Low-dose thiafentanil in combination with azaperone alone or azaperone and medetomidine for the immobilization of African buffalo *(Syncerus caffer)*

By

Vanessa FABER

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Supervisor: Prof Katja Koeppel, BVMS, MSc (Wildlife), PhD, CertZooMed, Dip ECZM (ZHM)

Department of Production Animal Studies

Faculty of Veterinary Science, Onderstepoort

University of Pretoria

Co-Supervisor: Dr Richard Burroughs, BSc, BVSc, MSc (Vet Trop Dis)

Department of Production Animal Studies

Faculty of Veterinary Science, Onderstepoort

University of Pretoria

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Declaration of originality

Full name of student: Vanessa Faber

Student number: u14397872

Topic of work: Low-dose thiafentanil in combination with medetomidine and azaperone for the immobilization of African buffalo (*Syncerus caffer*)

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Prof Katja Koeppel

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Abbreviations

ACTH	adrenocorticotropic hormone
α_2	-alpha two adrenoreceptor
%	percentage
°C	degree(s) Celsius
BCS	body condition score
Boma	holding pen, temporary enclosure
bpm	beat(s) per minute
BW	body weight
CNS	central nervous system
CRH	corticotropin-releasing hormone
CO_2	carbon dioxide
DAP	diastolic arterial pressure
et al.	et alia
$ETCO_2$	end-tidal carbon dioxide tension
FiO_2	fractional inspired oxygen concentration
FMD	foot-and-mouth disease
g	gram(s)
G protein	guanine nucleotide-binding protein
H1-4	Thiafentanil dosage groups of the TA combination
HR	heart rate
HCl	hydrochloride
HPA axis	hypothalamus-pituitary-adrenal-axis
IM	intramuscular
IV	intravenous
kg	kilogram(s)
L	litre(s)
L1-3	Thiafentanil dosage groups of the TMA combination
m	meter(s)

M1-3	Medetomidine dosage groups of the TMA combination
MAP	mean arterial pressure
mg kg^{-1}	milligram(s) per kilogram
mg	milligram(s)
min	minute(s)
ml	millilitre(s)
mmHg	millimetres Mercury
mmol	millimole(s)
N	novel regime (TMA)
n	number of animals
OIRD	opioid-induced respiratory depression
p	statistical significance
Patm	atmospheric pressure
$P(A\text{-}a)O_2$	alveolar-arterial oxygen tension gradient
$PaCO_2$	arterial carbon dioxide tension
PaO_2	arterial oxygen tension
pH	negative log of hydrogen ion concentration
PH_2O	water vapour pressure of saturated air in the alveoli
R	gas exchange ratio
RR	respiratory rate
S	standard regime (TA)
SAP	systolic arterial pressure
SC	subcutaneous
SD	standard deviation
SpO_2	peripheral oxygen haemoglobin saturation
T	time of data collection points in minutes (T0, T5, T10, T15, T20, T25, T30, T35, T40)
TA	thiafentanil-azaperone combination
TMA	thiafentanil-medetomidine-azaperone combination

Abstract

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By Vanessa FABER

Supervisor: Prof Katja Koeppel, BVMS, MSc (Wildlife), PhD, CertZooMed, Dip ECZM (ZHM)

Co-Supervisor: Dr Richard Burroughs, BSc, BVSc, MSc (Vet Trop Dis)

Department: Department of Production Animal Studies, Faculty of Veterinary Science, Onderstepoort, University of Pretoria

Objective

To determine the efficacy, safety and cost of a novel drug regime comprising of a low thiafentanil dose in combination with medetomidine and azaperone; to the more established thiafentanil and azaperone combination for the immobilization of African buffalo (*Syncerus caffer*)

Study design

Prospective, randomised, cross-over study.

Animals

Twelve adult African buffalo (*Syncerus caffer*) bulls.

Material and methods

This randomized cross-over study on twelve African buffalo (*Syncerus caffer*) was performed on a buffalo farm located in the Sekhukhune District Municipality, Limpopo, South Africa. The study consisted of two data collection periods. The two combinations used were the more conventional **thiafentanil-azaperone (TA)** drug combination comprising of thiafentanil oxalate at 6-7 mg/animal and azaperone at 40 mg/animal as described by Burroughs *et al.* (2012b), and the novel **thiafentanil-medetomidine-azaperone (TMA)** combination comprising of a relatively low dose of thiafentanil oxalate at 1 mg/animal, medetomidine hydrochloride at 3-4 mg/animal and azaperone at 40 mg/animal. During the first data collection period, each buffalo was immobilized once with either the TA or the TMA combination. Six buffalo received the TA combination and six buffalo received the TMA combination. After twenty-one days, each buffalo was immobilized with the alternate combination. One animal was immobilized at a time.

Before each data collection period, the animals were brought from the breeding camps into the boma where food and water was withheld for 24 hours and 12 hours respectively to minimize the risk of regurgitation during immobilization. For the immobilization and data collection, the respective buffalo was separated from the herd and moved into a separate pen. The buffalo were dosed according to its estimated weight and body condition score (BCS) as visualised by the immobilisation team.

After dart placement, a stopwatch was started and the time to immobilization as well as quality of induction was recorded. Once recumbent, the animal was instrumented with a digital rectal thermometer (HI98509 Checktemp 1, HANNA Instruments (Pty) Ltd., USA), a pulse oximeter (Veterinary Pulse Oximeter, Model 9847V, Nonin Medical, USA) to measure the peripheral oxygen haemoglobin saturation (SpO_2) and a multi-parameter monitor (M3T Mini vet, TooToo Meditech, China) which was used to measure the end-tidal carbon dioxide ($ETCO_2$). The caudal auricular artery was aseptically cannulated for continuous arterial blood pressure measurement using an intra-arterial blood pressure monitor (IntraTorr, IntraVitals, United Kingdom) and blood sampling. Blood was drawn at 10-, 20- and 35-min post immobilization and was analysed on site with a portable blood gas analyser (epoc® SIEMENS Blood Analysis System, Siemens Healthcare GmbH, Erlangen, Germany) using single-use epoc® BGEM Test Cards.

Physiological values and anaesthetic plane were recorded at 5-min intervals until 40 minutes. Variables measured were the heart and respiratory rate, body temperature, SpO_2 and $ETCO_2$. After 40 minutes, the buffalo was de-instrumented, antagonized and the recovery times were recorded.

The TA combination was antagonized using naltrexone hydrochloride intravenously at 10 mg/mg thiafentanil. The animals immobilized with the TMA combination received a standardized mixture of atipamezole and yohimbine intravenously at 0.5 ml/mg medetomidine hydrochloride followed by naltrexone hydrochloride at 10 mg/mg thiafentanil.

The immobilization costs were compared descriptively.

Results

The mean dosages (range) of the thiafentanil-azaperone combination were 0.0136 (0.011 to 0.0163) mg kg-1 thiafentanil and 0.0792 (0.063 to 0.093) mg/kg azaperone; and of the thiafentanil-medetomidine-azaperone combination were 0.00216 (0.0016 to 0.0023) mg kg-1 thiafentanil, 0.00688 (0.0047 to 0.0084) mg/kg medetomidine, and 0.0688 (0.047 to 0.084) mg/kg azaperone. The TA combination induced recumbency in a significantly shorter time compared to the TMA combination. Mean (range) induction times for the TA and TMA combinations were 5.7 (4 - 9.5) and 10.95 (6 - 20) minutes, respectively. Both combinations provided sufficient immobilization throughout the procedure of 40 minutes for routine veterinary and management procedures. Heart rates were significantly different ($p < 0.001$) between the two combinations with a mean heart rate of 139 bpm (± 25) and 70 bpm (± 27) in the TA and TMA combination, respectively. There was a significant difference in the PaO_2 ($p < 0.05$) between the two combinations. All buffalo were hypoxaemic during immobilization with a mean (SD, range) PaO_2 value of 44 mmHg (± 14, 24 – 77 mmHg) and 51 (± 13, 33 – 80 mmHg) in the TMA and TA combination, respectively. The A-a gradient was significantly different ($p < 0.01$) between the two combinations and was significantly wider in the TMA than in the TA combination: TMA (mean, ±SD): 40 (± 9) mmHg; TA (mean, ± SD): 27 (± 13) mmHg. The costs to chemically immobilize and antagonise an adult buffalo bull using the TA combination were ± R 593/buffalo. This is four times more expensive than the TMA combination which was calculated at ± R 146/buffalo.

Conclusions and clinical relevance

Both combinations were effective in providing a sufficient immobilization for routine veterinary and management procedures in African buffalo with quick recoveries and no mortalities. The TMA combination induced immobilization with only 1/7th of the higher dose of opioid and at only a quarter of the cost. Hypoxaemia was a concern in both combinations and resulted mainly from decreased pulmonary oxygen diffusion rather than hypoventilation. Importantly, despite respiratory rates and partial pressure of carbon dioxide (PaCO2) values being within

the normal expected physiological range, hypoxaemia was more severe in the TMA combination. Supplementary oxygen is considered mandatory during immobilisation with both combinations. The enormous reduction in costs with the TMA combination could be beneficial for the wildlife industry. However, the longer induction times, and risks from marked hypoxaemia need to be considered and addressed when this combination is used.

Keywords

African buffalo, *Syncerus caffer*, immobilization, medetomidine hydrochloride, thiafentanil-medetomidine, hypoxaemia

CHAPTER I: LITERATURE REVIEW

1.1 Background and rationale: Chemical immobilization of wildlife

South Africa is renowned for its biodiversity and world-famous wildlife and nature reserves such as the Kruger National Park (KNP) and the Kgalagadi Transfrontier Park (KTNP). While the KNP and KTNP are state-owned conservancy areas, various private game farms can be found throughout South Africa. In 1998, approximately 29% of South Africa's land was used for wildlife ranching - a billion-dollar industry that has developed over the last few decades. In the 1980s, a change in South Africa's legislation devoted the management rights for wildlife to private landowners. This was a key driver for the development of privately owned game reserves in South Africa (Kreuter *et al.* 2010). The increase in profitability in the game industry resulted in a greater demand for wildlife immobilizations. Translocation and reintroduction of wildlife are aimed at conserving species, managing populations and maintaining genetic diversity. As describes by Laubscher *et al.* (2015), in 2015, a yearly minimum of 300 000 head of game were captured and translocated by physical and chemical immobilization. In most instances, the chemical immobilization of wildlife is required. Opioids are the most important immobilizing drugs in the chemical immobilization of wildlife (Burroughs *et al.* 2012a). Opioids are frequently used in combination with tranquilizers or sedatives to mitigate opioid-induced side-effects and to reduce anaesthetic requirements. Of these, the most commonly used are medetomidine hydrochloride and azaperone tartrate. Capture related morbidities and mortalities are frequently seen during capture operations. Thus, for animal welfare and conservation reasons, it is in the interest of all parties involved to minimize capture-related morbidities and mortalities (Laubscher *et al.* 2015). Therefore, safe and readily repeatable immobilization protocols are important and in-depth understanding of the pharmacodynamics of the anaesthetic compounds is critical.

1.2 Chemical immobilization of wildlife: A short history

Before the development of remote drug delivery systems and suitable anaesthetic agents, wildlife capture was achieved by physical capture methods or by adding tranquillizers to the feed. These methods were often unsuccessful or dangerous for both handlers and animals (Pistey & Wright 1961).

Historically, chemical immobilization of wildlife can be traced back to South American hunters which used curare-dipped arrows to immobilize animals. The plant extract contained certain

alkaloids which induced muscle paralyses by neuromuscular blockage and eventually death by respiratory and cardiac arrest (Isaza 2007).

The difficulty of developing a drug of sufficient quantity which could induce the desired state of immobilization with an acceptable safety margin, and the lack of an appropriate remote drug delivery system, posed a challenge to the beginnings of game capture (Pistey & Wright 1961).

In the second half of the twentieth century, the development of remote drug delivery systems and a drug that successfully induced muscle paralysis following intramuscular injection (i.e., gallamine triethiodide) lead to a major change in the chemical immobilization of wildlife. Since then, great improvements have been made in the establishment of new drugs and delivery systems (Isaza 2007).

1.3 Chemical immobilization of African buffalo (*Syncerus caffer*)

The African buffalo (*Syncerus caffer*) is a large wild bovid that can be found throughout most of sub-Saharan Africa. Their population was decimated drastically during the Rinderpest outbreak between 1888 and 1899. Although recovered, African buffalo are listed as a threatened species due to habitat loss and poaching. As a flagship species and a member of the 'Big Five', African buffalo play an important economic role in both ecotourism and trophy hunting. However, they also act as reservoir hosts and vectors for various diseases. Diseases of economic and public health importance are foot-and-mouth disease, corridor disease, bovine brucellosis and bovine tuberculosis. Therefore, veterinary interventions and population health screening play an integral part in the wildlife medicine (Metzger *et al.* 2010, Michel & Bengis 2012). Routinely, chemical immobilization protocols for buffalo consist of an opioid and a tranquillizer or sedative, or both. The standard species-specific protocol that is frequently used combines etorphine hydrochloride (0.01 mg/kg) with azaperone (0.15-0.2 mg/kg) (Curro 2007). Thiafentanil oxalate combined with azaperone can be used instead of or in combination with etorphine hydrochloride to reduce induction time. Szabó *et al.* (2015) describe successful immobilization of African buffalo with thiafentanil oxalate at 6 mg/animal and azaperone at 40 mg/animal (Szabó *et al*. 2015). Burroughs *et al*. (2012b) describe the immobilization of an adult African buffalo with either etorphine hydrochloride at 8-10 mg/animal and azaperone at 50-150 mg/animal or thiafentanil oxalate at 6-10 mg/animal and azaperone at 50-100 mg/animal (Burroughs *et al.* 2012b). It is advised to withhold food and water for 24 and 12 hours, respectively, to minimize the risk of regurgitation of ruminal contents. Under field conditions, where fasting isn't possible, it is recommended to avoid

chemical immobilization in the late afternoon as the animals are more likely to consume water in the afternoon which makes them more prone to regurgitation and aspiration (Curro 2007). In general, xylazine is not recommended in ruminants as they can reduce rumen motility and increase the risk for bloat and regurgitation (Burroughs *et al.* 2012b). Hypoxaemia is frequently observed during chemical immobilization of ruminants. Therefore, buffalo should be placed in sternal recumbency as soon as possible with an extended head and neck to ensure a patent airway (Curro 2007). As buffalo are sensitive to hyperthermia, chemical immobilization during extreme temperatures should be avoided (Burroughs *et al.* 2012b). Hyperthermia is linked to an increased metabolic demand for oxygen which can lead to hypoxaemia with associated pathologies (Curro 2007).

1.4 Immobilizing dugs of interest

Depending on the species, two categories of immobilizing agents can be differentiated: opioids and cyclohexylamines. While opioids are predominantly used in herbivores, cyclohexylamines are primarily used in species belonging to the order Carnivora (Burroughs *et al.* 2012a).

1.4.1 Opioids

'Opioid' is the Greek word for 'juice' as they are derived from the resin of the flowering plant species *Papaverum somniferum* (Barber 1997). They have been used for thousands of years as powerful medicinal agents in human and veterinary medicine. Opioids can be divided into endogenous and exogenous opioids. Endogenous opioids are synthesized in the body (e.g., endorphine, encephaline, dynorphine, nociceptin); exogenous opioids can either be naturally extracted from the juice of the opium poppy (namely morphine, codeine etc.) or be synthesized from a natural opioid. Exogenous opioids exert their effects by opioid receptor binding. Opioid receptors are expressed in the peripheral and central nervous system including the kidney, heart, adrenal glands and gastrointestinal tract (Ahlbeck 2011). Three main receptor types are differentiated: mu (μ), kappa (κ) and delta (δ). The μ-receptor can be divided into μ_1 and μ_2 receptor subtypes (Grimm & Lamont 2007).

Opioids exert different effects on different species. While opioids cause severe central nervous system (CNS) depression in some species (e.g., dogs, primates), CNS stimulation can be observed in other species (e.g., horse, cow) (Meyer 2010). The species-specific differences are attributed to variations in receptor type and distribution among species. Furthermore, intraspecific differences exist determined by the physiological status,

psychological factors, age, dose, route of administration, and type of opioid used (Grimm & Lamont 2007).

