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LIST OF ABBREVIATIONS

Abbreviation(s)	Meaning
≈	Almost equal to or approximately equal to
©	Copyright sign
°C	Degree(s) Celsius
/	Divide by
=	Equal to
>	Greater than
<	Less than
-	Minus in arithmetic or to represent a negative number
X or x	Multiply by (product of)
%	Percent
+	Plus
±	Plus Minus
:	Ratio indicator or colon
;	Semicolon used to separate information
½	Half
^{2}	Squared, to the power of two
5-HT$_{1A}$, 5-HT$_4$, 5-HT$_7$, 5-HT	Serotonergic receptors
AG	Anion gap
AMPA	Alpha-amino-3-hydroxy-5methyl-4-isoxazolepropionic acid receptor
ANOVA	Analysis of variance
ARDS	Acute respiratory distress syndrome
Atot	Total weak acids
BE	Base excess
Beats min^{-1}	Heart beats per minute
Breaths min^{-1}	Breaths per minute
Ca^{++}	Calcium ion
cAMP	Cyclic adenosine monophosphate
Cl^-	Chloride ion
CO_2	Carbon dioxide
DA1 and DA2	Dopamine receptors type 1 and 2
DOP	Delta-opioid receptor
ET	Endotracheal
ET-tube	Endotracheal tube
et al.	*et alia* meaning "and others"
$F_E{'}O_2$	Fractional exhalation of oxygen
FiO_2	Fractional inspiration of oxygen
GABA	Gamma-aminobutyric-acid

$GABA_A$	Gamma-aminobutyric-acid receptors type A
$g\ dL^{-1}$	Gram(s) per decilitre
$g\ L^{-1}$	Gram(s) per litre
GPCRs	G-protein coupled receptors
H^+	Hydrogen ion
H1	Histamine receptors type 1
HCO_3^-	Bicarbonate ion
$[HCO_3^-]{:}[H_2CO_3]$	Bicarbonate ion to carbonic acid ratio
H_2CO_3	Carbonic acid
H_2O	Water
I1 and I2	Imidazoline receptors type 1 and 2
i.e.	id est meaning "that is" or "in other words" or "namely"
im	Intramuscular
IP_3	Inositol trisphosphate
IQR	Interquartile range
iv	Intravenous
K^+	Potassium ion
Ka	Effective equilibrium dissociation constant
KOP	Kappa-opioid receptor
kPa	Kilo Pascal(s)
Kg	Kilogram
$L\ L^{-1}$	Litre per litre
$L\ min^{-1}$ or $L\ minute^{-1}$	Litre(s) per minute
log_{10}	Logarithm
m	Meter(s)
m^2	Meter(s) squared
MAP	Mean arterial blood pressure
Max	Maximum
Mean	Average
mEq	Milliequivalence(s)
$mEq\ L^{-1}$	Milliequivalence(s) per litre
mg	Milligram(s) reported as a total dose
$mg\ dL^{-1}$	Milligram(s) per decilitre
$mg\ kg^{-1}$	Milligram(s) per kilogram
$mg\ kg^{-1}\ hour^{-1}$	Milligram(s) per kilogram per hour
$mg\ ml^{-1}$	Milligram(s) per millilitre
Min	Minimum
ml	Millilitre(s)
$ml\ kg^{-1}$	Millilitre(s) per kilogram
$ml\ kg^{-1}\ hour^{-1}$	Millilitre(s) per kilogram per hour

ml minute^{-1}	Millilitre(s) per minute
mmHg	Millimetre(s) Mercury
mmol	Millimole(s)
mmol L^{-1}	Millimole(s) per litre
MOP	Mu-opioid receptor
N	Number of (count of)
Na$^+$	Sodium ion
N/A	Not applicable
NFκB	Nuclear factor-*kappa* B (B indicates that it activates/signals B cells)
NMDA	*N*-methyl-*D*-aspartate receptors
nmol L^{-1}	Nanomole(s) per litre
NOP	Nociception/orphanin FQ peptide receptor
NSAIDs	Non-steroidal-anti-inflammatory drugs
OH$^-$	Hydroxide ion
p	Level of statistical significance
$PaCO_2$	Arterial partial pressure (or tension) of carbon dioxide
$PaCO_2$ - $P_{ET}CO_2$ or $PaCO_2$ - $P_E{'}CO_2$ or P(a-et)CO$_2$	Arterial to end-tidal carbon dioxide tension gradient
PAO_2	Alveolar partial pressure (or tension) of oxygen
PaO_2	Arterial partial pressure (or tension) of oxygen
P(A-a)o$_2$	Alveolar-to-arterial oxygen tension gradient or A-a gradient
P(A-a)O$_2$/PaO$_2$	Respiratory index
PaO$_2$/PAO$_2$	Arterial-to-alveolar oxygen tension ratio
PaO$_2$/FiO$_2$ or P:F ratio	Arterial tension to fractional inspired oxygen ratio
Patm or Pbar	Barometric (or atmospheric) air pressure
$P_{ET}CO_2$ or $P_E{'}CO_2$	Partial pressure of expired carbon dioxide
pH	Negative logarithm of the Hydrogen ion concentration
PhD	Doctor of Philosophy
PH$_2$O	Partial pressure (or tension) of water or alveolar vapour pressure
pKa (or pK$_1$)	Equilibrium dissociation constant (of carbonic acid)
R	Correlation coefficient
RAS	Reticular activating system
RDDS	Remote drug delivery systems
Ref	Reference
Ref Range	Reference range
RQ	Respiratory quotient
S	Solubility coefficient of carbon dioxide in plasma
SaO$_2$	Arterial oxygen-haemoglobin saturation (Calculated)
sc	Subcutaneous
SD	Standard deviation

SID	Strong ion difference
SIDa	Apparent strong ion difference
SIDe	Effective strong ion difference
SIG	Strong ion gap
sp.	Species
SpO_2	Peripheral oxygen-haemoglobin saturation
Temp	Temperature
TIVA	Total intravenous anaesthesia
$\mu g\ kg^{-1}$	Microgram(s) per kilogram
$\mu g\ kg^{-1}\ hour^{-1}$	Microgram(s) per kilogram per hour
$\mu mol\ L^{-1}$	Micromole(s) per litre
$\mu g\ ml^{-1}$	Microgram(s) per millilitre
Vd/Vt	Dead-space to tidal volume ratio
Vt	Tidal volume

THESIS SUMMARY

Practicality of using impala (A*epyceros melampus*) as a research model

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This thesis focus on providing information that furthers our understanding on the practicality of using impala (*Aepyceros melampus*) as a model for research regarding the effects of immobilisation and general anaesthesia on wildlife generally and wild ungulates specifically. Impala have been used as a research model for around seven decades, but deaths have been reported, especially in experiments where free-ranging impala are placed under captive conditions. There are currently no reports investigating the cause of these deaths. Therefore, the risks involved when using impala in research studies are not entirely known. This paucity in the literature magnifies plausible concerns regarding research ethics and animal welfare that require consideration when using free-ranging animals.

