]
SpO_2	%	88 ± 5	90 ± 4	91 ± 4	93 ± 2 [80–100]
SO_2	%	93 ± 4	90 ± 5	91 ± 4	92 ± 4 [79–95]
PaO_2	mmHg (kPa)	72 ± 3 (9.6 ± 0.4)	76 ± 2 (10.1 ± 0.3)	73 ± 3 (9.7 ± 0.4)	68 ± 9 [50–86] (9.1 ± 1.2 [6.7–11.5])
$PaCO_2$	mmHg (kPa)	33 ± 5 (4.4 ± 0.7)	34 ± 5 (4.5 ± 0.7)	32 ± 5 (4.3 ± 0.7)	33 ± 5 [29–45] (4.4 ± 0.7 [3.9–6.0])
pH[†]		7.35 ± 0.03	7.33 ± 0.02	7.32 ± 0.05	7.33 ± 0.02 [7.25–7.41]
Lactate	mmol L^{-1}	0.42 ± 0.4	0.30 ± 0.2	0.30 ± 0.2	0.3 ± 0.2 [0.30–0.88]

*Median ± standard deviation. [†]Corrected to the rectal temperature.
PaO_2, arterial partial pressure of oxygen (measured value, temperature corrected); $PaCO_2$, partial pressure of arterial carbon dioxide (measured value, temperature corrected); SpO_2, haemoglobin oxygen saturation measured by pulse oximetry.

drug combination which provides rapid, smooth recoveries is important, so as not to endanger the welfare of the animals when they are released into areas where they are vulnerable to attack by other predators. Moreover, rapid recoveries allow for more complete monitoring of recovery upon release of the animal.

In the current study, all the animals showed calm and smooth inductions that were rapid and without observable side effects. The lack of intramuscular and subcutaneous fat as well as the excellent muscular blood supply in this species likely contributed to the rapid inductions. Induction times were similar to those reported for cheetahs immobilized with TZ (Deem et al. 1998; Lewandowski et al. 2002; Walzer & Huber 2002) and much faster than those that have been reported for cheetahs immobilized with KM (9.2 ± 3.4 minutes), ketamine, midazolam and medetomidine (11.3 ± 10 minutes) or TZM (16.8 ± 18.1 minutes) (Stegmann & Jago 2006).

Seven animals presented with bluish coloured tongues or mild cyanosis. Cyanosis is caused by the presence of more than 5 g desaturated haemoglobin per 100 mL of blood and is generally accepted to develop when blood is insufficiently oxygenated in the lungs or when haemoglobin is unable to carry oxygen (Sinclair 2003). It has been reported in a number of species treated with medetomidine, and Miller et al. (2009) note that oral mucous membranes should consistently be monitored as an adjunct to pulse oximetry, specifically when low SpO_2 values are observed during immobilization with BAM. This is because of the peripheral vasoconstrictive effects of the alpha2-adrenoceptor, medetomidine, in the drug combination (Flacke 1992). Additionally, Sinclair (2003) theorizes that cyanosis may also develop because of the stagnation of blood within peripheral capillary beds, which results in increased oxygen extraction. Thus, the cyanosis observed with the administration of medetomidine may likely be due to low blood flow through peripheral capillary beds and an actual venous desaturation. This is substantiated by the fact that relatively low peripheral SpO_2 was observed in all the animals ($SpO_2 < 95\%$) while arterial blood gas analysis revealed that blood oxygenation was at an acceptable level throughout immobilization ($PaO_2 > 70$ mmHg). It must be kept in mind that pulse oximetry readings may often be problematic, particularly in animals treated with alpha$_2$-agonists that result in vasoconstriction, since the decreased peripheral blood flow may hamper accurate readings.

Respiration was good with all animals maintaining respiratory rates between 10 and 20 breaths minute^{-1}, consistent PaO_2 values > 70 and $PaCO_2$ values < 40 mmHg throughout immobilization. Although none of the animals developed apnoea, some apneustic-type breathing was noted in five of the animals. This abnormal breathing pattern was characterized by inhalation followed by a pause in breathing and eventual exhalation, although neither inhalation nor exhalation appeared abnormal in depth or duration. This occurrence may have been due to a respiratory disturbance as a result of both butorphanol and medetomidine, although no published reports are available that elucidate on the effect of butorphanol in combination with medetomidine on breathing patterns. Doses of 20−60 μg kg^{-1} of medetomidine have been reported to result in reduced respiratory rates for varying periods in a number of studies in dogs (Sinclair 2003). Conversely, butorphanol is also a sigma-receptor agonist which may stimulate respiratory drive. In the current study, respiratory rates and blood gas results indicated that respiration was adequately maintained throughout monitoring and so clinically, the occurrence of apneustic-type breathing in some of the animals could be considered irrelevant.