The need for an opioid that is potent enough to immobilize wildlife led to the development of etorphine hydrochloride and thiafentanil oxalate (Burroughs *et al.* 2012a). Etorphine hydrochloride is a semi-synthetic derivate of the opioid alkaloid thebaine which is 1000 x more potent than morphine. The latter is a non-specific μ-, δ- and κ-receptor agonist which produces catatonia on very low dosages and can therefore be used to chemically immobilize wildlife (Blane *et al*. 1966; Zeiler & Meyer 2017). Etorphine hydrochloride induces rapid immobilization, potent analgesia, muscle relaxation and sedation; and its effects are fully reversible. It is the most commonly used immobilizing agent worldwide for species of the Orders Proboscidea, Artiodactyla and Perissodactyla. (Burroughs *et al.* 2012a; Meyer 2010). Thiafentanil oxalate is a more recently developed immobilizing drug that is derived from the synthetic opioid fentanyl. It has a similar potency to that of etorphine hydrochloride but a faster induction time and shorter duration of action (Zeiler & Meyer 2017).

Opioids are known to induce several side effects which limit their clinical use. The analgesic effect, as well as the majority of side effects, are caused by μ-receptor binding (Grimm & Lamont 2007). The most common side effects associated with exogenous opioid administration are respiratory depression, CNS excitement and muscular hypertonicity with associated hyperthermia. Sub-optimal dosages or severe agitation may result in prolonged excitement and sympathetic exertion (Burroughs *et al*. 2012a; Grimm & Lamont 2007). This can lead to a fatal physiological cascade with hyperthermia, reduced tissue perfusion, hypoxaemia and metabolic acidosis (Paterson 2007).

Naltrexone is a pure antagonist and the drug of choice when a complete and long-acting opioid reversal is desired. It competes with the immobilizing agent at the receptor site and reverses its pharmacological effect (Burroughs *et al* 2012a).

Opioid-induced respiratory depression (OIRD)

Central control of respiration

One of the major concerns in wildlife immobilization and anaesthesia is opioid-induced respiratory depression (OIRD) (Meyer 2010). Respiration is controlled and regulated by a neuronal network that is found in the lower brainstem (Meyer 2010). Three basic elements can be differentiated: the sensors, the central controller and the effectors. The sensors are comprised of central and peripheral chemoreceptors which perceive and send information to

the respiratory centre, also referred to as the central controller, to adjust respiration (West 2012). The central respiratory centre consists of neuron groups situated in the medulla, pons and other parts of the brain. Ventral, dorsal and pontine respiratory groups can be differentiated and interact with each other to regulate respiration (Meyer 2010). The pre-Bötzinger complex, a group of cells situated in the ventrolateral medulla of the brainstem is considered as the respiratory rhythm generator (West 2012). Changes perceived by the chemoreceptors (sensors) are coordinated by the respiratory centre (central controller) and sent as impulses to the respiratory muscles (effectors). The dorsal respiratory centre sends impulses to the external intercostal muscles and diaphragm to generate inspiration. The ventral respiratory centre sends impulses to internal costal muscles for expiration (West 2012).

Opioids and respiration

Opioid-induced respiratory depression is mainly caused through activation of opioid receptors located in respiratory neurons, including the pre-Bötzinger complex. The main opioid receptors involved in OIRD are the μ_2-receptors (Meyer 2010). The involvement of μ-receptors in OIRD was confirmed in a study with mice in which a lack of μ-receptors did not induce respiratory depression after morphine administration (Montandon & Slutsky 2019). The severity of the respiratory depressions is influenced by the dosage, route, and type of opioid and receptor type involved (Meyer 2010; Van der Schier *et al.* 2014). OIRD is caused by various effects following opioid-receptor activation. One of the main reasons for OIRD is an altered chemoreceptor response of the ventral respiratory group neurons to hypercapnia and hypoxia (Meyer 2010, Pattinson 2008). Under normal conditions, the respiratory neurons situated in the ventral surface of the medulla, sense hypercapnia and spinal pH changes and consequently adjust respiration (Burroughs *et al.* 2012a; Guyenet *et al.* 2010). Respiratory rhythm is further influenced by μ-receptor activation at the pre-Bötzinger complex. Furthermore, the peripheral chemoreceptor response to abnormal oxygen levels in the blood is depressed by direct action of opioids on the carotid bodies (West 2012).

The ventral respiratory centre, which is responsible for inspiration, is inactivated by the use of opioids. In contrast, neurons responsible for expiration are insensitive to the action of opioids (Lalley 2003, Pattinson 2008). This inhibitory effect and the difference in sensitivity leads to an irregular rhythm with dropped inspirations (Pattinson 2008). Opioids further decrease the chest wall compliance and diaphragmatic muscle activity and increase upper airway resistance (Lalley 2003).

Species that are more sensitive to respiratory depression include giraffes, white rhinoceroses, impala, waterbuck, hippopotamus and mini-antelopes (Burroughs *et al.* 2012a). Furthermore, differences among individuals of the same species exist (Meyer 2010).

Opioid-induced muscle rigidity

Opioids are further known to induce general muscle hypertonicity. As with respiratory depression, the receptor group predominantly involved in muscle rigidity is the μ-receptor (Zeiler & Meyer 2017). At higher doses, respiration can be compromised by opioid-induced muscle rigidity. This is partially the result of an increase in the chest rigidity but also of a general increase in muscle metabolism and hence oxygen consumption. This effect can contribute to the development of hypoxaemia during the chemical immobilization of wildlife (Haw 2016).

Hypoxaemia and pulmonary hypertension

Hypoxaemia does frequently occur in chemically immobilized wildlife and can predispose the animal to other significant morbidities and mortalities such as capture myopathy, and can be the result of low inspired oxygen, hypoventilation, shunts, diffusion impairment or ventilation-perfusion mismatch (Read 2003). As mentioned, μ-receptor binding of etorphine hydrochloride or thiafentanil oxalate results in depression of the respiratory centre which is characterized by hypoventilation, hypercapnia and hypoxaemia. The hypoxia observed during opioid-induced immobilization can be compounded by an increased muscle rigidity and oxygen consumption (Haw *et al.* 2015). In a study with goats immobilized with etorphine hydrochloride, the recorded hypoxia did not only result from hypoventilation but also pulmonary hypertension (Meyer *et al.* 2015). Pulmonary hypertension following opioid administration can be the result of the vasoconstrictive effects on pulmonary circulation following an increased sympathetic activity (Heard *et al.* 1996, Zeiler & Meyer 2017).

1.4.2 Tranquillizers and sedatives

Opioids are frequently co-administered with tranquillizers or sedatives, or both, to reduce opioid-induced side-effects and anaesthetic requirements (Burroughs *et al*. 2012a).

1.4.2.1 Alpha$_2$-adrenergic agonists

The use of alpha$_2$-adrenergic agonists dates back to 1962 when the sedative xylazine was synthesized (Grimm & Lamont 2007). Medetomidine hydrochloride is a potent and highly specific alpha$_2$-adrenoceptor agonist which has synergistic and dose-sparing effects when used in combination with opioids. Some opioid-induced side effects are dose dependent and can be minimized by reducing the dose of the opioid (Swegle & Logemann 2016). Medetomidine is an imidazole-based compound that has anxiolytic properties and induces a reliable and dose-dependent sedation, muscle relaxation and analgesia. Animals sedated with alpha$_2$-adrenergic agonists can appear to be immobilized; however, they can react to stimuli which can result in spontaneous arousal (Burroughs *et al.* 2012a). Compared to xylazine, medetomidine hydrochloride has a 10 x higher affinity to alpha$_2$-adrenergic receptors. (Citino *et al*. 2001). Alpha$_2$-adrenoreceptors, a distinct subclass of alpha-adrenergic receptors, are G protein-coupled receptors which can be found pre-, post- or extrasynaptically in the central and peripheral nervous system including the cardiovascular, renal, endocrine, respiratory, gastrointestinal and haematological system (Grimm & Lamont 2007; Murrell & Hellebrekers 2005). Three main iso-receptors can be differentiated: α_{2A}, α_{2B}, α_{2C}. Species-specific differences regarding subtype, density and distribution of iso-receptors among animals exist. This results in a species-specific response to the administered drug and the different dosages and routes of administration. The two subtypes of clinical relevance are the α_{2A} receptors and α_{2B} receptors (Murrell & Hellebrekers 2005; Sinclair 2003). Alpha$_2$-adrenergic receptors can be found both pre- & postsynaptically. Presynaptic alpha$_2$-adrenergic receptor activation controls the release of norepinephrine by a negative feedback mechanism. In the absence of alpha$_2$-agonists, norepinephrine is released into the synaptic cleft where it binds to adrenoceptors of the effector cell which can induce arousal. In the presence of alpha$_2$-agonists, the sympathetic outflow is depressed via negative feedback which contributes to sedation but also to bradycardia. Postsynaptic alpha$_2$-agonist receptor binding in the vasculature of the smooth muscles can induce peripheral vasoconstriction (Grimm & Lamont 2007, Sinclair 2003). Stress and exertion can override the sedative effects of medetomidine due to an increased endogenous catecholamine release (Sinclair 2003).

Besides the beneficial sedative, muscle relaxing and analgesic effects, medetomidine can induce harmful cardiovascular side effects. Bradycardia is one of the most concerning

adverse effects and can be the result of both central and peripheral receptor activation. Central receptor activation reduces the release of norepinephrine which results in reduced sympathetic tone and bradycardia. Peripheral receptor activation results in increased systemic vascular resistance (SVR) which decreases the heart rate and increases arterial blood pressure (Sinclair 2003). The initial hypertension is followed by a fall in blood pressure as a result of the baroreceptor reflex and reduced cardiac output. The resting heart rate can fall below 50% in some individuals (Grimm & Lamont 2007). The extent of hypotension depends on the dose, route of administration and receptor type activation; and can be pronounced when administered in combination with other anaesthetics. The side effects brought about by medetomidine are typically dose-related. Lower doses induce predominantly central receptor activation while higher doses activate peripheral receptors. Respiratory side effects are not significant when administered alone but can be compounded when combined with opioids (Sinclair 2003).

The effects of medetomidine hydrochloride can be reversed by competitive antagonism with one of the following alpha$_2$-adrenergic antagonists: yohimbine, atipamezole or tolazoline. Reversal is important in case of cardiovascular side effects or other life-threatening situations following alpha$_2$-adrenergic receptor stimulation (Sinclair 2003). Atipamezole was developed as the preferred antagonist for medetomidine hydrochloride. The recommended dose is 2.5 - 5 times that of medetomidine hydrochloride (Burroughs *et al*. 2012a). Intramuscular injection is recommended to induce a gradual antagonism and awakening. Intravenous administration is associated with sudden cardiovascular changes which can result in severe opposite haemodynamic effects such as tachycardia, excitement, vasodilation and death (Sinclair 2003). As alpha$_2$-adrenergic antagonists also reverse the analgesic effects, sufficient analgesia needs to be provided following painful procedures (Grimm & Lamont 2007). Due to its longer half-life, re-narcotization is uncommon in animals reversed with atipamezole (Sinclair 2003). Although considered to be the antagonist of choice, the relatively high cost of atipamezole can limit its use. Yohimbine hydrochloride is an indole alkaloid that has been used effectively in antagonizing alpha$_2$-adrenergic agonist induced sedation. Yohimbine is approved for the reversal of xylazine hydrochloride in dogs and labelled for intravenous use only. Atipamezole has been found to have a higher affinity and selectivity for alpha$_2$-adrenergic receptors than yohimbine and some references state that atipamezole is the preferred alpha$_2$-receptor antagonist with lesser side effects (Janssen *et al.* 2017).

In summary, alpha$_2$-adrenergic agonists are commonly used in veterinary medicine to induce reversible and dose-dependent sedation, analgesia and muscle relaxation. Alpha$_2$-agonists are often co-administered with opioids due to their synergistic and drug-sparing effects. Given at a higher dose, alpha$_2$-agonists can induce an immobilizing state which can be

shaken off by audible and tactile stimuli. The latter should be taken into consideration when working with an immobilized animal. The use of alpha$_2$-adrenergic agonists is associated with several side effects. The cardiovascular adverse effects are most concerning. Bradycardia and hypotension are induced by central and peripheral receptor stimulation. Studies suggest that these side effects are dose-dependent. Respiratory depression can be severe when alpha$_2$-agonists are administered in combination with opioids. Alpha$_2$-agonists interfere with adrenergic neurotransmission which can reduce the sympathetic outflow by reducing the level of circulating catecholamines. The latter has been associated with a reduced stress response.

1.4.2.2 Butyrophenones

Azaperone is a neuroleptic drug belonging to the group of butyrophenones. Neuroleptics find their use as anti-psychotic drugs in humans but are also widely used in veterinary medicine (Grimm & Lamont 2007). Azaperone, manufactured under the name Stresnil® (Bayer) is predominantly used as a tranquillizer and anti-aggressive drug in pigs. Since 1980, it has been effectively used to tranquilize wildlife species as an adjunct with an opioid or in combination with butorphanol and medetomidine (BAM) (Burroughs *et al*. 2012a; Grimm & Lamont 2007). Similar to sedatives, tranquillizers interfere with adrenergic neurotransmission. At therapeutic dosages, azaperone antagonizes alpha$_1$-adrenoreceptors (Mentaberre *et al*. 2010) with anxiolytic, sedative and muscle-relaxing effects. Azaperone has minimal effects on respiration but is believed to reduce opioid-induced respiratory depression (Clarke, Trim & Hall 2014). Furthermore, azaperone induces vasodilation which may be useful in alleviating hypertension (Mentaberre *et al*. 2010). Due to its anti-dopaminergic and vasodilatory effects, azaperone is thought to impair thermoregulatory processes. However, no evidence was found on neuroleptics inducing heat lability or interfering with thermoregulation in wildlife (Fick *et al*. 2007). Doses exceeding therapeutic recommendations can induce adverse effects such as extrapyramidal effects and catalepsy (Burroughs *et al*. 2012a).

1.5 Capture related morbidities and mortalities

1.5.1 Chemical immobilization of wildlife: monitoring and complications

Morbidities and mortalities frequently occur during the chemical immobilization of wildlife. The mortality rate can be as high as 43% during some capture operations (Meyer 2010). One of the reasons for frequent capture-related pathologies is the difficulty of performing pre-anaesthetic assessments of the animals under field conditions (Read 2003). A majority of

losses can be attributed to stress-related injuries and capture myopathy (Breed *et al.* 2019). Furthermore, respiratory depression, regurgitation and bloat are common side effects observed during chemical immobilization of ruminants (Burroughs *et al.* 2012a). Anaesthetic monitoring is important in immobilized animals to detect and respond to physiological changes. When full anaesthetic monitoring is not possible, it is advised to minimally record temperature, heart rate and respiratory rate (Curro 2007).

1.5.2 Respiratory depression

As described earlier, opioids influence the respiratory rhythmicity and volume, the hypoxic and hypercapnic ventilator response, the expansion of the chest wall and the upper airway resistance (Lalley 2003). This can result in a decreased pump-action and hypoventilation characterized by hypercapnia, hypoxia and respiratory acidosis (Meyer 2010; Meyer et al 2015). The effect on the respiratory centre is dose-dependent - a higher dose can exacerbate respiratory depression. Sub-optimal doses can lead to a prolonged excitement phase which can result in fatal hyperthermia and hypoxaemia (Burroughs *et al*. 2012a).

The use of alpha$_2$-adrenergic agonists can reduce the respiratory rate by CNS depression. However, this is seldom of concern when medetomidine is administered on its own. Respiratory depression is compounded when co-administered with other anaesthetics, especially opioids (Sinclair 2003).

1.5.3 Hypoxaemia

Hypoxaemia is a frequent sequel of chemical immobilization and can predispose to other significant morbidities and mortalities. Blood oxygenation can be measured via pulse oximetry. However, arterial blood gas measurement is a more sensitive indicator of blood oxygenation and also measures the acid-base state (Heard 2007). The physiological partial pressure of oxygen (PaO_2) ranges between 80-110 mmHg in animals. Hypoxaemia is present when the PaO_2 value falls below 80 mmHg. The cause of hypoxaemia can be multifactorial. Hypoxaemia can be the result of low inspired oxygen, hypoventilation, shunts, diffusion impairment and ventilation-perfusion mismatch. Low oxygen inspiration is uncommon during chemical immobilization.

Hypoventilation can be secondary to respiratory depression caused by the immobilizing agent (Read 2003). Inadequate gas exchange resulting from hypoventilation can be

corrected by supplementary oxygen therapy and should be available at all times during chemical immobilization.

The alveolar-arterial gradient (A-a gradient) measures the difference of alveolar oxygen concentration and arterial oxygen concentration and is used to determine the source of hypoxaemia. In animals, an A-a gradient above 25 mmHg is considered as abnormal. During anaesthesia, a widened A-a gradient enables an assessment of the severity of pulmonary dysfunction and can indicate that the origin of hypoxaemia is more likely due to decreased pulmonary oxygen diffusion rather than hypoventilation (Harris & Massie 2019; Hantzidiamantis & Amaro 2020). Hypoxaemia caused by hypoventilation alone should reflect in a normal A-a gradient (Grimm 2010).