This thesis comprises of a series of studies which focus on 1) determining and evaluating the physiological effects of various drug combinations used to immobilise and anaesthetise impala, and 2) determining factors that place free-ranging impala at risk of injury or death when captured and placed into captivity under intensive research conditions. Fifteen adult female impala were enrolled into the 16-week long project. In the first study reported in this thesis, both thiafentanil-medetomidine and etorphine-medetomidine drug combinations successfully immobilised impala, however, despite seemingly normal heart and respiratory rates, all impala were hypoxaemic. Calculated ventilation and oxygenation indices suggested that the hypoxaemia was primarily attributed to right-to-left intrapulmonary shunt of blood. However, other causes of dysfunction in gas exchange such as increased dead space ventilation or ventilation to perfusion mismatch or alterations within the alveoli-capillary membrane diffusion, or combinations of all of the above aetiologies for hypoxaemia are plausible. Opioid-induced hypoventilation was not considered a primary cause of the hypoxemia

because measured minute volumes were within an appropriate range for medium sized antelope. The second study in this thesis determined if an etorphine-ketamine-medetomidine constant rate infusion was a practical method of maintaining general anaesthesia in impala for up to 120 minutes. However, hypoxaemia, hypercapnia and acidosis were sufficient to require intervention if this protocol is used in the field. The third study evaluated the acid-base status of impala undergoing immobilisation and general anaesthesia using two different drug combinations. In both cases, there was a moderately progressive respiratory acidosis compensated by a marked metabolic response. Both the Henderson-Hasselbalch and the Stewart approaches could explain the acid-base status in the impala. The fourth study determined and discussed the risk factors that resulted in morbidity and mortality in the impala throughout the study period. We found that darting injuries were the highest risk factor that resulted in the most impala deaths during our study. The last study reviewed chemical capture of impala to highlight all the major risks contributing to morbidity and mortality of impala in clinical practice. The greatest risks emanate from the drug and drug delivery factors, where potent opioids (etorphine and thiafentanil) cause profound respiratory compromise, that if left untreated often translates into fatalities. Furthermore, the procedure of darting, an essential tool in game capture, can cause irreparable fractures and other fatal injuries mainly through accidental misplacement of the dart into a long bone, thoracic or peritoneal cavity. Impala are anxious and flighty, and this demeanour (animal related factor) can contribute towards morbidity and mortality rates.

The findings of these studies highlight that impala serve as a useful model for improving immobilising and anaesthetic drug protocols, darting techniques or new methods of remote injection in wild antelope. In order to improve animal welfare and the success of similar studies in impala, it is essential that the risks and physiological effects of chemical capture and anaesthesia are well understood before embarking on similar studies.

Keywords: *Aepyceros melampus*, etorphine, general anaesthesia, immobilisation, impala, medetomidine, morbidity, mortality, research model, thiafentanil

CHAPTER 1

General introduction, literature review and scope of the thesis

1.1 General introduction

The demand to perform immobilisation and general anaesthesia in wild ungulates, in particular antelope, has surged in the recent past in South Africa. The growing demand is due to the increased value of these antelope in game ranching and zoological collections. Antelope remain a challenging species to immobilise and place under general anaesthesia, mostly due to their anxious disposition (Ball 2007; Ball & Hofmeyr 2014) and physiological response to immobilising drugs (Kock & Burroughs 2012). Impala (*Aepyceros melampus*) have often been used in research studies as a model for other antelope, especially in research fields focusing on behaviour (Jarman 1970; Cronje et al. 2002; Matson et al. 2004; Rduch 2016), thermoregulation (Maloiy & Hopcraft 1971; Meyer et al. 2008a; Shrestha et al. 2012), predator-prey relationships (Caro et al. 2004; Shorrocks & Cokayne 2005; Tarakini et al. 2014), parasitology in wild (Ezenwa 2004) and captive (Mooring et al. 1996) herds, reproduction (Jarman & Jarman 1973; O'Kane & Macdonald 2016) and physical or chemical capture (Pienaar et al. 1966; Ables & Ables 1969; Drevemo & Karstad 1974; Grootenhuis et al. 1976; Murray et al. 1981; Cheney & Hattingh 1987; Hattingh 1988; Gandini et al. 1989; Hattingh et al. 1990; Knox et al. 1990; Knox et al. 1991; Knox et al. 1992; Knox et al. 1993; Janssen et al. 1993; Pitts & Mitchell 2002; Bush et al. 2004; Meyer et al. 2008b; Meyer et al. 2010; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2017; Gerlach et al. 2017). Despite being a popular research model, little is known on what effects intensive research projects have on the impala's wellbeing. Furthermore, few publications report on physical and chemical capture of impala that have been confined in an outdoor antelope housing structure (boma) (Knox et al. 1992; Bush et al. 2004; Meyer et al. 2008a, b; Meyer et al. 2010). Only a few of these studies have reported mortalities in impala (Knox et al. 1992; Meyer et al. 2008a), but in practice, morbidity and mortality appear to occur more often than reported. Therefore, if impala are to be used in intense studies investigating novel immobilisation and general anaesthetic drug combinations, there is a need to clearly understand the physiological and welfare effects of the drugs and the procedures and management practices used during boma confinement of captured free-ranging impala during these studies. Understanding these aspects will identify the risks of morbidity and mortality associated with these species. Identifying these risks will allow for feasible practical recommendations to be made in

order to improve the health and welfare of impala enrolled in boma immobilisation and general anaesthesia related studies.