Hypertension was noted in all animals with MAP consistently exceeding 150 mmHg. Similar results were reported by Deem et al. (1998) in cheetahs immobilized with TZM. Lafortune et al. (2005) also noted hypertension in all the cheetahs they immobilized with a medetomidine-butorphanol-midazolam combination. The peripheral vasoconstriction caused by medetomidine has been shown to result in hypertension, although this effect has also been reported to be transient (Sinclair 2003). Additionally, this effect may vary between species and although the extent of the effect is not dose-dependent, its duration has been shown to increase with increasing doses of medetomidine (Kuusela et al. 2000) . In dogs, Kuusela et al. (2000) found that medetomidine doses exceeding 20 μg kg^{-1} resulted in a longer duration of hypertension associated with a persistent increase in systemic vascular resistance. In the current study, the mean dose of medetomidine in the cheetah was 120 μg kg^{-1} which could explain why hypertension was noted throughout monitoring. Furthermore, it has been suggested that cheetahs in captivity suffer from chronic stress which may, as it does in humans, produce hypertension (Cassia et al. 2015). Indeed, Stegman and Jago (2006) found that immobilized

captive cheetahs were hypertensive whether KM was used or whether K/MID was used, suggesting that medetomidine was not the only contributor to the high BP observed.

Overall, BAM at a dose of 0.010 ± 0.001 mL kg^{-1} or 0.5 mL per adult cheetah produced a safe and reliable immobilization in cheetahs with no re-dosing required to maintain immobilization for up to 50 minutes. Inductions and recoveries were smooth and uneventful, and no sudden arousals were observed in any of the animals during immobilization. Cardiovascular and respiratory parameters fell within acceptable ranges. Although hypertension was noted in most of the animals, this has been reported by a number of authors in cheetahs immobilized with different drug protocols.

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Conflict of interest statement

Authors declare no conflict of interest. Dr JP Raath is the owner of Wildlife Pharmaceuticals South Africa (Pty).

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Lynn Cathrine Bäckström, Degree in Veterinary Medicine, 2020, (sup) Aleksandr Semjonov; Toomas Orro. A novel protocol for anaesthesia of chacma baboons, Estonian University of Life Sciences.

Triin Edula, Degree in Veterinary Medicine, 2020, (sup) Ingrid Hang, Aleksandr Semjonov, Alo Tänavots. Opioidi kasutus koertel: äge pankreatiit, võõrkeha seedekulglas ja sooletuppumus (Opioid use in dogs: acute pancreatitis, gastrointestinal foreign body and intestinal intussusception), Estonian University of Life Sciences.

Sonja Krista Marianne Kneckt, Degree in Veterinary Medicine, 2019, (sup) Aleksandr Semjonov. Ability of MyotonPro to detect changes in the muscle tone of immobilized blesbok (*Damaliscus pygargus phillipsi*) as a diagnostic method of capture myopathy, Estonian University of Life Sciences.

Noora Maria Jantunen, Degree in Veterinary Medicine, 2018, (sup) Aleksandr Semjonov, Anna Mykkänen, Maria Raekallio. The cardiovascular effects of the peripheral α_2-adrenoceptor antagonist vatinoxan in captive markhors (*Capra falconeri heptneri*) immobilized with medetomidine and ketamine, Estonian University of Life Sciences.

Saara Alessandra Trimeloni, Degree in Veterinary Medicine, 2016, (sup) Aleksandr Semjonov. Human and animal safety during the chemical immobilization of large carnivores in captivity, Estonian University of Life Sciences.

David Sargsyan, Degree in Veterinary Medicine, 2016, (sup) Aleksandr Semjonov, Toomas Orro. Kraniaalse ristatisideme rebendi etioloogia, ravi ja postoperatiivne taastumine koertel (Cranial cruciate ligament rupture etiology, treatment and postoperative recovery in dogs), Estonian University of Life Sciences.