Ventilation-perfusion mismatch is commonly reported in ruminants during chemical immobilization and is the result of abnormal positioning of the animal and the resulting increased weight of the viscera on the diaphragm (Read 2003). Furthermore, etorphine hydrochloride has been associated with the development of pulmonary hypertension in some animals, especially in small domestic livestock. Pulmonary hypertension can be the result of increased pulmonary vascular resistance, increased pulmonary blood flow and/or increase in the left atrial pressure. An increased pulmonary vasoconstriction can result from an increased sympathetic activation following opioid administration, hypoxic pulmonary vasoconstriction or other opioid-induced effects on the pulmonary vasculature (Meyer *et al.* 2015). Pulmonary hypertension can result in vascular congestion and the development of pulmonary oedema (Heard *et al.* 1996, Zeiler & Meyer 2017) resulting in oxygen diffusion deficits. Furthermore, an increased cardiac output following sympathetic system activation results in an increased blood flow and therefore a decreased oxygen transit time from the alveolar blood to arterial blood (Haw *et al.* 2015).

Hypoxaemia observed during opioid-induced immobilization can be compounded by an increased muscle rigidity and tissue oxygen consumption (Haw *et al.* 2015*)*.

In summary, opioid-induced hypoxaemia is not only caused by hypoventilation and increased muscle metabolism, but also by functional changes in the pulmonary vasculature. To alleviate opioid-induced hypoxaemia, measures should not only be focused on reversing opioid-induced respiratory depression but also on reducing pulmonary vasoconstriction and improving gas exchange (Meyer *et al.* 2015). Furthermore, studies have shown that physiological and psychological stress does play a role in the severity of hypoxaemia (Haw *et al.* 2015). Therefore, measures which decrease the individual stress response are important in alleviating severe hypoxaemia.

The use of alpha$_2$-agonists as an adjunct in chemical immobilization has been associated with hypoxaemia. This can partly be the result of respiratory depression induced by the use of alpha$_2$-agonists in combination with opioids. Furthermore, the cardiopulmonary effects of an alpha$_2$-agonist result in bradycardia, reduced cardiac output and hypotension (Sinclair 2003). Medetomidine hydrochloride had also been reported to cause pulmonary hypertension and vasoconstriction of pulmonary vasculature exacerbating hypoxaemia (Read 2003).

1.5.4 Capture myopathy

Hypoxaemia is linked to the development of capture myopathy (Read 2003). Capture myopathy is a non-infectious, metabolic outcome of chemical immobilization of wildlife that has been documented in various species, including terrestrial & sea mammals, non-human primates, birds and humans. Since it has first been described in 1964 in a hartebeest in Kenya, capture myopathy has been documented in many African ungulates (Breed *et al.* 2019). Under normal circumstances, wild animals do not engage in excessive exercise over a prolonged time. However, during the capture process, animals can be faced with extreme exertion (Harthoorn 1976). Capture myopathy in wild animals is similar to exertional rhabdomyolysis in humans and myo-degenerative disorders in domestic animals (Paterson 2007).

The clinical presentations of capture myopathy vary substantially, with death occurring within minutes up to weeks after immobilization. Capture myopathy can either be classified according to the time to the onset of clinical signs (hyper-acute, acute, subacute and chronic) or according to the clinical syndrome (capture shock syndrome, myoglobinuric syndrome, ruptured muscle syndrome and delayed per-acute syndrome). Furthermore, the classification is linked to the amount of stress and exertion perceived during the immobilization process (Harthoorn 1976). In the hyper-acute form, early findings may be exertion, hyperthermia and muscle tremors and animals can die within an hour of capture as a consequence of shock and cardiac failure due to hyperkalaemia and metabolic acidosis. The acute or myoglobinuric syndrome, which is seen within hours to days after capture, is characterized by ataxia, myoglobinuria and kidney failure. The ruptured muscle syndrome is usually seen weeks after capture and its findings are lameness, weight shifting and rupture of damaged muscles, predominantly the gastrocnemius muscles. The chronic form is usually seen during re-capturing of the animals and death is usually attributed to heart failure of acute onset or following chronic myocardial degeneration (Breed *et al.* 2019, Harthoorn 1976).

Capture myopathy has been observed in both chemically and non-chemically captured animals regardless of the terrain, environmental conditions, and length of handling or transport (Bartsch *et al*. 1977).

Some species are more susceptible to the development of capture myopathy than others: ungulates – especially giraffe, impala, nyala, kudu and springbok - are more prone to capture myopathy than carnivores. Moreover, individual differences exist: young and old animals, as well as pregnant and diseased animals, are more frequently affected by this condition (Paterson 2007).

Capture myopathy is the malignant outcome of stress and/or exertion; this may be fatal as a consequence of shock and metabolic acidosis (Harthoorn 1976). Rhabdomyolysis is believed to be one of the primary pathophysiological factors in the development of capture myopathy (Breed *et al.* 2019). An increased adrenergic activity and catecholamine release following the capture process can result in an increased heart rate, hyperthermia and perfusion impairments (Mentaberre *et al*. 2010). Perfusion impairment and hypoxia can result in anaerobic glycolysis and lactic acid build-up. Prolonged hypoxia and lactic acid build-up are associated with muscle fatigue, cellular damage and the release of intracellular components into the blood circulation. Intracellular components released are myoglobin, creatine kinase, phosphate and potassium (Breed *et al.* 2019, Read 2003). Rhabdomyolysis and associated electrolyte imbalances can have fatal consequences: hyperkalaemia can affect neuronal cardiac conduction resulting in atrial fibrillation and death. Myoglobinaemia in the face of metabolic acidosis has been associated with myoglobinuria, myoglobin precipitation, tubular necrosis and kidney failure followed by multi-organ failure (Breed *et al.* 2019).

Extreme stressors can cause adrenergic exhaustion of the animal resulting in neurogenic shock. At this point, the vascular tone is lost resulting in a decrease in blood pressure and cardiac output, hypo-perfusion and hypoxia. At maximal exertion, the amount of lactic acid produced results in a critical decrease of the blood pH. Acidaemia in combination with hyperkalaemia can cause cardiac arrest and death. Furthermore, severe acidaemia in combination with increased adrenergic activity can result in pulmonary hypertension followed by pulmonary oedema (Harthoorn 1976).

Besides rhabdomyolysis, hyperthermia is a major feature of capture myopathy. Exertional rhabdomyolysis and exertional heatstroke are considered to be the human comparatives to capture myopathy. Exertional heatstroke can be observed after strenuous exercise or in untrained individuals. The syndrome is also referred to as ‘march myoglobinuria’ as it is frequently seen in the first months of military training. Prolonged hyperthermia can exacerbate muscle injury and the release of intracellular components into the circulation

(Breed *et al.* 2019). Environmental factors such as ambient temperature or humidity can predispose to the development of capture-induced hyperthermia which can increase the risk of capture myopathy. Furthermore, the extent of capture-induced hyperthermia is proportional to the amount of stress perceived during the capture process (Meyer *et al*. 2008a).

The choice of the immobilizing agent plays a role in the development of capture myopathy. Drugs and toxins are the major cause of rhabdomyolysis in humans with opioids being the most frequent drug associated with rhabdomyolysis (Efstratiadis *et al.* 2007). Opioid-induced side effects which may increase the risk of capture myopathy are hyperthermia, muscle rigidity and respiratory depression (Paterson 2007). These effects may be exacerbated by the use of alpha$_2$-receptor agonists (Breed *et al.* 2019).

In summary, various internal and external factors contribute to the development of capture myopathy. Rhabdomyolysis, hyperthermia, adrenal exhaustion and hypoxia are the primary factors increasing the risk of developing capture myopathy.

1.5.5 Stress response and sympathetic activation

During chemical immobilization, the animal is faced with various capture-related stressors such as unusual noises, presence of the capture team, prolonged chase, transport and environmental stressors. Stress can be defined as the response of the organism to any condition which threatens the body's compensatory mechanism to maintain normal homeostasis (Arnemo & Caulkett 2007). Stress induces the release of hormones that prepare the organism for the perceived danger. If stress is severe and cannot be escaped from, prolonged arousal can result in severe physiological and psychological changes resulting in trauma, hyperthermia, capture myopathy, shock and/or death (Meyer 2010).

The welfare of an animal is closely related to its ability to adapt to different situations and environmental factors. If the animal cannot adapt, it becomes stressed. Stress is controlled by two neuro-endocrine regulatory systems namely the sympathetic nervous system and the hypothalamic-pituitary-adrenal system. The activation of the sympathetic system results in a fight-or-flight response by triggering the release of catecholamines (epinephrine and norepinephrine) from the adrenal medulla. Depending on the intensity of the stressor, the catecholamine concentration can increase to 300-fold within seconds. In response, heart rate and respiratory rate increase to improve oxygenation. Central vasodilation results in increased blood flow to the cardiac and skeletal muscles while peripheral vasoconstriction decreases blood perfusion to visceral organs. Splenic contraction and intravascular

coagulation increase to prevent blood loss from injuries. The extent of the response correlates with the concentration and ratio of the catecholamine release (Arnemo & Caulkett 2007).

Sympathetic system activation can also result from opioid receptor activation, hypoxaemia or pain (Heard *et al.* 1996). Etorphine hydrochloride has been shown to stimulate catecholamine release via activation of the sympathetic nervous system which can result in tachycardia in many species (Buss *et al.* 2016).

Besides the activation of the sympathetic nervous system, stress also activates the hypothalamus-pituitary-adrenal-axis (HPA axis). Corticotropin-releasing hormone (CRH) is released from the hypothalamus which stimulates the secretion of the adrenocorticotropic hormone (ACTH) from the adenohypophysis. ACTH circulates in the blood where it stimulates the release of glucocorticoids (cortisol and corticosterone) from the adrenal cortex. Glucocorticoids mobilize glycerol, fatty acids and amino acids to fuel the body in times of distress (Arnemo & Caulkett 2007). These short-term endocrine changes are necessary for the body to return to homeostasis and to cope with life-threatening situations.

The stress response can be assessed in various ways. Plasma cortisol - as well as salivary, urinary and faecal cortisol, is frequently measured to gain an insight into an animal's stress levels and well-being. However, cortisol levels are influenced by many factors such as species-specific differences, circadian and seasonal patterns, exercise, starvation, physiological state, emotional stress, type & method of drug administration. It is therefore important not to solely rely on cortisol levels as indicators of stress, but to also observe clinical and vital signs during immobilization (Arnemo & Caulkett 2007).

Lactate is formed during anaerobic glycolysis and its concentration can increase as a response to exertion, shock, tissue hypoxia and rhabdomyolysis (Lorenzo *et al*. 2020). It can therefore be used as an indicator of stress, muscle fatigue and capture myopathy (Mentaberre *et al.* 2010). In veterinary medicine, a precise definition of hyperlactatemia and lactic acidosis is not established yet. In humans, hyperlactatemia is present when the lactic acid concentration exceeds 2 mmol/l. Furthermore, lactic acidosis is present when the pH is below 7.35 (Pang & Boysen 2007). Two forms of lactic acidosis are differentiated. 'Type A' lactic acidosis results from oxygen shortage causing hypoperfusion of cells and tissue. 'Type B' lactic acidosis is characterized by normal blood oxygenation with lactic acidosis resulting from congenital, toxic or other causes (Pang & Boysen 2007).

An increased adrenergic activity and catecholamine release following capture stress can result in oxygen debt and tissue perfusion impairment. Metabolism shifts to anaerobic

glycolysis where lactic acid is formed. In human and veterinary medicine, lactic acid concentration can be used as a prognostic indicator for survival. Here, the initial lactate level is not the prognostic indicator, but rather the change in lactate levels in response to therapy (Pang & Boysen 2007).

It has been described that the risk of capture myopathy is severe after a short and intense period of muscular exertion. This is because during maximal intermittent exercise, the lactic acid build-up is higher than during continuous exercise. As lactic acid increases, the pH decreases and metabolic acidosis can develop. The body initiates compensatory mechanisms as a response to metabolic acidosis and increases respiration. This compensatory effort can be counteracted by the use of immobilizing drugs which depress respiration (McAllum 1978).

Alleviation of stress is an important factor during capture operations. Extreme stress and exertion can result in injuries, shock, hyperthermia, capture myopathy and death (Meyer 2010). Studies have shown that the central alpha$_2$-adrenergic receptor stimulation decreases the sympathetic output via a negative feedback mechanism. This can result in an overall decrease in the circulating catecholamine level and reduced stress response (Ambrisko & Hikasa 2002).

1.5.6 Thermoregulation

The drugs used during chemical immobilization can influence the animal's thermoregulatory ability. Capture-induced hyperthermia and respiratory depression are the most common capture-related morbidities and can result in death. Chemical immobilization usually increases the body temperature and the latter should therefore be monitored closely. Although the pathophysiology and significance are not well known, hyperthermia can predispose to capture myopathy and death. The reason for hyperthermia during capture operations can be multifactorial, including strenuous exercise, stress, high ambient temperature and choice of drugs (Meyer 2010).

A study performed during the physical and chemical immobilization of impala suggests that the extent of hyperthermia correlates with the amount of stress perceived during the capture process irrespective of the ambient temperature, activity level and capture process (Meyer *et al*. 2008a). As part of that study, a miniature temperature recording device was implanted in impala and temperature was recorded. Hyperthermia was greater in naïve animals than in habituated animals. This finding suggests that hyperthermia is subject to the amount of stress perceived during capture (Meyer *et al*. 2008a). Furthermore, the extent of capture

induced hyperthermia correlates with the induction time. Animals with a shorter induction time had a lower spike in body temperature. This finding was irrespective of the pharmacological compound used but correlated with the duration of consciousness before immobilization. However, individuals with shorter induction times were more likely to develop hypoxaemia and respiratory depression. Therefore, it is crucial to use a drug combination which balances between thermal liability and respiratory depression (Meyer *et al*. 2008b). Although these studies suggest, that hyperthermia develops irrespective of ambient temperature (Meyer *et al.* 2008a), heat exchange is compromised with high environmental temperatures. If the ambient temperature exceeds 36°C, the heat exchange gradient is reversed and heat is gained rather than lost (Wendt *et al*. 2007). Therefore, it is advised to plan capture operations during the cooler hours of the day and on cooler days.

Opioids play a role in thermoregulatory processes by directly influencing the hypothalamic thermoregulatory system. The opioid receptors which play a role in thermoregulatory processes are μ-, δ- and κ-receptors. The binding of μ-receptors induces hyperthermia, while κ-receptor activation produces hypothermia (Rawls & Benamar 2011). Hyperthermia has been observed in ruminants and horses immobilized with opioids. This can be the result of direct effects on the hypothalamic system and an increased CNS excitement and muscular activity following opioid administration (Grimm & Lamont 2007).

Studies on mice and rats suggest that the influence of opioids on thermoregulation is dose-dependent. In the latter, low doses of etorphine hydrochloride have induced hyperthermia, medium doses did not change core temperature and high doses resulted in hypothermia (Meyer *et al* 2008b).

Alpha$_2$-adrenoreceptors agonists also have the potential to cause thermal lability and affect thermoregulation. Hyperthermia has been reported in ruminants following medetomidine hydrochloride administration and it is advised that body temperature should be monitored for 12 - 24 hours post-capture (Grimm & Lamont 2007).

CHAPTER II: BACKGROUND AND RATIONALE

2.1 Introduction

African buffalo (*Syncerus caffer*) are frequently immobilized for veterinary interventions, disease screening and translocations. Classically, African buffalo have been immobilized with a potent opioid in combination with a tranquillizer or sedative, or both. Opioids are one of the most important drugs used for chemical immobilization of wildlife and are used as the primary immobilization agent of which etorphine hydrochloride and thiafentanil oxalate are the most commonly used (Grimm & Lamont 2007). They induce their clinical effects by acting at a variety of opioid receptors centrally, but have significant side-effects, particularly respiratory depression and in extreme situations, capture myopathy (Breed et al. 2019). Furthermore, pulmonary hypertension and muscular hypertonicity can be observed in animals immobilized with opioids. Opioids are frequently used in combination with tranquilizers or sedatives to mitigate this and other side-effects. Of these, the most commonly used are medetomidine and azaperone. In combination, opioids and sedatives have synergistic effects; medetomidine hydrochloride potentiates opioids and hence allows for reduced anaesthetic requirements. When given in a high dose, medetomidine hydrochloride can induce anaesthetic effects and is not without risk – respiratory depression, blood pressure and thermoregulatory disturbances have been described (Burroughs *et al*. 2012a). Capture related morbidities and mortalities are frequently seen during capture operations. Thus, for animal welfare and conservation reasons, it is in the interest of all parties involved to minimize capture-related morbidities and mortalities. Therefore, safe and readily repeatable immobilization protocols are important and in-depth understanding of the pharmacodynamics of the anaesthetic compounds is critical (Laubscher et al. 2015). Species-specific anaesthetic protocols are available and are revised continuously.