1.2 Literature review

1.2.1 Chemical immobilisation and general anaesthesia defined

Chemical immobilisation and general anaesthesia are different states, and although definitions of each have been proposed, the specifics of these definitions are not always agreed upon in the literature. Nevertheless, the concepts of chemical immobilisation and general anaesthesia are reviewed and a definition of each will be synthesised from the available information and these will be used for the rest of the thesis. In general chemical immobilisation is when drugs are administered alone or in various combinations with the specific purpose of capturing an animal. The aspects that encompass the administration of the drug(s) and subsequent capture of the animal is termed chemical capture. The most common features used to describe a state of chemical immobilisation include: 1) an animal that, due to drug effects, does not ambulate easily and is often found recumbent or could be easily manipulated into a recumbent state by a member of the capture team; 2) the animal's level of conscious awareness is obtunded to the extent that they are often unaware of, or only mildly respond to noxious or other external stimuli; 3) the animal's autonomic and subconscious reflexes remain intact; and 4) chemical immobilisation can typically be reversed by the administration of pharmacological or physiological antagonist drugs (Kock & Burroughs 2012). General anaesthesia can be defined as a state of unconsciousness that is produced by a process of controlled, reversible, depression of the central nervous system, whereby the patient neither perceives nor recalls noxious or other external stimuli. Furthermore, the state of general anaesthesia must fulfil three criteria (called the triad of general anaesthesia), as follows: 1) render patient unconscious, whereby there is no perception or memory of any sensory or motor event; 2) supresses autonomic (haemodynamic, respiratory and thermoregulatory) and somatic (proprioception reflexes such as the righting reflex) reflexes to a point where the patient maintains normal physiological function (or as near to normal as possible) and ensures muscle relaxation; and 3) provides antinociception, whereby the responses to surgical stimulation (nociceptive sensory inputs) are supressed (Eke & Bell 2004).

To better understand the state of the obtunded conscious awareness, a number of anaesthesiologists have described scoring systems to try and identify the level of consciousness and suppression of

physiological processes while patients are under general anaesthesia. These scoring systems are used to gauge the depth of general anaesthesia. The depth of general anaesthesia ranges from when the patient is still alert and awake, conscious of its surroundings until they are unconscious with severe suppression of all vital organ system functions. John Snow was the first anaesthesiologist to describe the various stages of anaesthesia in 1847 (Eke & Bell 2004). He described five stages and indicated that only the fourth degree (fourth stage in his proposed system) provided an adequate depth of anaesthesia that allowed safe surgery. Considering all the scoring systems described, the signs of general anaesthesia, described by Arthur Guedel was first proposed in 1937 (**Table 1.1**) and is perhaps the best tool used to date to describe where the states of chemical immobilisation and general anaesthesia are in the bidirectional transition between consciousness and unconsciousness (Guedel 1937).

Table 1.1 The signs of general anaesthesia, as described and tabulated by Arthur Guedel in 1937.

The scanned copy of the table was sourced from the University of California San Francisco online source: https://blogs.library.ucsf.edu/broughttolight/tag/arthur-e-guedel-anesthesia-collection/

Guedel described the clinical signs of people being induced into general anaesthesia and maintained by inhaling ether as the sole anaesthetic drug, alone or with premedication with an opioid (morphine) or anticholinergic (atropine or scopolamine). The clinical signs were grouped into common patterns, which allowed a way to determine the depth of anaesthesia (Dugdale 2010; Grimm et al. 2015). A patient, based on the clinical signs at the time of evaluation, was placed into an appropriate stage (I to IV) and plane (stage III was further divided into four planes). The patient was considered to be in danger if they moved from stage III plane 3 to a deeper plane 4. Stage IV was considered early stages of respiratory arrest and cardiovascular collapse. Guedel continued his work in small stock and dogs and noticed a similar predictable pattern in clinical signs. However, some species of animals, like horses and cats, do not always follow all of the predictable signs of transition, especially the eye movement and pupil size (Grimm et al. 2015). Pupil diameter is not a useful indicator in veterinary science due to the variable response to different drugs among species (Dugdale 2010; Grimm et al. 2015). Important to this discussion is the change in respiratory pattern, eyeball movement, eyelash reflex (palpebral reflex), swallowing and vomiting as the patient transitions from a conscious state to unconsciousness. Modern versions of Guedel's signs of anaesthesia have also included changes in the arterial blood pressure, which will also be considered important in this discussion.

Stage I was originally termed the stage of analgesia, but modern versions tend to term this stage as the stage of voluntary excitement. This stage is where, once an anaesthetic drug has been administered (or during administered) there is a decrease in the perception of pain if there are pre-existing injuries that the patient is suffering from. The pain relief would make the patient "calm" and more compliant during the induction phase of anaesthesia (Guedel 1937). Most notable is that the respiratory rate and pattern is considered normal and that people reported an altered state of mind, where they could respond to verbal commands, yet not always recall these events on reawakening (Dugdale 2010). Therefore, there is an altered state of consciousness, opposed to a loss of conscious awareness of their surroundings. Autonomic and somatic reflexes remain intact, but could be mildly supressed (Dugdale 2010; Grimm 2015). As the patient progresses in stage I towards the start of stage II, evidence of voluntary excitement could be unmasked, especially in anxious patients unaccustomed to drug induced alterations of brain function and cognitive processing. These patients, while experiencing phases of excitement, still respond to verbal commands, therefore indicating that consciousness is still present (Grimm et al. 2015). Common to stage I, whether there is voluntary excitement or not, is that

the patient is not able to ambulate easily and can be manipulated into different recumbences (Sedgwick 1986; Spraker 1986). These descriptions of stage I are commonly observed during induction (of chemical immobilisation, especially when potent opioid drugs (etorphine, thiafentanil, fentanyl and carfentanil) are used as the primary drug for immobilisation (Kock & Burroughs 2012) and when peripheral muscle relaxants are not used. Untamed animals that experience involuntary excitement could be a dangerous hazard to themselves and to the veterinarian. The animal could thrash around in a transport crate and cause damage to their muscular or skeletal system that could even result in death. Large animals that experience involuntary excitement are difficult to control and pose a very dangerous threat to veterinarians who might need to attend to them. Often involuntary excitement can be minimised by reducing external stimulation using such tecniques as blindfolding and blocking the external ear canals (Harthoorn 1968). These provisions minimise the "spontaneous arousal" or voluntary excitement or early attempts to stand, especially in animals where the level of consciousness is only obtunded and not lost.

Stage II has been termed the stage of excitement, or more accurately the stage of involuntary excitement (Guedel 1937; Dugdle 2010; Grimm et al. 2015). People who transition through this phase do not have any recollection (anterograde amnesia) of transitioning through this stage. Furthermore, people experiencing this excitement do not respond to verbal commands or physical stimulations to encourage arousal. Therefore, these patients have been said to be in a state of unconsciousness, or at least dissociated from their immediate surroundings (Grimm et al. 2015). Erratic and irregular breathing pattern, systemic hypertension, nyastagmus, rapid blinking of the eyelids, vocalisation, defecation, urination, repeated swallowing attempts, vomiting or regurgitation and repeated spasmodic limb and neck movements (similar to a person or animals suffering syncope or an ictal episode) are all possible manifestations of the involuntary excitement (Harthoorn 1967; Tranquilli 1986; Dugdale 2010; Grimm et al. 2015). The clinical signs associated with the manifestation of involuntary excitement are observed frequently during chemical immobilisation, especially of antelope (Pienaar 1969; Haigh 1977). Therefore, an important question arises, are these animals experiencing these symptoms because of drug effect (opioids and cyclohexylamine drugs, frequently used in chemical capture, cause catatonia, muscle hypertonia and seizure-like activity) or because they are resisting the effects of the drugs in an attempt to remain ambulatory and conscious? Regardless, these animals are considered cognitively dissociated from their environment, and if still standing or wandering around with an ataxic gait, can be

hand captured and placed into sternal or lateral recumbency. Once they are blindfolded and the ear canals blocked then the animal will become calmer and allow handling, sometimes without further drug administration (Kock & Burroughs 2012).