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2017– …	Eesti Maaülikooli veterinaarmeditsiini ja loomakasvatuse instituudi kliinilise veterinaarmeditsiini õppetooli kliiniline juht
2016– …	Veterinaar- ja Toiduameti ravikvaliteedi hindamise eksperdirühma esimees
2016–2017	Eesti Maaülikooli veterinaarmeditsiini ja loomakasvatuse instituudi kliinilise veterinaarmeditsiini osakonna juhataja

Teadustöö põhisuunad

Veterinaaranestesioloogia, loomaaia- ja metsloomade meditsiin, veterinaarkirurgia

Osalemine uurimisprojektides

2011–2016	Haridus- ja Teadusministeeriumi rahastatud projekt „Kõhr- ja luukoe formeerumise ja degeneratsiooni mehhanismid erinevate patoloogiate korral“ (SF0180012s11)
2010–2013	Eesti Teadusagentuuri rahastatud projekt „Luumurru regeneratsiooni protsessi stimuleerivate faktorite uuring uue fiksaatori ROD-THROUGH-PLATE kasutamise puhul. Kompleksuuring: luuregeneratsiooni füsioloogiliste, biokeemiliste ja morfoloogiliste näitajate hindamiseks“ (ETF8513)

Juhendatud väitekirjad

Lynn Cathrine Bäckström, loomaarstikraad, 2020, (juh) Aleksandr Semjonov, Toomas Orro. „A novel protocol for anaesthesia of chacma baboons“ (Uudne protokoll karupaavianide anesteesiaks), Eesti Maaülikool.

Triin Edula, loomaarstikraad, 2020, (juh) Ingrid Hang, Aleksandr Semjonov, Alo Tänavots. „Opioidi kasutus koertel: äge pankreatiit, võõrkeha seedekulglas ja sooletuppumus“, Eesti Maaülikool.

Sonja Krista Marianne Kneckt, loomaarstikraad, 2019, (juh) Aleksandr Semjonov. „Ability of MyotonPro to detect changes in the muscle tone of immobilized blesbok (*Damaliscus pygargus phillipsi*) as a diagnostic method of capture myopathy“ (MyotonPro võime tuvastada immobiliseeritud blesboki (*Damaliscus pygargus phillipsi*) lihase toonuse muutusi püüdmise müopaatia diagnoosimiseks), Eesti Maaülikool.

Noora Maria Jantunen, loomaarstikraad, 2018, (juh) Aleksandr Semjonov, Anna Mykkänen, Maria Raekallio. „The cardiovascular effects of the peripheral α_2-adrenoceptor antagonist vatinoxan in captive markhors (*Capra falconeri heptneri*) immobilized with medetomidine and ketamine“ (Perifeerse α_2-adrenergilise antagonisti vatinoksaani kardiovaskulaarne mõju medetomidiini ja ketamiiniga immobiliseeritud tehistingimustes peetavatel markuuridel (*Capra falconeri heptneri*)), Eesti Maaülikool.

Saara Alessandra Trimeloni, loomaarstikraad, 2016, (juh) Aleksandr Semjonov. „Human and animal safety during the chemical immobilization of large carnivores in captivity“ (Inimeste ja loomade turvalisus tehistingimustes peetavate suurte kiskjaliste keemilisel immobiliseerimisel), Eesti Maaülikool.

David Sargsyan, loomaarstikraad, 2016, (juh) Aleksandr Semjonov, Toomas Orro. „Kraniaalse ristatisideme rebendi etioloogia, ravi ja postoperatiivne taastumine koertel“, Eesti Maaülikool.

Erialane enesetäiendamine

Kursus „Anaesthesia and Pain Management I“, European School for Advanced Veterinary Studies (ESAVS), Austria 27.–31.01.2020.

Konverents „Joint Leibniz-IZW/EAZWV/ECZN Zoo and Wildlife Health Conference 2019“. Rootsi 12.–15.06.2019.

Konverents „Joint EAZWV/AAZV/Leibniz-IZW Zoo and Wildlife Health Conference 2018“. Tšehhi Vabariik 06.–12.10.2018.

LIST OF PUBLICATIONS

1.1. Scholarly articles indexed by Web of Science Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index and/or indexed by Scopus

Laubscher, L. L., Pfitzer, S., Rogers P. S., Wolfe L. L., Miller, M. W., **Semjonov, A.**, Raath J. P. (2020). Evaluation the use of butorphanol-azaperone-medetomidine fixed dose combination for standing sedation in African elephant (*Loxodonta africana*). J Zoo Wildlife Med. (accepted for publication).