2.2 Problem statement

For the immobilization of buffalo, the conventional thiafentanil-azaperone (TA) combination has widely been used in the past and is described in Burroughs *et al.* (2012b). Due to the frequency, and associated costs, at which chemical immobilisations occur, as well as for safety reasons and previous difficulties in opioid supply, alternative and less potent immobilisation protocols have gained popularity over the last few years. An alternative anaesthetic regime on buffalo that is now being used extensively in many species of ruminants and non-ruminants in southern Africa as routine immobilizing regime, with the exception of megaherbivores, is based on a low thiafentanil dose in combination with

medetomidine and azaperone (TMA). The opioid doses that are used are 20-30% of the current standard species-specific protocols and is combined with medetomidine (which other published protocols do not) and are considered to be a more affordable combination. As there is little information available on the efficacy and safety of these alternative combinations, this study was to evaluate and compare the physiological effects of two different immobilization protocols in African buffalo.

2.3 Aims and objectives

This study aimed to evaluate and compare the induction times, quality of immobilization and physiological effects of the thiafentanil-azaperone (TA) versus thiafentanil-medetomidine-azaperone (TMA) immobilization in African buffalo. The objective of the above was to establish if either combination could induce a safer and more efficient immobilization in African buffalo.

2.4 Hypothesis

This study was aimed at comparing the induction times and physiological effects of two immobilization protocols in African buffalo (*Syncerus caffer*).

H0: The induction times and cardiopulmonary effects in African buffalo (*Syncerus caffer*) immobilized with the thiafentanil-medetomidine-azaperone combination will not be significantly different from those of the thiafentanil-azaperone combination.

H1: The induction times and cardiopulmonary effects in African buffalo (*Syncerus caffer*) immobilized with the thiafentanil-medetomidine-azaperone combination will be significantly different from those of the thiafentanil-azaperone combination.

CHAPTER 3: MATERIALS AND METHODS

The study was approved by the Animal Ethics Committees of the University of Pretoria (REC241-19) (APPENDIX II). Funding was granted from the University of Pretoria, as well as from V-Tech (Pty) Ltd Pharmaceutical Company.

3.1 Experimental design

This was a prospective, randomized cross-over study.

3.2 Animals and model design

Twelve African or Cape buffalo (*Syncerus caffer*) bulls between two-and-a-half to four years of age were used for this project. The study took place in October/November 2020 at Lekkerleef Wild Stoet located in the Sekhukhune District Municipality, Limpopo, South Africa (S:24˚57”50.6’, E 29˚11”12.1’) (Figure 1).

Figure 1: Location of Lekkerleef Wild Stoet near Marble Hall, Limpopo Province, South Africa.

All study animals are permanently kept in breeding camps at Lekkerleef Wild Stoet. The immobilization and data collection were carried out under a roofed boma of 30 x 50 m. The boma consists of twenty-two pens of 5 x 5 m and three pens of 3 x 5 m and three passageways. The reason for the immobilization in the boma was to create a controlled environment where the buffalo could be weighed and where variables and bias could be kept at a minimum. Immobilization took place throughout the day. Only one animal was immobilized at a time.

The study was conducted over three periods in October and November 2020. The data which was collected during the first period was not included in this study as the immobilization took place in a different pen which was believed to have caused unnecessary stressors and possible errors. However, the accurate weight of each buffalo could be obtained during the first period, which was used to calculate the exact drug dosage per kilogram body weight, and is therefore referred to as the weighing period.

During the second and third period, the data collection periods, each buffalo was immobilized once with either the thiafentanil-azaperone (TA) or thiafentanil-medetomidine-azaperone (TMA) combination. Six buffalo received the TA combination and six buffalo received the TMA combination. After a three-week washing out period the animals received the alternative combination. The two combinations used included:

- Combination TA: Buffalo of this combination were immobilized with the conventional **thiafentanil-azaperone (TA)** immobilization protocol as described by Burroughs *et al.* (2012) with each animal receiving 6-7 mg thiafentanil oxalate (Thianil®, 10 mg/ml, Wildlife Pharmaceuticals, White River, Mpumalanga, South Africa) and 40 mg azaperone (Azaperone, 100 mg/ml, V-tech (Pty) Ltd., Johannesburg, South Africa) intramuscularly.

- Combination TMA: Buffalo of this combination received a **thiafentanil-medetomidine-azaperone (TMA)** immobilization protocol combining a relatively low dose of thiafentanil oxalate (Thianil®, 10mg/ml, Wildlife Pharmaceuticals, White River, Mpumalanga, South Africa) at 1 mg/animal with medetomidine hydrochloride (Medetomidine HCl, 50 mg/ml, V-tech (Pty) Ltd., Johannesburg, South Africa) at 3-4 mg/animal and azaperone (Azaperone, 100 mg/ml, V-tech (Pty) Ltd., Johannesburg, South Africa) at 40 mg/animal (Table 3).

The individual dart mixture was made up according to the estimated weight and body condition score (BCS) of each buffalo as visualized by the immobilization team

3.3 Buffalo immobilization

The preparation and application of the dart were implemented by an experienced veterinarian registered with the South African Veterinary Council (SAVC). The buffalo were darted using a Pneu-dart cartridge-fired dart projector (Model 389, Pneu-dart®, Williamsport, Pennsylvania, USA). A 1.5 cc Motsumi Stinger dart (Motsumi Darts®, Pretoria, South Africa) with a 1 ¼ inch wire barbed needle was used for drug delivery. All darts were filled up with physiological saline (Sodium Chloride Fresenius 0.9%, 1000ml, Fresenius Kabi Manufacturing SA (Pty) Ltd, Port Elizabeth, South Africa) to ensure that each animal received the same volume.

3.4 Experimental procedure

One day before each immobilization period, the buffalo were brought from the breeding camps into the boma and food and water were withheld for 24 and 12 hours, respectively, to prevent regurgitation.

Weighing period: Due to the management capabilities of the farm, the weight of the buffalo could only be obtained once the buffalo were immobilized. Therefore, the buffalo were dosed according to their estimated weight and body condition score (BCS). During the weighing period each buffalo was immobilized separately in a weighing pen of 3 x 5 m dimension. The weighing pen was enclosed by a 2.4 m solid and non-transparent wall (Figure 2a). At the end of each procedure, the buffalo were placed on a scale. The scale consisted of a steal plate (Figure 2b) which was placed onto two load bars and which was connected to a digital readout instrument (Gallagher TW-1 Livestock Scale Indicator) (Figure 2c). The obtained true body weights of the buffalo were used to calculate the exact drug dosage per kilogram body weight.

Figure 2: *a-c*: Weighing pen with scale, immobilized buffalo on steel plate, Gallagher Scale Indicator.

Data collection period: Each buffalo was immobilized twice during the two data collection period using each immobilization protocol once, allowing a twenty-one-day washing-out period between the two immobilizations. Each animal was immobilized for a minimum of 40 min before the full reversal was administered. The immobilization process consisted of the induction phase, immobilization phase and recovery phase. Quality of induction and immobilization were recorded subjectively with a scoring system (Table 1 & 2).

Before each immobilization, the specific buffalo to be worked on was separated from the rest of the herd and moved into an adjoining pen of 5 x 5m. In this way, the buffalo could be worked on safely.

Induction

After dart preparation, the buffalo was darted from a 3-5 m distance into the right or left gluteal muscle area. Once the drugs were administered, the time to induction and recumbency were recorded. The time to induction was defined as the time between dart placement and the time to the first visible signs of sedation. The time to recumbency was defined as the time between dart placement and the time the buffalo became recumbent and could be approached safely

Table 1: Induction score 1-4.

		Characteristics	
Score	1	Excellent	No CNS excitation. Slight ataxia. No or minimal movement after dart placement. Smooth and quick transition to recumbency.
	2	Good	Mild CNS excitation. Moderate ataxia and physical activity (pacing). Smooth transition into recumbency.
	3	Fair	Severe CNS excitation. Extensive physical activity (circling) and ataxia. Attempts to jump over the fence. Prolonged time to reach recumbency.
	4	Poor	Mild CNS excitation. Moderate physical activity (walking) and ataxia. Failure to reach immobilization but can be assisted into recumbency.

Immobilization

Once the animal was immobilized and/or could be approached safely, the animal was placed into and maintained in sternal recumbency and instrumented within five minutes. Blindfolds were used to reduce exposure to external stimuli. The left or right caudal auricular artery was aseptically prepared and cannulated by a 22-gauge catheter (Jelco®, Smiths Medical, Lancashire, United Kingdom) to allow for ongoing arterial blood pressure measurement and blood sampling (Figure 3a). The systolic (SAP), mean (MAP), and diastolic (DAP) arterial pressure were measured in five-minute intervals using a pressure monitor (IntraTorr, IntraVitals, United Kingdom) (Figure 3b) which was connected to a pressure transducer with non-compliant tubing (Deltran disposable pressure transducer DPT 200, Utah medical products incorporated, USA). The pressure transducer was zero-calibrated at the level of the heart.

Before each sample collection, 0.5-1 ml waste blood was drawn and discarded. Blood samples were then collected into sodium heparinized 1 ml syringes at 10-, 20- and 35-min post immobilization (Figure 3c). The sample was analysed immediately with a portable blood gas analyser (epoc® SIEMENS Blood Analysis System, Siemens Healthcare GmbH, Erlangen, Germany) (Figure 4c) using single-use epoc® BGEM Test Cards. The blood values

measured during immobilization were the partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), pH, blood lactate and glucose. The values were not corrected for body temperature.

The following physiological values were measured additionally at a five-minute interval for 40 minutes:

- Systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure (mmHg) was measured using an intra-arterial blood pressure monitor (IntraTorr, IntraVitals, United Kingdom) that was connected with non-compliant tubing to an electronic pressure transducer (Deltran disposable pressure transducer DPT 200, Utah medical products incorporated, USA) (Figure 3b).

Figure 3: *a-c*: Sterile catheterization of the caudal auricular artery, intra-arterial blood pressure monitor, arterial blood sampling for blood gas analysis.

- The heart rate was determined by auscultation over sixty seconds
- The respiratory rate was measured by counting chest movements or air movements at the nares for sixty seconds
- Peripheral oxygen haemoglobin saturation (SpO_2) was measured using a pulse oximeter (Veterinary Pulse Oximeter, Model 9847V, Nonin Medical, USA) with the probe placed on the 3rd eyelid (Figure 4a).
- A multi-parameter monitor (M3T Mini vet, TooToo Meditech, China) was used to measure end-tidal carbon dioxide ($ETCO_2$) with a 10 mm internal diameter cuffed endotracheal tube placed into the nasal cavity (Figure 4a).

- A digital thermometer (HI98509 Checktemp 1, HANNA Instruments (Pty) Ltd., USA) was inserted into the rectum to measure body temperature (C°). The same device was used for the environmental temperature before each immobilization (Figure 4b).

Figure 4: *a-c*: Placement of pulse oximeter and veterinary monitor, HANNA digital rectal thermometer and epoc® SIEMENS Blood Analysis System.

The quality of immobilization and anaesthetic plane was recorded every 5 minutes using a 1-4 scoring system and was determined subjectively by the primary investigator (Table 2).

Table 2: Immobilization score 1-4.

		Characteristics	
Score	1	Deep	Buffalo remains recumbent without any attempts to stand up. Palpebral reflex reduced or absent. Reduced muscle rigidity, no involuntary movements of head and tail.
	2	Moderate	Buffalo remains recumbent without any attempts to stand up. Response to tactile/audible stimuli (e.g., when cannulated). Palpebral reflex reduced or absent. Mild muscle tremors and rigidity observed. Spontaneous muscle movements possible.
	3	Light	All characteristics of 2. Palpebral reflex intact. Voluntary head movements.
	4	Awake	Minimal immobilization. Attempts to stand up. Head movement.

Recovery

At the end of each procedure (T40), each buffalo was de-instrumented, immobilization drugs antagonized and the recovery times recorded.

- The **TA combination** was antagonized with naltrexone hydrochloride (Naltrexone HCl, 40 mg/ml, Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) intravenously in the caudal auricular vein. For each mg of thiafentanil oxalate (Thianil®, Wildlife Pharmaceuticals, 10mg/ml), 10 mg of naltrexone hydrochloride (Naltrexone HCl, 40mg/ml, Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) was administered
- The **TMA combination** received a standardized mixture of yohimbine (Yohimbine, 6.25 mg/ml, Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa) and atipamezole (Atipamezole, 20 mg/ml, V-tech, Johannesburg, South Africa) intravenously followed by naltrexone hydrochloride (Naltrexone HCl, 40 mg/ml, Kyron Laboratories (Pty) Ltd, Johannesburg, South Africa). The atipamezole-yohimbine combination contained 2 mg of atipamezole for each ml of yohimbine. Each animal received 0.5 ml atipamezole-yohimbine mixture per mg medetomidine. Naltrexone hydrochloride was given at 10 mg per 1 mg thiafentanil oxalate. The atipamezole-yohimbine combination was the method of choice by the supervising veterinarian, and is commonly used by practitioners in SA.

After antagonist administration, the recovery times (time to first signs of recovery, time to standing up) were recorded. All buffalo were observed for 24 hours post-recovery for signs of re-narcotization.

3.5 Data collection

All data collected was recorded in individual data sheets which included the date, time, and identification, BCS, body weight and age (APPENDIX I). The doses of the immobilizing agent and recovery drugs were recorded. The physiological values were measured and recorded every 5 minutes for the duration of 40 minutes. The quality of induction, as well as the induction time, was noted on the sheets. Arterial blood was drawn at 10-, 20- and 35-minutes post immobilization, analysed and printed on-site with a mobile printer (Zebra iMZ320 Mobile Printer, Zebra Technologies Asia Pacific (Pty) Ltd., Singapore).

3.6 Data analysis

Data analysis was performed using commercially available IBM SPSS Statistical software (IBM SPSS Statistics Version 25.0; International Business Machines Corp; Released 2017; NY, USA) and evaluated for normality by calculation of descriptive statistics, plotting histograms, and by performing Shapiro-Wilk test. Quantitative data were described using mean, ± standard deviation (SD) and ranges. Results were interpreted at a 5% significance level ($p < 0.05$). Paired sample t-tests were used to compare the clinical and physiological data obtained between the two combinations over 40 minutes.

The alveolar oxygen tension (PAO_2) was calculated from the alveolar gas equation $PAO_2 = (Patm - PH_2O)\ FiO_2 - PaCO_2/RQ$.

FiO_2 is the fractional concentration of inspired oxygen (21%), Patm is the atmospheric pressure calculated at an altitude of 910 m for the Marble Hall district (683 mmHg), PH_2O the water vapour pressure of saturated air in the alveoli (47 mmHg) and R the gas exchange ratio (Sharma *et al.* 2020). Total costs of drugs associated with the chemical immobilization of the buffalo with the TA and TMA regime were calculated and compared descriptively.

3.7 Biosecurity

Full safety with respect to handling the animals and immobilizing drugs were observed, with appropriate COVID-19 safety protocols. All immobilizations were be done by a competent and experienced veterinarian. All hazardous waste will be disposed of according to standardized protocols.

CHAPTER 4: RESULTS

4.1 General research variables

The data from ten adult African buffalo bulls are shown in Table 3. Age ranged between 2 ½ and 4 years and the mean body weight (range) was 511 (428.5 to 635) kg at the time of the study. All buffalo were deemed healthy on visual examination before dart placement and during chemical immobilization. The sample collection took place on three occasions over a period of six weeks in October/November 2020. The study process during the data collection period progressed according to the study design. Ten of the twelve buffalo completed the trial. One buffalo had to be excluded due to an inability to place an arterial catheter and one buffalo due to inadequate dart placement. The individual drug dosage (mg/kg) could be calculated with the true body weight obtained during the weighing period. The actual mean dosage (range) of the TA combination was 0.0136 (0.011 to 0.0163) mg/kg thiafentanil and 0.0792 (0.063 to 0.093) mg/kg azaperone; and 0.00216 (0.0016 to 0.0023) mg/kg thiafentanil, 0.00688 (0.0047 to 0.0084) mg/kg medetomidine and 0.0688 (0.047 to 0.084) mg/kg azaperone for the TMA combination.