Stage III is termed the stage of surgical anaesthesia (general anaesthesia). A patient is said to have transitioned into this stage when their breathing pattern becomes regular and their arterial blood pressure returns to their normal resting values (Guedel 1937). Furthermore, the patient is relaxed (muscle relaxation), unconscious and does not respond to surgical stimulation (depending on the invasiveness of the stimulation, traditionally more painful surgeries require a deeper plane of anaesthesia) (Dugdale 2010; Grimm et al. 2015). A chemically immobilised animal which has a regular breathing pattern, normal blood pressure and is non-responsive to physical stimulation, could be catagorised as being within the Stage III of anaesthesia. This stage could be associated with the use of dissociative anaesthetic agents (cyclohexylamine) or opioid based drug combinations that include an alpha$_2$-adrenoceptor agonist and/or benzodiazepine drug (Bush et al. 2004; Meyer et al. 2008b). Drug effects (potent opioids and alpha$_2$-adrenoceptor agonists and cyclohexylamines) could cause a drug-induced increase in arterial blood pressure (Kästner 2006; Meyer et al. 2015). Potent opioids and cyclohexylamines could cause erratic and irregular breathing patterns (Bush et al. 2004, Pattinson 2008; Meyer et al. 2005). These drug effects on the cardiovascular and respiratory systems make determining the depth of chemically immobilised patients a challenge. Regardless, stage III plane 1 should be considered a "maximum" depth achieved during chemical immobilisation. In stage III plane 1 the animal will lose its righting reflex. Therefore, to minimise aspiration of saliva and regurgitate, antelope should be supported in sternal recumbency, their head should be raised above shoulder height, and their nose pointed downwards (Kock & Burroughs 2012; Ball & Hofmeyr 2014). Patients, especially the domesticated ruminants, are considered in a surgical plane of anaesthesia when they are in stage III plane 2. In this stage and plane the patient will have a regular breathing pattern, a normal arterial blood pressure and be unresponsive to surgical stimulation. Stage III plane 3 is a difficult plane to determine. Patients are said to be in this stage and plane if their respiration rate begins to slow and the arterial blood pressure begins to decrease.

Occasionally, the depth of immobilisation may progress rapidly to deeper surgical anaesthesia planes, where signs of bradypnoea, hypoventilation, bradycardia and hypotension may be prequels to respiratory arrest and cardiovascular collapse. Therefore, stage III plane 4 and stage IV should be

recognised early and animals should receive emergency interventions, such as administering physiological or pharmacological antagonists, to revive the chemically immobilised or anaesthetised animal to prevent cardiac arrest and death (Dugdale 2010; Grimm et al. 2015).

The Guedel signs of anaesthesia are helpful for describing the various states of chemical immobilisation and general anaesthesia. However, the major limitation in extrapolating findings to animal models is the definition of consciousness. Consciousness if not fully understood and therefore it is difficult to predict how "conscious" an animal truly is after being chemically immobilised or anaesthetised (Alkire 2008). During chemical immobilisation, there is drug induced central nervous system depression that mimics the early stages of general anaesthesia; this depression can progress quickly to dangerously deeper stages and therefore monitoring the physiological state of immobilised animals is essential. The Guedel signs of anaesthesia attempted to compartmentalise the depth of anaesthesia into incremental changes. The system was able to predict the depth of anaesthesia in patients that were subjected to "old world" anaesthetics like ether. These patients did progress through the predictable stages and planes. However, nowadays, animals are often anaesthetised with a combination of drugs where the end clinical point might not follow the predictable compartmentalised scoring system like Guedel had suggested. Therefore, the transition from wakefulness to surgical anaesthesia should be viewed as a continuum. If common cardiovascular of respiratory parameters, such as arterial blood pressure, heart and respiratory rate, decrease to suboptimal ranges then an anaesthetic overdose should be suspected and treated. Failure to recognise these parameters could increase the risk of morbidity and mortality.

1.2.2 Physical and chemical techniques used to capture impala

Various physical and chemical capture techniques used in medium sized antelope have been evaluated, mainly in impala, over the last seven decades. These techniques are studied either in free-ranging impala in the wild or in impala captured from the wild, relocated to a boma, and kept in captivity during the investigations.

Prior to the introduction of the potent opioid etorphine in the early 1960s, physical capture techniques were commonly used to capture wild antelope, and often they were the only method available (Harthoorn 1967; Haigh 1990). Hand-capture and net-capture techniques have been described for medium sized antelope.

Maloiy & Hopcraft (1971) describe a novel approach where they hand-captured two neonatal impala which they hand-raised until they were adults prior to using them in an investigation to determine water balance under various environmental temperatures. Hattingh et al. (1988b) described a method of hand-capturing individual free-ranging impala at night. The method used was to suddenly shine a bright light (spotlighting technique) in the direction of the impala that was targeted for captured. This sudden increase in light intensity stunned the impala (perhaps by temporarily blinding it) which could subsequently be hand-captured and physically restrained for jugular blood sampling. Densham (1974) was one of the first to report a technique of mass capturing free-ranging herds of impala using a helicopter-assisted approach to temporarily confine impala. The helicopter shepherded the impala into a concealed boma (Densham 1974), a technique known as the "*Oelofse helicopter-boma method*" (Pienaar 1973). Murray et al. (1981) used the same helicopter assisted method to mass capture free-ranging impala herds (size 7 to 141 impala). At first, Murray et al. (1981) attempted to hand-capture individual impala in a group of impala that were confined in a small boma; this was not successful and they resorted to using net-capture techniques.