Rannamäe E, Andrianov V, Järv E, **Semjonov A**, Haak A, Kreem J (2019). A month in a horse's life: healing process of a fractured third metatarsal bone from medieval Viljandi, Estonia. Int J Paleopatholog. 24:286-292.

Semjonov A, Raath JP, Laubscher L, Orro T, Pfitzer S, Tiirats T, Rogers PS, Andrianov V (2019). Evaluation of butorphanol-azaperone-medetomidine in captive cheetah (*Acinonyx jubatus*) immobilization. Vet Anaesth Analg. 46:90-95.

Semjonov A, Andrianov V, Raath JP, Orro T, Laubscher L, Pfitzer S, Tiirats T (2018). Evaluation of butorphanol-azaperone-medetomidine (BAM) in captive blesbok (*Damaliscus pygargus phillipsi*) immobilization. Vet Anaesth Analg. 45:496-501.

Semjonov A, Andrianov V, Raath JP, Orro T, Venter D, Laubscher L, Pfitzer S (2017). Evaluation of BAM (butorphanol-azaperone-medetomidine) in captive African lion (*Panthera leo*) immobilization. Vet Anaesth Analg. 44:883-889.

1.2. Peer-reviewed articles in other international research journals with an ISSN code and international editorial board, which are circulated internationally and open to international contributions

Semjonov A, Andrianov V (2013). Tehistingimustes peetavate ulukkaslaste keemiline immobiliseerimine (Chemical immobilization of wild feline species in captive conditions). Agraarteadus. 24:79-85.

1.3. Scholarly articles in Estonian and other peer-reviewed research journals with a local editorial board; peer-reviewed scientific articles in journals important for Estonian culture or scholarly articles in Akadeemia, Looming, Vikerkaar

Semjonov A (2016). Looduskaitse meditsiin ja loomaarsti roll selles (The role of veterinarian in conservation medicine). Eesti Loomaarstlik Ringvaade. 2:20-22.

Semjonov A, Andrianov V (2013). Düstookia roomajatel (Dystocia in Reptiles). Eesti Loomaarstlik Ringvaade. 4:11-15.

6.3. Popular sciences articles

Rannamäe E, Haak A, Kreem J, Järv E, **Semjonov A**, Andrianov V (2019). Ühe keskaegse hobuse viimane elukuu. (The last month in the life of a medieval horse). Tutulus: Eesti arheoloogia aastakiri. 8:34-35.

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FACTORS AFFECTING APPLE JUICE QUALITY AND MYCOTOXIN PATULIN FORMATION
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31. august 2020

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MAPPING LANDSCAPE ORGANIZATION CONDITIONS, ENABLING USE OF CULTURAL ECOSYSTEM SERVICES, BY MEANS OF REMOTE SENSING AND LOCATION-BASED SOCIAL MEDIA DATA: A RESOURCE-DRIVEN APPROACH
ÖKOSÜSTEEMI KULTUURITEENUSEID KAARDISTADA VÕIMALDAVAD MAASTIKUTUNNUSED KAUGSEIRE JA KOHAPÕHISE SOTSIAALMEEDIA ANDMETES – RESSURSIPÕHINE LÄHENEMINE
Professor **Mart Külvik**, professor **Ihor Chervanyov**
31. august 2020

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DIVERSITY IN BALTIC POPULATIONS OF POTATO LATE BLIGHT PATHOGEN *PHYTOPHTHORA INFESTANS*
KARTULI-LEHEMÄDANIKU TEKITAJA *PHYTOPHTHORA INFESTANS* BALTIMAADE POPULATSIOONIDE MITMEKESISUS
Professor **Marika Mänd**, dotsent **Eve Runno-Paurson**
10. september 2020

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SUREMUS FARMIS JA SELLEGA SEOTUD RISKITEGURID EESTI PIIMA- JA LIHAVEISEKARJADES
16. september 2020
Dotsent **Kerli Mõtus**, professor **Arvo Viltrop**

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EVALUATION OF FOREST MANAGEMENT IN THE CONTEXT OF CARBON FLUXES: EDDY-COVARIANCE METHOD
METSADE MAJANDAMISE MÕJU HINDAMINE SÜSINIKU KONTEKSTIS: TURBULENTSE KOVARIATSIOONI MEETOD
Vanemteadur **Kalev Jõgiste**, vanemteadur **Marek Metslaid**
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