4.2 Induction times and quality of induction

All buffalo were successfully immobilized for a minimum of 40 minutes regardless of which protocol had been used. No buffalo required any additional drugs to reach immobilization. A high-stepped gait and forward pacing could be observed as the most consistent sign of induction (Figure 5). CNS excitation was mild to moderate in most buffalo and was scored on a scale from 1-4 (Table 1, Table 4).

Figure 5: Buffalo with gait changes as first signs of induction.

Table 3: ID, age, body weight, BCS and drug dosages administered to each buffalo during the two immobilization periods.

		Age	BW (kg)	BCS	TA Combination Dosages				TMA Combination Dosages					
Animal	ID				*Thiafentanil (mg, mg/kg)*		*Azaperone (mg, mg/kg)*		*Thiafentanil (mg, mg/kg)*		*Medetomidine (mg, mg/kg)*		*Azaperone (mg, mg/kg)*	
1	B 999	3	517.5	4	7	0.0135	40	0,077	1	0.0019	4	0.077	40	0.077
2	B 36	3 1/2	467.5	4	7	0.0149	40	0,085	1	0.0021	3	0.0064	30	0.064
3	B 12	3 1/2	441.5	4	6	0.0136	40	0,091	1	0.0023	3	0.0067	30	0.067
4	B 45	3	523.5	4	7	0.0134	40	0,076	1	0.0019	4	0.0076	40	0.076
5	G 44	4	635	5	7	0.0110	40	0,063	1	0.0016	3	0.0047	30	0.047
6	G 42	4	596	4	7	0.0118	40	0,067	1	0.0017	4	0.0067	40	0.067
7	O 3	2 1/2	515.5	4	7	0.0135	40	0,078	1	0.0019	3	0.0058	30	0.058
8	G 56	4	510	4	7	0.0137	40	0,078	1	0.0019	4	0.0078	40	0.078
9	B 24	3 1/2	428.5	4	7	0.0163	40	0,093	1	0.0023	3	0.0070	30	0,07
10	O 7	3	477.5	4	7	0.0146	40	0,084	1	0.0021	4	0.0084	40	0,084

Only two buffalo, both of them immobilized with the TMA combination, did not reach immobilization on their own within a time-frame of twenty minutes post dart placement. However, these buffalo could be approached safely and could be assisted into recumbency. After immobilization, these animals were considered to be adequately anaesthetized and were therefore not excluded from the study.

A difference in behaviour was observed between the two combinations after dart placement. Some buffalo immobilized with the TMA combination showed a relatively smooth induction with a wide-based stance and reduced physical activity, while others showed visible excitement after drug administration with marked pacing throughout induction and stumbling into recumbency. Buffalo immobilized with the TA combination showed a more consistent induction characterized by a high-stepping gait and hypermetria. Some buffalo were pushing into the enclosed fence before falling into recumbency. It was observed that all buffalo that showed excitement during induction with the TA combination were also more likely to show excitement during induction with the TMA combination and vice versa.

Significant differences ($p < 0.001$) were found when comparing induction times between the two combinations. The induction times for the TA combination were significantly shorter compared to the TMA combination (Figure 6, Table 4).

Table 4: Induction times & scores during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

Immobilization				
Combination		First Signs (sec)	Immobilized (sec)	Induction Score
TMA	Mean (SD)	**264** ± 31	**657** ± 235	**2.50** ± 0.9
	Range	210 - 360	360 - 1200	
TA (	Mean (SD)	**127.5** ± 25.7	**342** ± 95	**1.90** ± 0.7
	Range	90 – 150	240- 570	

The average time (range) to immobilization was 5.7 (4 – 9.5) minutes and 10.95 (6 – 20) minutes for the TA and TMA combination, respectively. It took on average twice as long to

immobilize a buffalo with the TMA vs the TA combination. There was an increased range and SD observed for the induction times for the TMA combination, indicating that the TMA combination was more variable and less consistent than the TA. Induction quality tended to be superior in the TA compared to the TMA combination as represented by the induction score.

4.3 Quality of immobilization

The quality of immobilization was assessed every 5 minutes for 40 minutes based on a scoring system. The immobilization score is based on the subjective evaluation of the primary investigator (Table 2).

In general, all buffalo were immobilized successfully for 40 minutes with either of the two combinations. All buffalo went or could be assisted into sternal recumbency and remained recumbent for the duration of the procedure. There was no significant difference between the two combinations with regards to the immobilization score. The mean immobilization score was 1 and 1.4 for the TMA and TA combination, respectively. More buffalo immobilized with the TA combination showed an immobilization score of greater than 1 (n = 5). One animal immobilized with the TA combination showed a light and insufficient plane of immobilization towards the end of the procedure (T35). Visual examination revealed differences in characteristics of immobilization between the two immobilization protocols. Buffalo immobilized with the TA combination showed generalized muscular rigidity and noticeable muscle tremors predominantly in the ears and tail area. Furthermore, involuntary movements of tail, limbs and head were more frequently observed in the TA combination. One animal of the TA combination showed signs of regurgitation.

4.4 Physiological variables

The time was recorded from the moment a buffalo was immobilized and could be approached and instrumented safely (T0). All buffalo went into sternal recumbency. The buffalo (n = 2) that did not reach immobilization on their own were assisted into sternal recumbency. After blindfolding, the buffalo were instrumented and physiological variables were taken at five-minute intervals.

Anaesthetic monitoring data:

The heart rate was significantly different ($p < 0.001$) between the two immobilization protocols and was on average lower in the TMA combination. An average heart rate (SD) of 139 bpm (± 25) was measured in the TA combination with a transient, non-significant decrease over time. An average heart rate (SD) of 70 bpm (± 27) was recorded in the TMA combination with stable values over time (Figure 8, Table 6).

The immobilization took place in a wide range of environmental temperature (24.5 – 37.4 °C). No significant differences were found when comparing the body temperature between the two immobilization protocols. A mean temperature (SD, range) of 40.5 (± 1.3; 38.8 – 43.8) °C for the TMA and 40.3 (± 0.8; 39.2 – 41.8) °C for the TA combination was recorded. All buffalo developed hyperthermia (> 39.5 °C). In both combinations, the body temperature increased non-significantly over the first 5 - 10 minutes and then remained stable over time (Table 6). No statistically significant linear relationship was found between environmental temperature and body temperature.

The respiratory rates were similar between the two combinations. The mean (SD, range) breaths per minute were 33 (± 22; 13 – 83) and 37 (± 18; 9 - 64) for the novel TMA and standard TA combination, respectively (Figure 9, Table 6). The increased SD and range in the TMA combination can reflect variable results and individual differences in response to the drugs. However, respiration in the TMA combination appeared subjectively more regular with even chest expansions compared to the TA combination.

The SpO_2 showed a significant difference between the two combinations ($p = 0.024$). All buffalo were considered hypoxaemic. However, buffalo were significantly less hypoxaemic [SpO_2: 73 (± 12)%] when the TA combination was used compared to the TMA combination [SpO_2: 67 (± 17)%]. In the TMA combination, SpO_2 decreased significantly ($p = 0.037$) between T5 and T20 from 73% to 64% (Table 6).

No significant difference was found when comparing the $ETCO_2$ results - values appeared to be stable over time in the TA combination and increased insignificantly in the TMA combination over time.

There was no significant difference in the systolic, diastolic and mean blood pressure between the two combinations and the pattern of change was considered the same for SAP,

DAP and MAP. Hypertension was present with both immobilization protocols but was clinically more profound (although not statistically significant) in the TMA combination at the beginning of immobilization. At T5 the measured systolic arterial blood pressure was 170 (± 31) mmHg and 159 (± 35) mmHg in the TMA and TA combination, respectively. All blood pressure values decreased significantly in the TMA combination [SAP (p = 0.029), DAP (p = 0,018), MAP (p = 0.043)] over the first twenty minutes and then increased insignificantly again at the end of immobilization. The systolic arterial blood pressure at 40 minutes (T40) was 160 (± 37) and 163 (± 22) in the TMA and TA combination, respectively. The blood pressure variables of the TA immobilized buffalo decreased insignificantly in the first fifteen minutes and then increased significantly for the DAP (p = 0.006) and MAP (p = 0,04) and transiently for SAP over time to a value higher than initially recorded (Figure 10 - 12, Table 7).

Blood gases:

The arterial blood gas analysis revealed the presence of hypoxaemia and a widened alveolar-arterial (A-a) gradient in all buffalo regardless of the combination used. There was a significant difference in the PaO_2 ($p < 0.05$), A-a gradient ($p < 0.01$) and pH ($p < 0.01$) between the two combinations (Table 8). All buffalo were hypoxaemic during immobilization but were significantly more hypoxaemic in the TMA combination [PaO_2: 44 (± 14; 24 – 77) mmHg] compared to the TA combination [PaO_2: 51 (± 13; 33 – 80) mmHg] ($p = 0.032$).

Table 5: Classification and extend of hypoxaemia during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

Classification	TMA	TA	Total
No hypoxaemia (>80 mmHg)	0	0	0
Mild hypoxaemia (60 – 79 mmHg)	1	3	4
Moderate hypoxaemia (40 – 59 mmHg)	4	5	9
Severe hypoxaemia (< 40 mmHg)	5	2	7

Seven buffalo were considered as severely hypoxaemic (<40 mmHg), nine as moderately hypoxaemic (40-59 mmHg) and four as mildly hypoxaemic (60-79 mmHg) (Table 5, Figure 7). The mean PaO_2 level in the TMA combination increased insignificantly over time and remained stable in the TA combination (Figure 13).

There was no significant difference between the $PaCO_2$ values between the two combinations and values appeared stable over time. According to the recorded $PaCO_2$ values, eleven out of twenty buffalo - five of the TMA combination and six of the TA combination - were considered hypercapnic (> 42 mmHg). The mean $PaCO_2$ level (SD) was 44 (± 10) mmHg in the TA and 39 (± 11) mmHg in the TMA combination (Figure 7).

The A-a gradient was significantly different between the two combinations ($p < 0.001$) with a mean (SD) of 27 (± 13) mmHg in buffalo immobilized with the TA, and 40 (± 9) mmHg with the TMA combination. Over time, the A-a gradient decreased significantly ($p = 0.004$) in the TMA combination from 42 mmHg at T10 to 36 mmHg at T35. In the TA, the A-a gradient decreased insignificantly from 27 mmHg at T10 to 25 mmHg at T20 and then increased significantly ($p = 0.027$) to 30 mmHg at T35 (Table 8, Figure 15). In general, the A-a gradient was significantly wider ($p < 0.001$) in the TMA combination when compared to the TA combination.
A significant difference ($p < 0.001$) was found in the blood pH between the two treatment groups. All buffalo were acidotic with a mean (SD) pH value of 7.39 (± 0.54) and 7.33 (± 0.52) in the novel TMA and standard TA combination, respectively. pH values increased insignificantly over time in both treatment groups.

There was no significant statistical difference between the two combinations with regards to the glucose and lactate levels. The mean glucose level (SD) was 9.24 (± 2.91) mmol/l and 7.96 (± 2.49) mmol/l in the TMA and TA combination, respectively (Table 8). Lactate concentration was on average (SD) 4.76 (± 4.19) mmol/l in the TA and 3.99 (± 3.82) mmol/l in the TMA combination (Table 8).

Table 6: Physiological variables during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo. Results are reported as mean and ± standard deviation. Statistical significant difference between the two combinations is marked as *p<0.05 or **p<0.01.

		Temperature (°C)		Heart Rate**		Respiratory Rate		SpO_2*		ET CO_2	
Time		TMA	TA	TMA	TA	TMA	TA	TMA	TA	TMA	TA
5	Mean	40.5	40.1	67	148	32	40	73	74	44	53
	SD	*1.4*	*0.7*	*29*	*32*	*18*	*16*	*16*	*11*	*10*	*10*
10	Mean	40.6	40.3	69	146	32	38	65	73	46	56
	SD	*1.4*	*0.7*	*25*	*26*	*21*	*18*	*14*	*12*	*15*	*10*
15	Mean	40.6	40.4	71	142	34	38	64	71	47	55
	SD	*1.3*	*0.8*	*26*	*24*	*22*	*19*	*19*	*14*	*18*	*13*
20	Mean	40.5	40.3	70	142	32	36	63	71	53	56
	SD	*1.3*	*0.8*	*28*	*22*	*22*	*18*	*19*	*14*	*16*	*11*
25	Mean	40.5	40.3	73	134	33	36	64	71	52	54
	SD	*1.3*	*0.8*	*31*	*22*	*23*	*19*	*20*	*14*	*18*	*11*
30	Mean	40.5	40.3	73	135	35	36	68	73	52	53
	SD	*1.3*	*0.8*	*31*	*25*	*27*	*19*	*20*	*12*	*17*	*12*
35	Mean	40.4	40.2	70	135	35	37	70	74	54	52
	SD	*1.3*	*0.8*	*29*	*25*	*25*	*20*	*19*	*13*	*17*	*12*
40	Mean	40.3	40.2	68	127	34	36	71	75	53	54
	SD	*1.3*	*0.8*	*25*	*24*	*23*	*18*	*15*	*11*	*18*	*14*
Total	Mean	40.5	40.3	70	139	33	37	67	73	50	54
	SD	*1.3*	*0.8*	*27*	*25*	*22*	*18*	*17*	*12*	*16*	*11*

Table 7: Blood pressure (SAP, DAP and MAP) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo. Results are reported as mean and ± standard deviation.

		SAP		DAP		MAP	
Time		TMA	TA	TMA	TA	TMA	TA
5	Mean	170	159	128	122	147	137
	SD	*31*	*35*	*24*	*31*	*30*	*31*
10	Mean	163	145	112	111	131	128
	SD	*27*	*39*	*29*	*30*	*29*	*33*
15	Mean	162	145	107	113	126	129
	SD	*32*	*41*	*35*	*30*	*35*	*30*
20	Mean	161	152	105	121	125	134
	SD	*32*	*35*	*34*	*37*	*34*	*31*
25	Mean	161	157	107	124	126	137
	SD	*33*	*37*	*33*	*32*	*31*	*34*
30	Mean	165	159	114	123	130	139
	SD	*31*	*30*	*28*	*32*	*31*	*30*
35	Mean	158	162	112	126	130	142
	SD	*35*	*22*	*33*	*27*	*33*	*25*
40	Mean	160	163	113	126	131	144
	SD	*37*	*22*	*37*	*22*	*38*	*19*
Total	Mean	163	155	113	121	131	136
	SD	*31*	*32*	*31*	*29*	*32*	*29*

Table 8: Blood gases (pH, PCO_2, PO_2, A-a Gradient, Lactate, Glucose) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo. Results are reported as mean and ± standard deviation. Statistical significant difference between the two combinations is marked as *p<0.05 or **p<0.01.

		pH**		pCO_2		pO_2*		A-a Gradient**		Lactate		Glucose	
Time		TMA	TA	TMA	TA	TMA	TA	TMA	TA	TMA	TA	TMA	TA
10	Mean	7.38	7.31	38	42	42	53	44	28	4.28	5.55	8.65	7.77
	SD	*.044*	*.046*	*11*	*9*	*15*	*14*	*9*	*15*	*3.91*	*4.58*	*2.79*	*2.10*
20	Mean	7.39	7.32	39	46	43	50	42	25	3.73	4.35	9.36	8.38
	SD	*.055*	*.048*	*12*	*10*	*15*	*14*	*8*	*12*	*3.48*	*3.58*	*2.86*	*2.56*
35	Mean	7.40	7.36	41	43	47	51	36	30	3.97	4.33	9.72	7.74
	SD	*.065*	*.053*	*12*	*12*	*13*	*13*	*7*	*13*	*4.52*	*4.60*	*3.27*	*2.95*
Total	Mean	7.39	7.33	39	44	44	51	40	27	3.99	4.76	9.24	7.96
	SD	*.054*	*.052*	*11*	*10*	*14*	*13*	*9*	*13*	*3.81*	*4.20*	*2.91*	*2.49*