A variety of net-capture techniques have been used to capture free-ranging or confined medium sized ungulates, and include the use of drop-nets, drive-nets and net-guns (Kock et al. 1987). Drop-netting is a mass capture technique where a suitable capture area is defined and baited for a few weeks or months to encourage the targeted species of animal to frequent the area. Once the target specie frequents the capture area, a large net is erected by elevating the net on poles (or other suitable structures to elevate the net, like trees). Weights are often placed on the perimeter seam of the net. A remote controlled system is put in place to allow remote triggering of the devices holding the net in an elevated position. Once a sizable group of the target species of animal is under the elevated net, the remote device is activated to drop the net over of the animals to capture them. The weights placed on the seam of the net causes the net to drop downwards without major wind interference (Jessup et al. 1988; Kock et al. 1987). Drop-netting is a plausible method to mass-capture a large herd of impala, however, the prolonged baiting period is often not practical, but this method could be used in impala confined in a boma. The current literature lacks reports detailing the use of drop-netting to mass capture impala.

Drive-netting is another mass capture technique that has been used to capture free-ranging and boma confined impala (Murray et al. 1981; Hattingh et al. 1990; Knox et al. 1990; Knox et al. 1992; Meyer et al. 2008a). Drive-netting is a technique where standard linear net is spanned across the narrow end of

a funnelled capture zone (Kock et al. 1987). The funnel is designed to direct and drive the impala towards the net. The net is designed to make escape from the funnelled capture zone difficult (Jessup et al. 1988; Kock et al. 1987). Naturally occurring areas could be used as a funnel such as small valleys, bushy areas with natural narrow pathways (Kock et al. 1987); or man-made funnels using solid wall screenings (Murray et al. 1981). Impala are driven into the net by helicopter (free-ranging impala), by vehicle (free-ranging impala or impala confined in a large camp; Meyer et al. 2008a), or on foot by shepherding them into the funnel area with (Murray et al. 1981) or without (Knox et al. 1990) using solid walled screens (boma confined impala). Once the impala are trapped in the net, they can be hand-caught and physically restrained with or without sedation. Drive-netting to capture and handle impala by physical restraint can be safely done, but only for a very short period of time (Murray et al. 1981). However, capture and physical restraint in free-ranging and boma confined impala does cause a stress response (Hattingh et al. 1990). The stress response is profound, especially in naïve impala, and readily causes death (Murray et al. 1981; Knox et al. 1992; Meyer et al. 2008a).

The use of a net-gun is a plausible technique to capture individual impala, although no reports of its application in impala capture was found. Net-guns that deploy large nets are mounted on a helicopter or vehicle, while smaller nets (up to 5 x 5 meters) can be deployed using a handheld net-gun. In both cases, weights are attached to the perimeter of the square net. During deployment, the weights are projected from the net-gun and carry the opened net towards the targeted animal with great accuracy (Kock et al. 1987).

The physiological response to single (Murray et al. 1981; Hattingh et al. 1990; Meyer et al. 2008a) and repeated (Knox et al. 1992) physical capture of impala have been well documented. All of these studies report deaths of impala, therefore a summary recommendation of the findings reported in these studies is that physical capture should only be considered in impala that are to be captured and physically restrained for the shortest possible time (under 10 minutes) to minimise the risk of death (Murray et al. 1981). The aetiology of the deaths have been reported to be due to capture myopathy (Meyer et al 2008a) and horning injuries sustained during or after physical capture and restraint (Murray et al. 1981). Knox et al. (1992) monitored blood lactate, glucose, cortisol, catecholamines, total lipids, total proteins and haematocrit in impala that were repeatedly captured physically and determined that individuals at greater risk of dying tended to have values that were significantly different from those in individuals that survived. These variables could be incorporated into future intensive studies investigating repeated

capture of impala. Due to the high risk of mortality, of up to 30%, especially during prolonged physical capture, these techniques cannot be advocated for capturing impala requiring veterinary examination and intervention (Murray et al. 1981). Instead, chemical capture techniques should be used, where impala are sedated or immobilised enough to allow a thorough veterinary examination to take place (physiological responses to chemical capture are complex and are discussed in point 1.2.5).

Chemical capture techniques involve the methods of administrating a drug, or combination of drugs, with the purpose of immobilising an animal (chemical immobilisation is defined in point 1.2.1). The typical route of administrating the drug(s) is by intramuscular injection (Bush 1992; Kock & Burroughs 2012). Several techniques have been described to administer the drug(s) intramuscularly and can be divided into direct and remote techniques.

Direct techniques are when the drug(s) are injected using a hand-held device, therefore the operator is in direct control of the device. The hand-held syringe connected to a needle is the simplest example of a hand-held device (West et al. 2014). Impala that are net-caught or restrained within a confined purpose-built transport crate can be injected using a hand-held syringe (Murray et al. 1981; Knox et al. 1990). The pole-syringe is another example of a hand-held device and there are several types available for clinical practice (Kock & Burroughs 2012; West et al. 2014). The simplest type of pole-syringe is where the plunger is joined to, or incorporated into the pole and is called a manual pressure pole-syringe. The needle of the syringe is aimed at an appropriate muscle group and the entire device is advanced towards the animal, the pole is continually pushed towards the animal, against the resistance of the animal's body, to administer the contents of the syringe (West et al. 2014). A modification of the manual pressure pole-syringe is available and differs in that there is a movable internal extension within the pole to allow independent manual pushing of the plunger at the discretion of the operator. Thus, once the needle is placed then the operator manually advances the plunger by pushing the internal extension mechanism (West et al. 2014). Other, more technical versions of the pole-syringe are available whereby the method used to administer the syringe contents, once the needle is placed, is automated. The two most common methods of automation are spring-loaded mechanisms and rapid expansion of compressed air (Bush 1992). Once the needle penetrates the skin and muscle tissue layers, they alter the momentum of the pole-syringe, which triggers the automated mechanism to rapidly depress the syringe plunger. These hand-held devices are always under the control of the operator and therefore the risk of injury is usually limited to muscle bruising and haematoma formation (Bush 1992;

West et al. 2014). However, if the animal reacts violently to the intramuscular injection, as is usually the case in impala, then other injuries can be sustained during a profound fight or flight reaction.

Remote techniques involve the use of devices that project a "projectile syringe" or "flying syringe", now-a-days simply referred to as a dart, to administer the drug(s) to an animal that is, normally, outside the administration range of direct hand-held devices (Crockford et al. 1958; Montgomery 1961). These techniques are grouped as remote drug delivery systems (RDDS) (Bush 1992). The operator has control over the projector, but not the projectile or the targeted animal. There are many types of projectors and they typically include blowpipes, hand guns and rifles that are designed to project a dart towards the targeted animal. Additionally, there are many types of darts available and they are classified according to the mechanism used to discharge the drug(s) placed into the dart.

Blowpipes are extremely effective at projecting darts 1 ml in volume or less and can be used up to a range of 10-15 meters with ease in the hands of a skilled operator (Buesh 1992; West et al. 2014). Veterinarians working in animal sanctuaries, zoological gardens or quarantine facilities will often prefer to use this device because it is: 1) inexpensive, 2) lightweight, 3) easy to clean and maintain, 4) is virtually silent when projecting darts, 5) does not have mechanical parts that can breakdown or fail during operation, 6) can be used indoors and outdoors (when there is little to no wind), 7) can be easy to hide from intelligent animals, like those from the primate family, and 8) the darts are small in volume, lightweight and often projected at lower velocities compared to hand guns and rifles which often translates into less tissue injury (West et al. 2014). Therefore, a blowpipe could be used to dart impala that are habituated to captivity, where the operator can stand in relatively close proximity to project a dart (Bush et al. 1992). One of the limitations to consider when darting impala is the slow projection velocity. Impala are experts at evading darts, therefore the fastest velocity that does not cause profound tissue injury should be used. In the simplest form, a blowpipe is a fixed length smooth bore pipe that is fitted with a rubber mouthpiece on the operator's side. The rubber mouthpiece is designed to allow the operator to push their lips tightly up against it, creating a seal, to minimise escape of air during the "blow" attempt (Kock & Burroughs 2012). Once the operator's lips are sealed against the mouthpiece, they inhale maximally through their nose and then hold the inhalation (inspiratory pause, operator airway pressure greater than atmospheric air pressure). Thereafter they aim the device at the desired part of the target animal, and exhale forcefully to generate a maximum airflow thereby pushing the dart out of the blowpipe (Bush et al. 1992; Kock & Burroughs 2012). Instead of the operator directly providing

the gas flow to move the dart, blowpipes can also be fitted with a compressed gas chamber and trigger mechanism (Kock & Burroughs 2012). Release of compressed gas into the blowpipe provides the impetus for the dart. The compressed gas chamber can be filled with air by a foot-pump or hand-pump. It can also be filled with carbon dioxide from a larger, reserve CO_2 canister. The distinct advantage of compressed gas systems is that the chamber can be primed with compressed gas prior to aiming the device at the animal. The trigger mechanism for the compressed gas is similar to that of a handgun, and is used to discharge the contents of the compressed air chamber when the operator is ready to shoot the dart (Kock & Burroughs 2012). A blowpipe fitted with a compressed gas chamber and trigger is preferred to dart impala because: 1) The dart velocity can be adjusted to reduce flight time and minimise the opportunity for the impala to evade the dart, and 2) The flight trajectory is more predictable than it is with operator blowing methods.

Hand guns and rifles are similar in construct among the available projectors, with the obvious exception of barrel length (West et al. 2014). Hand guns have shorter barrels compared to rifles and are therefore easier to manoeuvre and aim in confined spaces. The bore of the barrel is either smooth or rifled and ranges in diameter (11 or 13 millimetres) depending on the type and size of dart that needs to be projected (West et al. 2014). Rifling is employed in an attempt to improve dart stability during flight, but it is of limited success with the darts that are currently available in South Africa. In some rifles, the operator can alter the barrel length and diameter by swopping between barrels on an as-needed basis, depending on the planned darting distance, dart type and dart size. The dart is projected from the barrel using either a gas-charged or powder-charged method (Cattet et al. 2006; Kock & Burroughs 2012). Gas-charged mechanisms typically involve the use of compressed carbon dioxide canisters that are attached to the projector, similar in fashion to a gas-charged blowpipe with a trigger assembly (Bush 1992; Kock & Burroughs 2012; West et al. 2014). There is usually a priming switch that allows the compression chamber to be filled to a desired pressure that is determined by the dart's weight in relation to the flight distance. Some models of projectors allow the operator to rapidly change the pressure in the compression chamber to accommodate changing distance between the operator and the target. In some cases, particularly in hand guns, a foot-pump, hand-pump or crank-pump (a crank lever is used to compress air within the chamber by decreasing the volume of the chamber) can be used to prime the compression chamber, similar to the blowpipes. Once the compression chamber is primed to the desired pressure, and the barrel is aimed at the animal, then the operator will activate a trigger

mechanism (button, or usually a gun trigger type mechanism) to discharge the compressed gas into the barrel to push the dart rapidly out of the barrel (Kock & Burroughs 2012). Powder-charged mechanisms make use of blank 0.22 calibre ammunition rounds which do not have a bullet, but just a gun powder filled casing and a primer (Cattet et al. 2006). Projectors using a powder-charged mechanism are very similar in construct to a regular firearm in that the chamber housing (to hold the blank round) and trigger mechanism are virtually the same. Furthermore, these projectors can take either a single round, or hold multiple rounds within a cartridge, depending on the make and model (Kock & Burroughs 2012). All projectors rely on manual loading of the dart and the powder-charged round. The powder-charged round is usually loaded using a bolt-action type loading mechanism, common to most manual regular firearm rifles. After cocking the loaded projector's hammer, the operator aims the barrel at the animal and pulls the trigger which activates a hammer designed to strike a firing pin at the back of the casing where the primer is placed (Kock & Burroughs 2012). Once the firing pin strikes the back of the casing it compresses the primer causing it to ignite, which in turn ignites the powder within the casing. Combustion of the powder causes a rapid increase in chamber pressure due to expansion of gas. This gas expansion forces the dart out of the barrel. The casing of the round can be filled with different types of gun powder that combust at different rates and intensities (Cattet et al. 2006). The faster the combustion rate and, higher the intensity of combustion, the more rapidly the gas within the chamber will expand and therefore, the faster the dart will be pushed out of the barrel (Kock & Burroughs 2012). In South Africa, some of the powder-charged projectors have an adjustable gas outlet port which allows variable amounts of the gas to leak from the combustion chamber without being channelled through the barrel. This allows the pressure within the combustion chamber to be regulated; the larger the outlet port aperture, the lower the pressure within the combustion chamber, and *vice versa* (Kock & Burroughs 2012). In turn this allows dart velocity to be regulated. The effect of this is similar to that of the priming switch used to adjust the pressure within a compression chamber found in the gas-charged hand guns and rifles. Hand guns and rifles using both gas-charged and powder-charged mechanisms have been used to project darts at impala. Rifles are often preferred in the field because they are superior for projecting a dart accurately over a long trajectory distance. The faster dart velocity that they generate, and consequent reduced flight time, also tends to minimise the opportunity for impala to evade the dart.