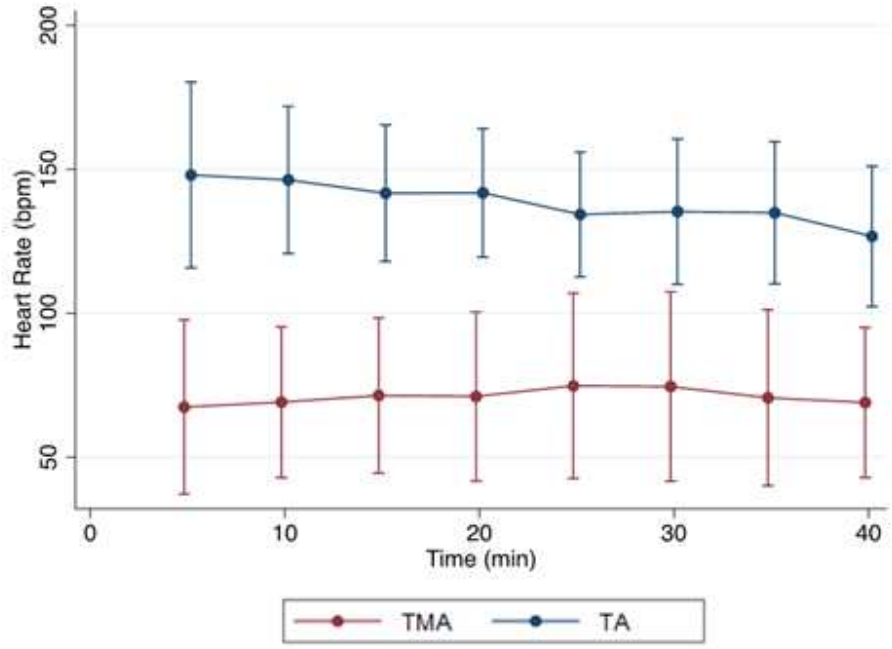

Figure 6: Comparison of the mean heart rate (bpm) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

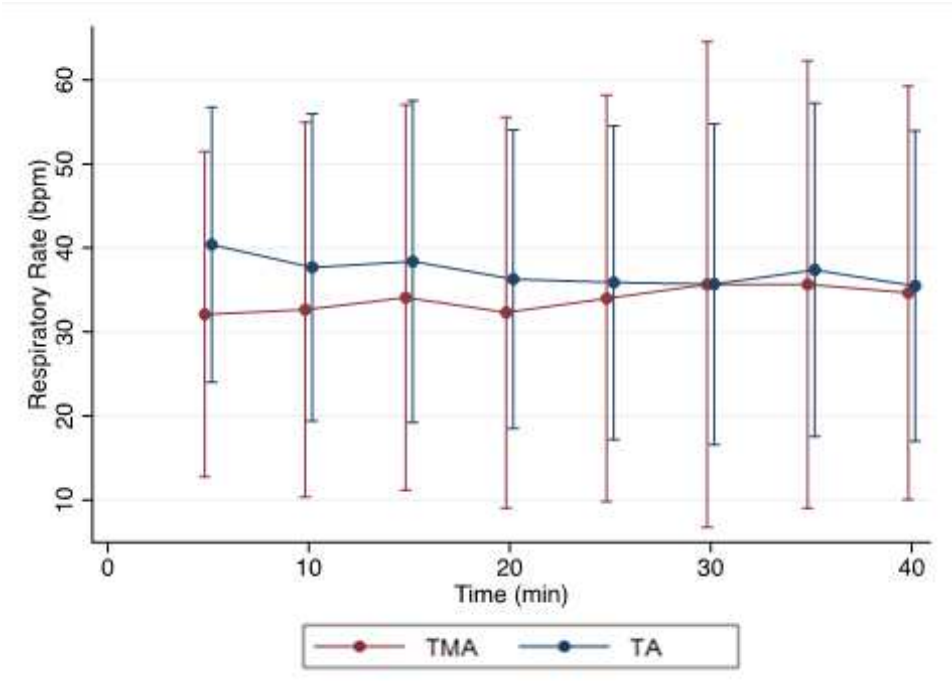

Figure 8: Comparison of the mean respiratory rate (bpm) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

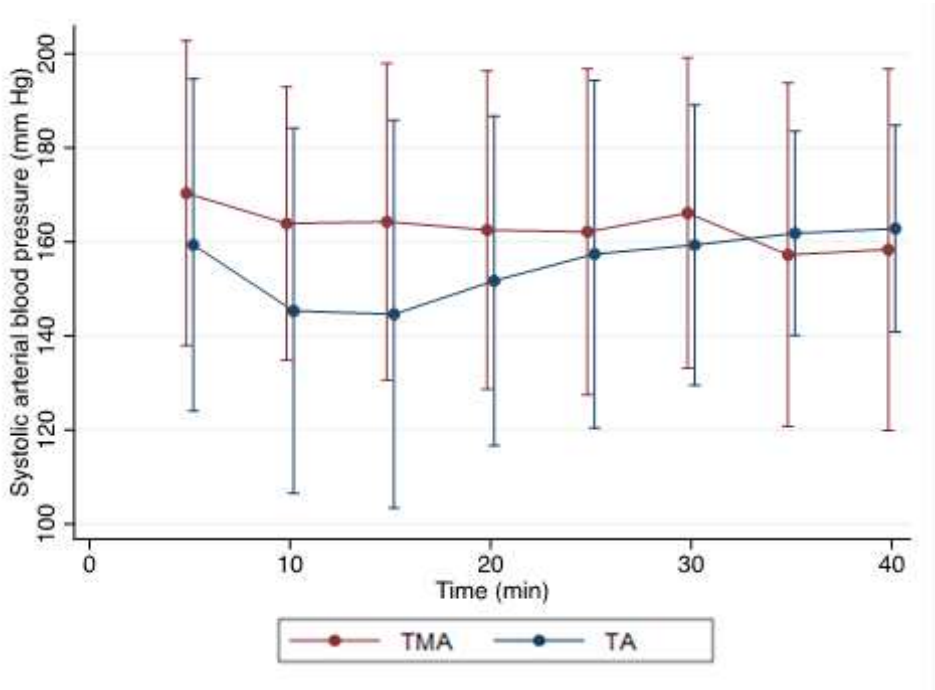

Figure 7: Comparison of the systolic arterial pressure (SAP) (mmHg) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

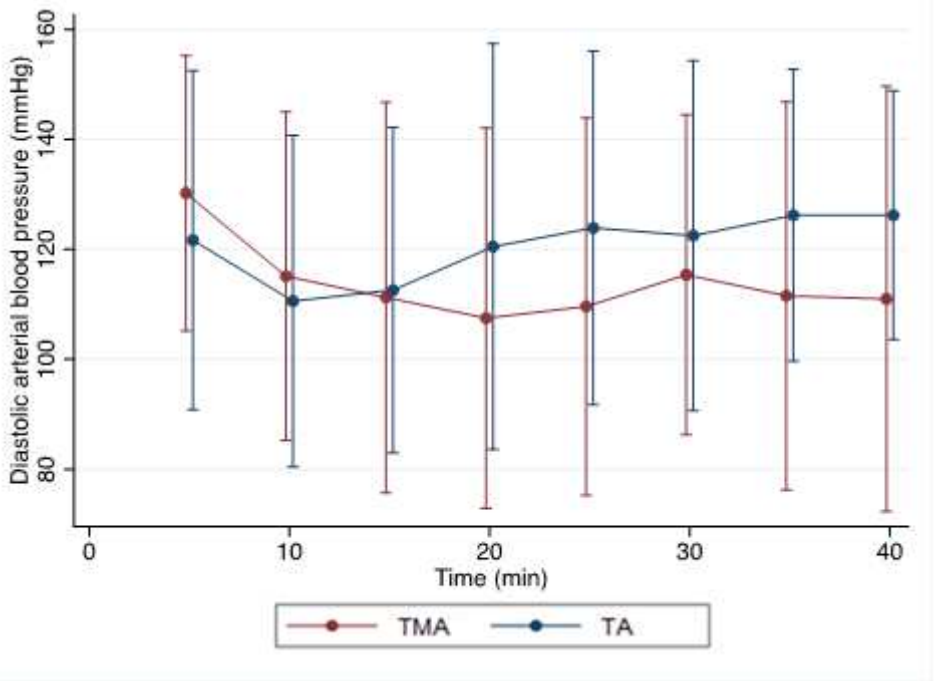

Figure 9: Comparison of the diastolic arterial pressure (DAP) (mmHg) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

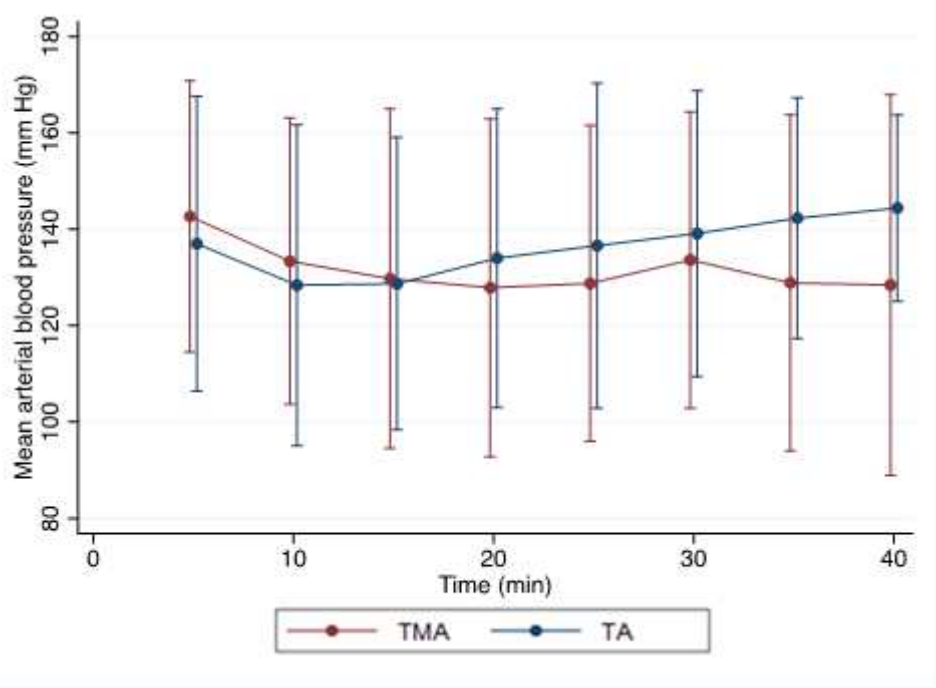

Figure 10: Comparison of the mean arterial pressure (MAP) (mmHg) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

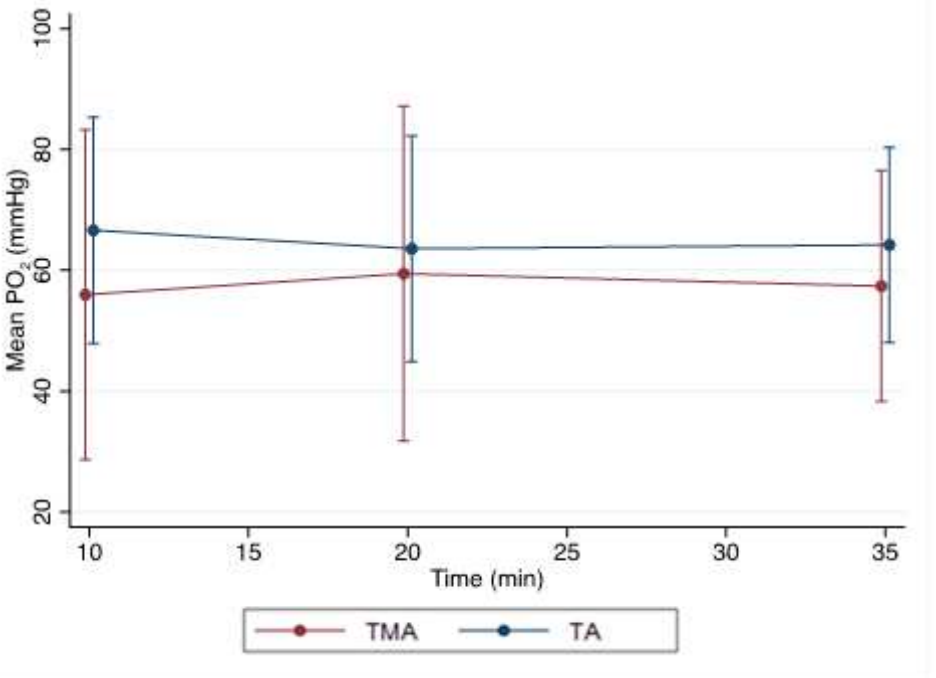

Figure 11: Comparison of the mean PaO_2 (mmHg) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

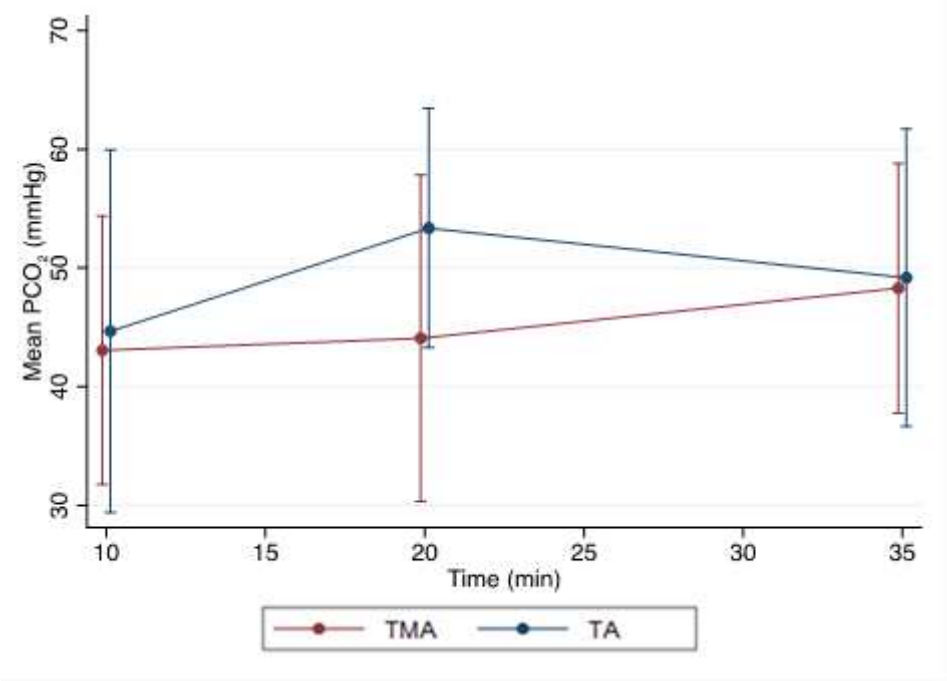

Figure 12: Comparison of the mean $PaCO_2$ (mmHg) during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

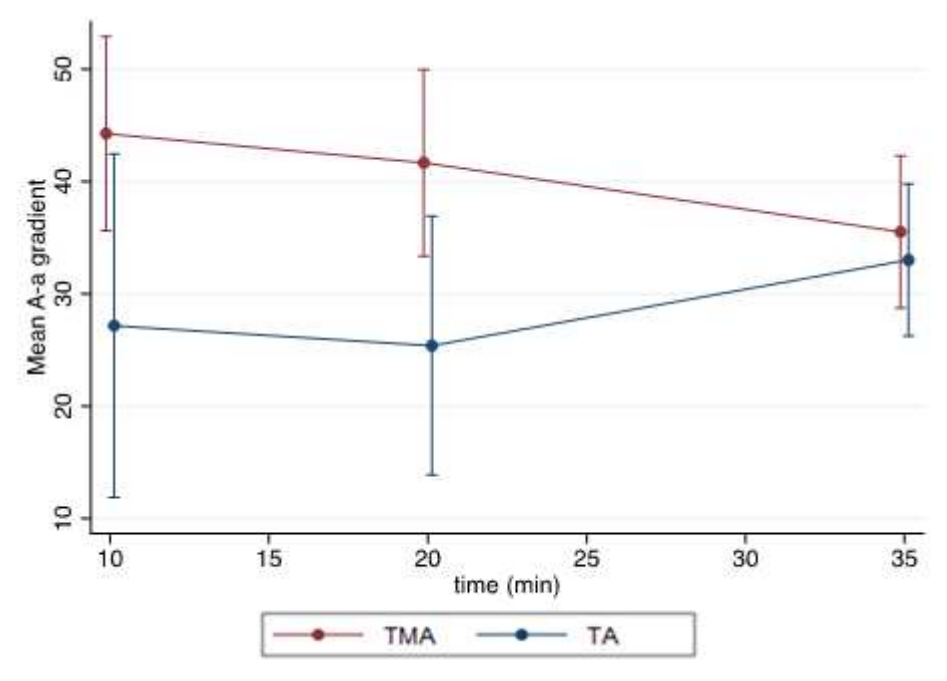

Figure 13: Comparison of the mean A-a gradient during immobilization with the novel TMA (thiafentanil-medetomidine-azaperone) or standard TA (thiafentanil-azaperone) combinations in African buffalo.