Darts are broadly classified as single-use or reusable types (Bush 1992; Kock & Burroughs 2012; West et al. 2014). Regardless of the type of dart, they all have the same essential components that includes

1) a needle, 2) a drug chamber in front of the plunger, 3) a discharge mechanism behind the plunger, and 4) a tail piece or flight (Kock & Burroughs 2012). The discharge mechanism is designed to rapidly advance the plunger through the drug chamber to expel all of its contents into the animal via the needle. This is analogous to the action of a hand-held syringe where the plunger is pushed, by hand, to empty the syringe contents via the needle. The volume of the drug chamber determines the size of the dart and this can range from 0.5 to 10 ml (Bush 1992). However, despite the drug volume of the dart, the weight of the dart is depended on the drug chamber size and the material used to make the dart (Bush 1992; Kock & Burroughs 2012; West et al. 2014). Therefore, for example, 1 ml darts made by different companies will not have the same mass. Therefore, the operator should be familiar with the mass (an important aspect to consider is dart trajectory kinetics, discussed later on in this section) and the volume of the darts that they use most often (Cattet et al. 2006). The needle is often permanently fixed to single-use darts but reusable darts can be fitted with many different types of needles depending on the operator's requirements (Kock & Burroughs 2012). Therefore, when purchasing a single-use type dart, the correct length and bore needle should be ordered. With reusable darts, the operator usually carries a range of needles of different length and bore so that they can match an appropriate needle to the circumstances. A maximum needle length of 1 inch (25 millimetres) should be used in impala to avoid unnecessary darting injuries like accidental intraosseous injections or bone fractures. The needle has its injection port at the patient end of the needle in most single-use darts (Bush 1992). The injection port of reusable needles is often at the side (side-port needles; important for gas-powered discharge mechanisms whereby a plastic sleeve covers the injection ports to allow the dart to be pressurised, see below where reusable darts are discussed further) (Kock & Burroughs 2012). However, custom built needles can be fitted to a single-use dart, where the operator could ask for side-ported needles (Kock & Burroughs 2012). Furthermore, needles can be plain, or have collars (plastic or metal) or have one or more wire barbs (Bush 1992; Kock & Burroughs 2012; West et al. 2014). The collars and wire barbs are designed to fix the needle in the muscle and counter the tendency for the dart to be forced out of the muscle in reaction to the force generated as injectate is propelled out of the needle tip. This tends to keep the dart assembly from dropping out and allows the drug to be injected at a constant depth. Also, if the dart is still embedded, the operator can approach the animal and retrieve the dart after it becomes immobile. Retrieving the dart is particularly important if potent, high concentration formulations of capture drugs are used (for safety reasons), and desirable if the dart is a reusable type (for cost

reasons), and for environmental reasons, as most commercial darts are not biodegradable (Kock & Burroughs 2012). The tail piece, or flight, of the dart is specific to the manufacturing company and could be made of plastic, metal or cloth (Kock & Burroughs 2012). The tail piece is designed to create drag to prevent the dart from tumbling during flight, thus ensuring a predictable trajectory and that the needle hits first. In South Africa, the two most common dart discharge mechanisms are powder-charged or gas-charged (Kock & Burroughs 2012). Single-use darts use powder-charged mechanisms. In these darts, a low tension spring that is slightly smaller in diameter and length compared to the cylindrical discharge chamber is placed to keep the primer and the weighted firing pin out of operational range from each other during transport, dart loading, dart firing, and dart flight. The primer is fixed (often glued or tension fitted) at the plunger end of the discharge chamber and a weighted firing pin is placed at the tail piece end (Bush 1992). When the dart is fired from the barrel, the spring ensures that the primer does not move towards the tail piece (and come into contact with the firing pin) as this would cause the dart to discharge in-flight. When the dart hits the animal, there is sudden deceleration of the dart, however, the momentum of the firing pin is still directed towards the animal, compressing the spring and subsequently allowing it to make contact with the primer, causing the primer to ignite. Once the primer ignites there is rapid expansion of the gases within the discharge chamber that pushes the plunger towards the needle, thus emptying the drug chamber into the animal (Bush 1992; Cattet et al. 2006). The entire process is rapid and the drug(s) are discharged with a force that can cause tissue expansion, produce a wound cavity, and induce injury (Cattet et al. 2006). The smallest possible volume of drug(s) should be used in this type of dart to minimise such tissue injury. If the operator can select dart discharge speed, then slower injection minimises cavitation at the injection site. (Cattet et al. 2006). Single-use darts of up to 1 ml in volume can be safely used to administer drug(s) to impala. Reusable darts make use of gas-charged mechanisms to discharge the drug(s) form the drug chamber (West et al. 2014). First, needle with a side-port is prepared by placing a tight fitting plastic sleeve onto the needle to cover the side-port(s). Second, the drug chamber is filled (with the plunger moved as far back within the chamber towards the tail piece) with the drug(s), then a sleeved side-ported needle is tightly fitted to the needle end of the dart (West et al. 2014). Third, while covering the needle end of the dart, the discharge chamber is filled with air by using a 20 ml syringe to push enough air into this chamber to charge the dart; the dart manufacturer recommends the amount of air required to charge the dart for optimal performance). A one-way valve mechanism prevents the compressed air in the discharge

chamber from escaping (Kock & Burroughs 2012). Then, lastly, the tail piece is fitted to the dart (West et al. 2014). The dart is primed and ready for firing. When the needle of the dart penetrates the skin of the animal on its way into the muscle layers, tissue drag pushes the sleeve along the needle uncovering the side-port(s). Once the side-port(s) are open, the pressure at the port(s) suddenly decreases to that of the surrounding tissue. The resulting pressure gradient allows the compressed air in the discharge chamber to expand pushing the plunger to empty the contents of the syringe through the needle (Kock & Burroughs 2012; West et al. 2014). With this approach, the discharge rate of the drug(s) is generally slower than it is with powder-charged mechanisms, and is therefore less likely to cause cavitation and injury in the injected tissues (Cattet et al. 2006). The 1.5 and 3 ml reusable darts have been used to capture impala (Meyer et al. 2008a; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2017; Gerlach et al. 2017).

The advantage of using RDDS to capture impala, is that the element of surprise is often in favour of the operator, especially if the impala are within a herd. During field darting, the impala are often indifferent to the presence of the operator and therefore do not suffer from pre-darting anxiety and stress. Impala that have been habituated to a boma tend to mount less of a stress response and suffer less pre-darting anxiety compared to naïve impala (Meyer et al. 2008a). Also, in boma habituated impala, no animal handling is required before administering the immobilising drug(s), therefore, administering them can be safely done over distances of up to 50 m (Bush 1992; Cattet et al. 2006). The author notes that, despite claims of impala being darted from >80 m away, in general it is hardly ever necessary to dart impala over distances greater than 35 m. Despite the advantage of the RDDS, they are not without disadvantages and the most important one being trauma related to dart placement (Cattet et al. 2006). Therefore, the operator must understand some physical science and mathematical principles to use RDDS safely and minimise injury to the animal due to dart placement. The kinetic energy of the dart during flight and the stopping momentum of the dart are important considerations and are discussed here.