4.5 Recovery

After the last recording was taken (T40), all instruments were removed and the animal was antagonized with the respective antidote.

There was a statistically significant difference (p = 0.022) between the two combinations with regards to the recovery times. In general, the mean time to recovery (SD, range) was 80 (± 33; 30 - 130) seconds and 95 (± 30; 50 – 140) seconds in the TA and TMA combination, respectively (Figure 24, Table 13).

All buffalo were monitored for 24 hours post immobilization. No signs of re-narcotization were observed. There was no difference observed with regards to the quality of recovery between the two combinations.

Table 9: Mean recovery times (seconds), SD and range in the novel TMA (thiafentanil-medetomidine-azaperone) and standard TA (thiafentanil-azaperone) combination in African buffalo.

Recovery Time in Seconds				
Regime		First signs	Standing	Walking
TMA	Mean	**58**	**84**	**95**
	SD	21	26	30
	Range	30 - 90	45 - 120	50 - 140
TA	Mean	**38**	**69**	**80**
	SD	16	34	34
	Range	20 - 75	26 - 120	30 - 130

4.6 Costs

The current cost to chemically immobilize and antagonise an adult buffalo bull using the TA combination was ± R 593/buffalo. This is four times more expensive than the TMA combination which was calculated at ± R 146/buffalo on average.

Table 10: Comparison of current costs associated with the chemical immobilization of an adult buffalo bull with the novel TMA (thiafentanil-medetomidine-azaperone) and standard TA (thiafentanil-azaperone) combination.

Drugs/Regime	TA	TMA
Thiafentanil	R 498	R 71
Azaperone	R 14	R 14
Medetomidine HCl		R 4
Naltrexone HCl	R 81	R 12
Atipamezole/Yohimbine Mix		R 45
Total	**R 593**	**R 146**

CHAPTER 5: DISCUSSION

5.1 General research findings

In the present study, we compared the induction times and physiological variables in African buffalo (*Syncerus caffer*) immobilized during two different immobilization protocols.

Both combinations were effective in providing a reliable immobilization for a minimum of 40 minutes with quick recoveries and no mortalities. Both combinations would be suitable for routine veterinary and management procedures. However, there were differences between the two combinations, particularly with regards to the induction times, costs and certain physiological variables.

The induction times with the TMA combination were significantly longer than with the TA combination. Cardiopulmonary side effects occurred with both combinations. The marked hypoxaemia was a matter of great concern in both combinations but was more significant in the TMA combination.

The TMA combination induced immobilization with only $1/7^{th}$ of the thiafentanil dose of the standard TA combination which may allow for a significant reduction in costs which could be of great importance for the wildlife industry.

5.2 Inductions times and quality of induction

Immobilization with the TA combination resulted in significantly shorter induction times with a superior induction score when compared to the TMA combination. Szabó *et al.* (2015) described similar induction times and effectiveness of the TA combination in African buffalo. Under field conditions, short induction times are preferred and can be of clinical relevance. Shorter time to immobilization can mean less distance travelled per animal before immobilization and therefore minimize injuries, and reduce exertion and stress. Non-domestic and domestic cattle are sensitive to capture stress which can increase the risk of capture myopathy, a fatal metabolic shock-like outcome of stress which can result in death within hours. Furthermore, severe stress can override the anxiolytic properties of alpha$_2$-adrenergic agonists requiring a higher dose for sufficient immobilization (Caulkett & Haigh 2007, Curro 2007). Therefore, these drugs should preferably be administered in calm environments where stressors can be kept at a minimum.

For above-mentioned reasons, the reduced induction times and higher quality of induction with the TA combination could reduce the risk of capture-related morbidities and mortalities such as capture myopathy and hyperthermia. The relation between induction times and body temperature has been studied by Meyer *et al.* (2008b). Their study suggests that capture-induced hyperthermia is primarily an anticipatory factor that is proportional to the amount of stress received and irrespective of the pharmacological compound, physical activity and environmental temperature (Meyer *et al.* 2008b).

The increased SD and range in the TMA combination reflects less consistent results and could also indicate a difference in the individual response to the capture process and drugs. Therefore, induction with the TA combination is believed to be more reliable.

5.3 Quality of immobilization

The quality of immobilization in buffalo immobilized with the novel TMA combination seemed superior compared to the TA combination characterized by less observed head movements and muscle rigidity. Opioid-induced muscle rigidity and involuntary tremors can be concerning as increased tissue metabolism can result in oxygen depletion, lactic acidosis and associated consequences (De Lange *et al.* 2017). The muscle relaxing and sedative properties of the alpha$_2$-receptor agonist may have resulted in the appearance of a superior immobilization.

Despite withholding food and water for 24 and 12 hours, respectively, one animal of the TA treatment group showed signs of regurgitation. The oral cavity was cleared and the tongue pulled out to prevent aspiration. No further incidences were observed.

5.4 Physiological variables

Hyperthermia was recorded with both combinations with a mean (SD, range) of 40.5 (± 1.3; 38.8 – 43.8) °C and 40.3 (± 0.8; 39.2 – 41.8) °C in the TMA and TA combination, respectively. The origin for the observed hyperthermia with both combinations could be multifactorial. Although opioids and alpha$_2$-adrenergic agonists are known to cause thermal lability and modulate the thermoregulatory response to temperature changes, it is believed that the role of the pharmacological compound in the development of capture-induced hyperthermia is not primary (Meyer *et al.* 2008b). Studies have shown that the primary factor associated with the development of hyperthermia during chemical immobilization is the amount of stress perceived during capture and is not related to environmental temperature,

physical activity or drugs used (Meyer *et al.* 2008a). The mechanisms of stress-induced hyperthermia are not fully understood but studies have shown that the magnitude of stress-induced hyperthermia is directly related to the amount of stress perceived (Meyer *et al.* 2008b). As hyperthermia was recorded in both treatment groups, it is hypothesized that stress was induced in all buffalo regardless of which combination was used. However, the anticipated stress in both treatment groups may have been caused and aggravated by different underlying factors. The significantly longer induction times observed with the TMA combination and therefore longer time of consciousness and physical activity before recumbency could have contributed to the hyperthermia in those buffalo (Meyer *et al.* 2008b). In the TA combination, the increased sympathetic activity following opioid administration - as well as an increase in tissue metabolism, heat production and oxygen consumption associated with opioid-induced muscle rigidity, may have contributed to the observed hyperthermia (Haw *et al.* 2015). Furthermore, as individual differences exist among animals in a group, the stress response experiences during the study may not have been uniform (Meyer *et al*. 2008b).

Although studies suggest that capture-induced hyperthermia is primarily an anticipatory factor, severe environmental temperatures and extreme physical activities can contribute to and compound capture-related hyperthermia (Meyer 2010). In this study, no significant correlation was found between environmental temperature and body temperature, however, a larger sample size and repeated individual measurements are necessary to compensate for errors.

Hyperthermia is frequently observed during capture operations and is considered to be one of the main contributing factors in the development of capture myopathy and capture-related deaths (Meyer 2010). Hyperthermia in the face of hypoxaemia can result in cellular disruption and predispose to organ failure and capture myopathy (Meyer *et al*. 2008b). Therefore, supplemental oxygen supply should be considered during capture operations to offset hypoxaemia. Furthermore, capture operations should be planned in a way to minimize/avoid severe stress, extreme physical activity and high ambient temperatures.

The heart rate showed a significant difference ($p < 0.01$) between the two combinations. Due to the lack of scientific papers on the physiological variables in African buffalo (*Syncerus caffer*), the average heart rate was calculated allometrically. Aleixo *et al.* (2019) studied the allometric relationship between heart rate and body weight in dogs and suggests that heart rate can be estimated by formula $HR = 241 \times BW^{-0,25}$. According to the latter, an average heart rate (range) of 52 (48 – 53) bpm was estimated in our study with a mean weight (range) of 465.25 (428.5 – 635) kg. A similar average (range) heart rate of 57 (53 – 59) bpm

could be estimated according to Mortola and Lanthier (2004) which proposed the formula $HR = 320 \times BW^{-0.28}$ for calculating the heart rate in mammals.

According to above calculations, both treatment groups were considered to be tachycardic with a mean heart rate (SD) of 70 (± 27) bpm in the TMA combination and 139 (± 25) bpm in the TA combination. This significant lower heart rate observed in buffalo immobilized with the TMA combination could be the result of both central and peripheral alpha$_2$-adrenergic receptor activation. Central activation reduces the release of norepinephrine which results in reduced sympathetic tone and therefore reduced heart rate. Peripheral receptor activation results in increased systemic vascular resistance (SVR) which decreases the heart rate and increases arterial blood pressure (Sinclair 2003). Alpha$_2$-adrenergic agonists are known to induce cardiovascular side effects such as bradycardia with associated bradyarrhythmia, 2nd-degree arterio-ventricular blocks, reduced cardiac output and transient hypertension followed by hypotension (Sinclair 2003). Compared to the above calculated physiological allometric heart rates, none of the buffalo of the TMA combination were found to be bradycardic. The individual stress response during capture could have overridden the cardiovascular effects brought about by medetomidine and therefore counteracted the development of a bradycardia. Furthermore, the cardiovascular side effects of medetomidine are dose-dependent (Sinclair 2003). It is therefore not known if a higher dose of medetomidine would result in bradycardia in buffalo immobilized with the TMA combination, and warrants further research. The hypotensive effect of azaperone and sympathomimetic effect of thiafentanil could have further prevented the development of bradycardia in this group (Buss *et al.* 2016).

In this study, most buffalo immobilized with the TA combination were tachycardic. Exogenous opioid administration increases the sympathetic nervous system activity and catecholamine release which can result in tachycardia in many species (Buss *et al.* 2016). Furthermore, the cardiovascular response could also result from opioid-induced respiratory depression. Hypoxaemia can activate arterial chemoreceptors, especially the carotid bodies which increase the heart rate and blood pressure (Schultz *et al.* 2007; Prabhakar & Kumar 2010). Persistent tachycardia in the face of hypoxaemia can be of great concern as the myocardium only has a limited anaerobic capacity (Buss *et al*. 2016).

To the best of the author's knowledge, the blood pressure of conscious African buffalo has not been established yet. Therefore, recorded blood pressure measurements of this study were compared to that of domestic cattle. In cattle, the mean arterial blood pressure is 143 mmHg and the systolic and diastolic blood pressure range between 100 – 140 mmHg and 50 – 85 mmHg, respectively.

Surprisingly, there was no significant difference in the systolic, diastolic and mean blood pressure between the two combinations and the pattern of change was considered the same for SAP, DAP and MAP. The mean (SD, range) systolic blood pressure value was 163 (± 31; 109 – 209) mmHg and 155 (± 32; 119 – 203) mmHg in the TMA and TA combination, respectively. A difference in individual response was recorded with both combinations and reflected by the higher standard deviations and wide range. Therefore, when evaluating blood pressure, it is important to take individual differences, day-to-day variations and environmental factors into consideration (Olsen & Booth 1971).

Compared to the physiological blood pressure variables of domestic cattle, all buffalo were considered to be hypertensive. In the TMA combination, the initial blood pressure values recorded at T5 decreased significantly [SAP ($p = 0.029$), DAP ($p = 0{,}018$), MAP ($p = 0.043$)] over the first twenty minutes. Administration of alpha$_2$-adrenergic agonists can result in a biphasic response in blood pressure. An initial hypertension can be the result of peripheral receptor activation resulting in an increased peripheral vasoconstriction. Thereafter the blood pressure can drop to normal or slightly lower levels mediated by the vagal baroreceptor reflex. The latter is accompanied by a decrease in heart rate and cardiac output (Sinclair 2003). This effect could explain the significant decrease in blood pressure values in all buffalo immobilized with the TMA combination. The extent of hypotension depends on the dose, route of administration and receptor type activation (Grimm & Lamont 2007).

In the TA combination, the blood pressure values decreased insignificantly between T5 and T10 and then increased significantly [DAP ($p = 0.006$) and MAP ($p = 0{,}04$)] and transiently (SAP) over time to a value exceeding T5. Hypertension following etorphine hydrochloride administration has been described in non-domestic ungulates. It is believed that hypertension is caused by an increased vascular tone and vasoconstriction following an increased adrenergic activity (LeBlanc *et al.* 1987; Buss *et al*. 2016). Increased blood pressure values could also be explained by hypoxaemia or by a lighter plane of anaesthesia towards the end of immobilization (Ozeki & Caulkett 2014).

Azaperone could have partially reduced the extent of hypertension in both combinations due to its alpha$_1$-antagonistic activity resulting in vasodilation (Burroughs *et al.* 2012a).

Hypertension is commonly observed during anaesthesia in ruminants but without baseline values, it is difficult to assess the severity of observed hypertension in our study. Acute systemic hypertension can cause damage and oedema in vital organs and should therefore be monitored during chemical immobilization (Curro 2007).

Although respiratory minute volume was not recorded, ventilation was evaluated by monitoring chest movements and by measuring blood gases. There was no significant difference between the two combinations with regards to the respiratory rates and values were found to be within normal range for most of the buffalo. The mean (SD) respiratory rate was 33 (± 22) and 37 (± 18) breaths per minute in the TMA and TA combination, respectively. The $PaCO_2$ value can be a good indicator of alveolar ventilation within the lungs and therefore efficiency of breathing. Normal values range between 35 to 45 mmHg (Messina & Patrick 2020). Hypercapnia was recorded in eleven of the twenty immobilized buffalo with more individuals of the TA combination being hypercapnic. The $PaCO_2$ values were never critical in any of the individuals with values never exceeding 60 mmHg. Opioids can decrease the sensitivity to carbon dioxide in the central chemoreceptors resulting in hypercapnia and hypoxia (Pattinson 2008). This effect has also been observed when $alpha_2$-agonists are administered in combination with other drugs, especially opioids (Caulkett & Haigh 2007).

One of the main findings of this study was the development of profound hypoxaemia (mean, SD) following the administration of either combination: PaO_2 (TA): 51 (± 13) mmHg; PaO_2 (TMA): 44 ± 14 mmHg. Despite recorded respiratory rates being within the normal expected range in both combinations, all buffalo were hypoxaemic during immobilization. Seven buffalo were considered as severe hypoxaemic (< 40 mmHg), nine as moderate hypoxaemic (40-59 mmHg) and four as mild hypoxaemic (60-79 mmHg). Hypoxaemia was more significant ($p < 0.05$) in buffalo immobilized with the TMA combination when compared to the TA combination. Despite the marked hypoxaemia, the $PaCO_2$ values of the majority of buffalo of the TMA combination were within the normal range.

The presence of hypoxaemia was further mirrored by the widened alveolar-arterial gradient (A-a gradient), especially in the TMA combination. The significantly widened A-a-gradient ($p > 0.01$) in the TMA combination, as reflected by the significantly lower PaO_2 value, can indicate the degree of venous admixture following medetomidine administration. Due to the widened A-a gradient in the face of normocapnia and a respiratory rate within the normal expected range, the origin of hypoxaemia was more likely due to ventilation/perfusion mismatch, oxygen diffusion impairment and/or shunting rather than hypoventilation (Celly *et al.* 1997; Hantzidiamantis & Amaro 2020). This finding is similar to that in Celly *et al.* (1997) where sheep sedated with medetomidine were found hypoxaemic but not hypercapnic (Celly *et al.* 1997). In the abovementioned study, intravenous medetomidine administration in sheep resulted in an increase in the airway pressure and intrapulmonary shunt fraction. The latter suggests that hypoxaemia was primarily caused by a change in pulmonary mechanics i.e. a decrease in compliance or increase in resistance, or both (Celly et al. 1997).

An increase in airway pressure mediated by alpha$_2$-agonist receptor activation was also reported after the use of xylazine in sheep (Nolan *et al.* 1986). It is believed that this effect is caused by an alteration in the airway dynamics which could result in decreased oxygen delivery to the alveoli due to an increased airway resistance or an altered pulmonary circulation decreasing the blood transit time.

Furthermore, pulmonary hypertension and vasoconstriction following alpha$_2$-agonist receptor activation have been observed in sheep following medetomidine administration (Read 2003). An increased pulmonary resistance and bronchoconstriction can result in pulmonary congestion and pulmonary oedema associated with gas diffusion deficits on the alveolar level (Celly *et al*. 1997).

Opioids have also been linked to the development of pulmonary hypertension in some animals, especially goats. Meyer *et al.* (2015) studied the origin of respiratory compromise in goats following etorphine administration. In their study, the widened A-a gradient was strongly correlated with the mean pulmonary artery pressure indicating that pulmonary hypertension was the primary reason for the oxygen diffusion deficits. Pulmonary hypertension can be the result of increased pulmonary vascular resistance, increased pulmonary blood flow and/or increase in the left atrial pressure. Although no further measurements were taken in this study, an increased pulmonary vascular resistance and left atrial pressure were the main reasons for the development of hypoxaemia in Meyer *et al.* (2015). Increased pulmonary vasoconstriction can result from an increased sympathetic activation following opioid administration, hypoxic pulmonary vasoconstriction or other opioid-induced effects on the pulmonary vasculature (Meyer *et al.* 2015).

Pulmonary hypertension and rapid blood flow across the capillary bed in the lungs can decrease the blood transit time and therefore reduce oxygen exchange in the alveoli resulting in hypoxaemia and a widened A-a gradient (Zeiler & Meyer 2017).

Another factor that could have contributed to the observed hypoxaemia in the TA combination is an increase in oxygen consumption. The increased sympathetic activity following exogenous opioid administration can increase cellular metabolism and oxygen consumption (Buss *et al.* 2018). Opioid-induced muscle rigidity could have further contributed to the development of hypoxaemia in the TA combination as increased muscle metabolism and oxygen demand can result in hypoxia and hyperlactatemia (De Lange *et al.* 2017).

Furthermore, ventilation-perfusion mismatch (V/Q ratio) is commonly reported in ruminants during chemical immobilization and can also be the result of abnormal positioning of the animal and the resulting increased weight of the viscera on the diaphragm (Read 2003). A

low V/Q ratio for a specific lung area can result in reduced perfusion and gas exchange and a reduced PaO_2 (Buss *et al.* 2015).

Based on the measured respiratory rates and $PaCO_2$ values, it is important to emphasize that despite an adequate breathing efficacy, hypoxaemia was significantly more profound in the TMA combination. Supplementary oxygen should be considered essential during immobilization with either combination. Furthermore, as cardiopulmonary side effects are dose dependent, the lowest possible required dose should be administered (Shah *et al*. 2014). Measures should not only be focused on improving oxygen delivery to the alveoli but also to improve the oxygen exchange between the alveoli and arterial blood by preventing pulmonary vasoconstriction (Meyer *et al.* 2015).

The TA combination may benefit from the addition of the mixed opioid antagonist butorphanol tartrate post recumbency as this could counteract the undesired μ-receptor effects such as oxygen diffusion deficits and muscular hypertonicity while maintaining the sedative and analgesic κ-receptor-mediated effects (Bush *et al.* 2011). Using the TMA combination, the addition of vatinoxan (MK-467), a peripheral alpha2-receptor antagonist, may improve the anaesthetic safety during the immobilization of ruminants. The latter could improve the cardiovascular function by alleviating hypertension and bradycardia brought about by alpha2-receptor agonists (Einwaller *et al.* 2020).

There was no significant difference in the lactate levels between the two combinations and the mean (SD, range) was 4.76 (± 4.12; 1.16 – 12.82) and 3.99 (± 3.81; 0.73 – 11.86) in the TA and TMA combination, respectively. Serum lactate levels can be used as a prognostic marker in both human and veterinary medicine. Hyperlactatemia is present when serum levels exceed 1.5-2 mmol/l and results from anaerobic metabolism following tissue hypoxia, hypoperfusion and/or poor oxygen delivery (Lorenzo *et al*. 2020).

Hyperlactatemia can reflect hypoxia and muscle fatigue and can be a good indicator for stress and capture myopathy (Mentaberre *et al.* 2010). The hyperlactatemia observed in all buffalo was interpreted as an outcome of an increased stress response during the induction phase. The high standard deviation could indicate a difference in individual stress response similar to that observed in the induction times. The presence of lactic acidaemia throughout the entire capture process indicates that anaerobic glycolysis continued throughout immobilization. The hyperthermia and hypoxaemia could have exacerbated and contributed to this. Harthoorn (1976) found that animals produced more severe hyperthermia and hyperlactatemia during maximal exertion of short duration rather than during prolonged activity of moderate intensity (Harthoorn 1976). Intensive exercise of short duration can

frequently be observed as part of the chase and dart placement during chemical game capture.

The lactic acid concentration had a higher tendency in the TA combination, this was not significant. Opioids can induce a cataleptic immobilization with increased excitement and arousal during induction. This results in higher adrenergic activity and lactic acid production. This excitement is believed to be dose dependant and is mostly observed at lower dosages. Higher dosages can override excitement but can also cause increased muscular hypertonicity (Heard *et al.* 1996). Furthermore, opioid-induced muscle rigidity, associated with an increase in tissue metabolism and oxygen depletion, may contribute to an elevation in the serum lactate concentration during chemical immobilization (Buss *et al.* 2015). In contrast, the sedative and muscle-relaxing effects of medetomidine could have prevented a further increase in the serum lactate levels. Alpha$_2$-agonist receptor activation inhibits norepinephrine release by a negative feedback mechanism (Grimm & Lamont 2007). However, Ranheim *et al.* (2008) studied the effectiveness of medetomidine on cortisol and noradrenaline levels in cattle and sheep and found that medetomidine does not seem to reduce the endocrine stress response in those species. This is because stressors such as hypoxaemia following medetomidine administration may override the inhibitory effect on the endocrine stress response (Ranheim *et al.* 2008). Therefore, neuroendocrine indicators of stress may not be accurate in quantifying the stress response when alpha$_2$-agonists are used (Grimm & Lamont 2007). As this may be true for cattle and sheep, further investigations are necessary to evaluate the effects of alpha$_2$-agonists on the stress response in non-domestic animals.

The acidemia recorded in our study was mild in most buffalo. The pH was lower in the early stages of immobilization in both combinations but was significantly lower with the TA combination. The pH and lactate values increased insignificantly over time in the TA combination despite persistent hypercapnia in most buffalo. This could indicate that compensation was rather of metabolic than respiratory origin.

Acidaemia during chemical capture is important as it has been associated with capture-related morbidities and mortalities due to electrolyte disturbances resulting in hyperkalaemia and myocardial damage. Furthermore, acidaemia has been linked to the development of pulmonary hypertension and pulmonary oedema (Harthoorn 1976). It is therefore important to prevent acidaemia resulting from severe stress, increased physical and sympathetic activity. As acidaemia has been associated with capture-related pathologies, further studies on prevention and treatment are necessary.

Glucose concentration was elevated in both combinations but had on average a higher tendency with the TMA combination. Alpha$_2$-adrenergic agonists can exert an inhibitory effect on insulin production following receptor activation located on pancreatic beta cells resulting in hyperglycemia and hypoinsulinaemia (Grimm & Lamont 2007).

5.5 Recovery

Both reversals were given intravenously for a quick and comparable recovery. Recovery was rapid in both combinations, however, the recovery times were significant ($p = 0.022$) longer in the TMA combination compared to the TA combination with a mean (SD, range) of 95 (± 30, 50 – 140) and 80 (± 34, 30 - 130) seconds, respectively. The recovery times in the TA combination were similar to those recorded by Szabó *et al.* (2015) where the mean time to recovery was 99 seconds.

Atipamezole has a higher affinity for the alpha$_2$-receptors compared to yohimbine and can therefore induce a quicker awakening than yohimbine (Janssen *et al.* 2017). The reduced affinity of yohimbine to the alpha$_2$-receptors could have contributed to the slower than expected recovery times in the TMA combination altogether. The atipamezole/yohimbine combination as reversal for the TMA group was chosen by the supervising veterinarian, who has held several workshops on that protocol, and has now become the standard for many practitioners in SA. However, its pharmacological advantages and/or disadvantages, as well as the difference in recovery time between atipamezole alone and the atipamezole/yohimbine combination has yet to be established. Furthermore, although atipamezole is effective by intravenous, intramuscular and subcutaneous administration, intravenous administration of atipamezole can induce severe haemodynamic changes (e.g., tachycardia, vasodilation and death) as described by Sinclair (2003). Due to the lack of data on the absorption of yohimbine after intramuscular administration (Janssen *et al.* 2017), the atipamezole/yohimbine combination was given slowly intravenously. Despite a reduction in costs when the atipamezole/yohimbine combination is used, atipamezole alone remains the reversal of choice for medetomidine hydrochloride (Sinclair 2003). However, it is important to find a balance between striving for a quick recovery time and minimizing the risk of severe haemodynamic changes when deciding on the route of administration.

No signs of re-narcotization were observed within 24 hours post immobilization.

Based on the acquired results, the alternative hypothesis can be accepted for the induction times, heart rate, SpO_2, PaO_2, A-a gradient and pH.

CHAPTER 6: CONCLUSION

Based on the obtained results, the novel thiafentanil-medetomidine-azaperone combination may provide a cost-effective and suitable chemical immobilization for routine veterinary and management procedures in African buffalo. Due to the high variability in induction times and quality of inductions in the TMA combination, the TA combination may induce a more predictable and shorter induction and therefore may reduce the risk of capture-related morbidities and mortalities.

The quality of immobilization appeared to be superior in the TMA combination with good muscle relaxation and less spontaneous movements. However, it is important to stress that, - despite a seemingly superior immobilization, and respiratory rates and $PaCO_2$ values within the normal expected range in most of the buffalo; the hypoxaemia was significant and severe in the TMA combination. As stressors can override the sedative effects of medetomidine, immobilization with this combination should only be recommended in boma habituated or free-ranging animals with accessible terrain and where capture stress can be kept at a minimum.

A great concern of the use of ultra-potent opioids is the human safety hazard following accidental exposure. Accidental injection or splashing can result in mortality if the antagonist naltrexone is not administered in time (Curro 2007). The use of alternative or non-opioid protocols could reduce the hazard to human safety. However, the concentrated medetomidine hydrochloride solution (50mg/ml) used in this study is extremely potent and should be handled with an equal degree of respect as the ultra-potent opioids. As there is currently no approved $alpha_2$-antagonist for humans, accidental exposure is treated with supplementary oxygen and ventilator support (Caulkett & Shury 2014).

Hypoxaemia was of great concern with both combinations. As the widened A-a gradient indicates, the observed hypoxaemia was predominantly caused by pathophysiological effects resulting in poor oxygen exchange/diffusion rather than hypoventilation. Supplementary oxygen should be considered essential during immobilization with either combination. Furthermore, measures should also be focused on improving gas diffusion deficits by preventing pulmonary vasoconstriction.

When deciding on an immobilization protocol, it is important to weigh up the advantages and disadvantages of each combination as either combination may be more or less beneficial for different capture objectives or setups. Nevertheless, irrespective of which combination is used, their cardiorespiratory function, especially blood oxygenation, should be monitored carefully.

Limitations

Although the novel TMA combination has recently been used widely among wildlife veterinarians, comparative data and in-depth studies on their physiological effects in African buffalo were not available at the time of this study. Due to the lack of published physiological variables in conscious African buffalo, the measured values were compared to that of domestic ruminants or calculated allometrically. Substantially large standard deviations and variability between the two combinations were observed for most of the physiological variables. The large standard deviation could indicate a different individual response to certain combinations or to the capture process itself. Some buffalo could have suffered a higher stress response which could have overridden the anaesthetic effects causing erratic results. To compensate for errors of measurement or to quantify individual responses, a larger sample size or repeated individual measurements are necessary. Furthermore, cortisol and catecholamine measurements were not obtained. The latter could have been useful to assess the impact of the individual stress response on drug immobilization efficacy and physiological response. A dose-dependent effect of the drugs on the physiological parameters is assumed. A difference in body weight could have also contributed to the substantially large standard deviations. This study took place in a controlled environment. It would be ideal to repeat the trial under field conditions with a capture process that would normally occur. This study was carried out in a boma to establish a controlled environment where variability and bias could be reduced. However, the unnatural environment of the boma may have caused unnecessary stressors which could have affected the results of this study. Furthermore, only male buffalo were used in this study to reduce variables. It would be important to repeat the study on all sexes, weight and age groups. As this was not a blind study, qualitative values could be biased.

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APPENDIX I: Data Collection Form

BUFFALO RECORD SHEET

DATE:	**LOCATION:**	**PROCEDURE NO:**
VET DARTING:	**TIME:**	**TEMP:**

ANIMAL DATA				
SPECIES:	ID /TAG NO:	SEX: *m w*	AGE:	WEIGHT:
LAST FEEDING: Fasted? ☐		ACTIVITY LEVEL: CALM ☐ ACTIVE ☐ EXCITED ☐		

DART DATA

REGIME:
STANDARD ☐
NOVEL ☐

NO of DART	DRUG	DOSE (mg)	DART TYPE	LOCATION/ROUTE OF ADMIN	COMMENTS

TIMES			
TIME OF 1st DART:			
TIME OF 2nd DART	*(if applicable):*		
RECUMBENCY:	LATERAL ☐ STERNAL ☐		
	FIRST EFFECT	**FIRST DOWN**	**PARTIAL REVERSAL (NOVEL)**
ELAPSED TIME			

QUALITY OF INDUCTION		
1	**Excellent**	No CNS excitation. Slight ataxia. No or minimal movement after dart placement. Smooth and quick transition to recumbency.
2	**Good**	Mild CNS excitation. Moderate ataxia and physical activity (pacing) and ataxia. Smooth transition into recumbency.
3	**Fair**	Severe CNS excitation. Extensive physical activity (circling) and ataxia. Attempts to jump over the fence. Prolonged time to reach recumbency.
4	**Poor**	Mild CNS excitation. Moderate physical activity (walking) and ataxia. Failure to reach immobilization but can be assisted into recumbency.

1

MEASUREMENTS									
TIME FROM DOWN	5	10	15	20	25	30	35	40	45
TEMP °C									
HEART RATE									
RESP RATE									
SPO2									
ET CO2									
BP SYS/DIA									
BP MEAN									
ARTERIAL SAMPLE									
QUALITY ANAESTHESIA *									

RECOVERY DATA			
TIME OF REVERSAL DRUG:			
	FIRST SIGNS	STANDING	WALKING
ELAPSED TIME			
DRUG ADMINISTERED			
COMMENTS ON REVOCERY			

QUALITY OF IMMOBILIZATION		
1	Deep	Buffalo remains recumbent without any attempts to stand up. Palpebral reflex reduced or absent. Reduced muscle rigidity, no involuntary movements of head and tail.
2	Moderate	Buffalo remains recumbent without any attempts to stand up. Response to tactile/audible stimuli (e.g. when cannulated). Palpebral reflex reduced or absent. Mild muscle tremors and rigidity observed. Spontaneous muscle movements possible.
3	Light	All characteristics of 2. Palpebral reflex intact. Voluntary head movement.
4	Awake	Minimal immobilization. Attempts to stand up. Head movement

Comments:

APPENDIX II: Animal Ethics Approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

6 July 2020

Approval Certificate
New Application

AEC Reference No.:	REC241-19
Title:	The comparative physiological and cardiovascular effects during the chemical immobilization of buffalo (Syncerus caffer) of two anaesthetic protocols
Researcher:	Dr VE Faber
Student's Supervisor:	Dr KN Koeppel

Dear Dr VE Faber,

The **New Application** as supported by documents received between 2020-03-30 and 2020-07-03 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-07-03.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number**
African Buffalo (Syncerus caffer)	12
Samples Whole Blood	 12 (3 ml per sample)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-07-06.
3. Please remember to use your protocol number (REC241-19) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.
Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8483
Fax +27 12 529 8321
Email aec@up.ac.za
www.up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

9 December 2020

Approval Certificate
Amendment 1

AEC Reference No.:	REC241-19
Title:	The comparative physiological and cardiovascular effects during the chemical immobilization of buffalo (Syncerus caffer) of two anaesthetic protocols
Researcher:	Dr VE Faber
Student's Supervisor:	Dr KN Koeppel

Dear Dr VE Faber,

The **Amendment** as supported by documents received between 2020-11-09 and 2020-12-04 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-12-04.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	**Number Available**
African Buffalo (Syncerus caffer)	12
Samples Whole Blood	12 approved 12 additional added (3 ml per sample) **24 Total**

2. Please note that the approved date(s) from the original application certificate / annual renewal certificate will be applicable to this amendment.

3. Please remember to use your protocol number (REC241-19) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.

6. As part of your approval, the committee requires that you record a **short video footage** of major animal procedures approved in your study. The committee may request them for monitoring purposes at any later point.

Room 6-13, Arnold Theiler Building, Onderstepoort
Private Bag X04, Onderstepoort 0110, South Africa
Tel +27 12 529 8434
Fax +27 12 529 8321
Email: marleze.rheeder@up.ac.za

Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Boogakadiruiwa