The kinetic energy of a dart in flight is described by the following equation (Kock & Burroughs 2012):

Kinetic energy (joules) = ½ mass (kg) x velocity^2 (m s^{-1})

The mass of the dart is dependent on the material from which it is made (metal darts tend to have more mass than those made from nylon or plastic), and the volume and specific gravity of the injectate in the

dart (typically between 0.5 and 10 ml) (Bush 1992; West et al. 2014). The specific gravity of most drug solutions varies little and approximates 1.0, thus it can be treated as a constant for the purposes of this discussion. The aforementioned kinetic equation predicts the amount of energy that can be transferred to an animal that is standing stationary. The energy that is transferred is the cause of much of the local tissue damage at the site of impact. Regardless of the dart volume, if the drugs do not fill the dart, then sterile water for injection or physiological saline should be added to fill the dart. Filled darts are less likely to tumble during flight because air bubbles within the drug compartment could move location during acceleration and deceleration of the dart and thus change the centre of gravity of the dart causing it to tumble. Large air bubbles within the drug compartment could shift the centre of gravity even if the bubble itself does not move (Bush 1992). The mass of darts with similar capacity differs among manufacturers, therefore the operator must be familiar with the mass of the darts that they use. Compared to older technology, modern darts are generally lighter and, therefore, the dart mass tends to play a less significant role in impact trauma. Rather, the speed of injection (rate of drug administration) plays the most significant role (Cattet et al. 2006). The projection velocity of the dart is arguably the most critical component of the kinetic energy equation, where high projection velocities result in increased tissue trauma (Valkenburg et al. 1999; Jessup 2001). The velocity of the dart is related to the trajectory path; the higher the velocity, the flatter (straighter) the trajectory and the higher the impact velocity. Therefore, ideally the dart should fly through an arced trajectory which will decrease the projection velocity required and minimise the amount of impact trauma (Bush 1992). Recommendations of ideal maximum safe projection velocity (measured using a chronograph placed 5 m from the projector's barrel muzzle) have been proposed; for example Valkenburg et al. (1999) stated that an ideal projection velocity is <45 m s^{-1} when firing a 1 ml dart (Cap-Chur dart) over a distance of 10 m. Because the mass of the dart is a constant variable, the manufacturers of RDDS publish ideal projector settings to ensure a safe projection velocity to achieve accurate darting over the desired distance (Cattet et al. 2006). The mass and velocity chosen for a dart therefore represent a compromise between conflicting factors: the need for minimal tissue damage versus the need for accuracy. Most articles and textbooks make reference to the importance of the dart's kinetic energy and how this relates to tissue trauma. However, little attention is given to the net forces required to stop a dart i.e. the actual energy that is transferred to the animal's tissues during dart impact in order to stop the dart from moving. The reason for this is perhaps because the mathematics behind the physical science of inelastic collisions

(two objects stick together after a collision) is not as intuitive as that of the kinetic energy equation (Backman 1976). In inelastic collisions, the momentum of the objects are conserved, but the kinetic energy is not conserved and is rather transferred into other types of energy. An important aspect is that the total energy is conserved and that the kinetic energy is a component of the total energy. If the dart comes to rest when it impacts an animal, the total energy must be conserved and therefore there must be a transfer of kinetic energy to another types of non-kinetic energy (heat, friction, potential energy, elastic or shock waves), which the tissue is liable to absorb (Backman 1976). This energy transfer is a major component responsible for the potential tissue damage during dart impact. The other components of tissue damage include the volume and speed of injection of the drug combination (Cattet et al. 2006).

Despite much research into dart ballistics (Montgomery 1961; Bush 1992; Valkenburg et al. 1999; Jessup 2001; Cattet et al. 2006) injuries still occur. Many experienced wildlife veterinarians (and operators) have attempted to define what acceptable dart impact trauma is. Valkenburg & Tobey (2001) noted that a general assumption among veterinarians is that if the barrel (needle end of the drug chamber) of the dart does not penetrate the skin then the dart velocity could be considered acceptable. This approach only examines the easily visible external tissue damage, but does not indicate the severity of injury to the tissues deeper than the subcutaneous layer. Extensive muscle damage may not be immediately obvious and can progress to extensive muscle damage that can hinder the animal's ability to ambulate and defend itself during intra- and interspecies animal interactions (Valkenburg et al. 1999). Furthermore, other vital tissues could be damaged if the dart is misplaced, e.g., bones may sustain fractures, or organs in the thoracic or abdominal cavity may be ruptured. These injuries often result in mortality (West et al. 2014). Dart site infections are a concern, especially if there has been extensive soft tissue injury (muscle damage) which decreases perfusion of the injury site and thus decreases the animal's innate ability to ward off infections. Recommendations such as treating the dart site with a single infiltration of oil-based antibiotics (e.g., teat preparations), or a single injection of long acting antibiotics have been proposed, despite an absence of controlled studies to support the practice (Isaza 2014). The practice of using clean (sterile) darts and needles should minimise the opportunity for infection. However, hair and skin surface debris can be pushed deep into the injection site and result in infection, therefore inspecting and cleaning the dart site is a sensible recommendation (Cattet et al. 2006).

Despite the distinct advantage of using RDDS that administer drug(s) to chemically capture animals there remains a tremendous amount of variability. The variability is attributed to the different RDDS, darts and needle types. Therefore, once the animal is darted and the dart is thought to have injected its contents into the animal, the operator can never be certain that all of the drug was deposited in muscle. Therefore, ideally, the RDDS should be tested against hand injection using otherwise similar circumstances (same drugs in animals randomly chosen from the same herd) (Smith et al. 1993) when studying drug effects. Furthermore, injuries are frequently observed in impala capture when RDDS are used, therefore a comprehensive review of the current knowledge and methods of mitigating these injuries is required to improve the welfare of impala capture.

1.2.3 General anaesthesia techniques used to anaesthetise impala

Most of the drugs used to capture wild animals do not usually cause an adequate depth of anaesthesia for surgical intervention (Stage 3 Plane II and III). If general anaesthesia is required, anaesthetic drugs can be given to induce the necessary plane of general anaesthesia once the animal is chemically immobilised (Dugdale 2010; West et al. 2014; Grimm et al. 2015). General anaesthesia can be induced by administering a hypnotic (propofol, thiopentone, alfaxalone) or dissociative (ketamine) drug intravenously (most common technique in impala) or intramuscularly (ketamine or alfaxalone; only necessary in impala that are not effectively immobilised). In practice, the end-point of induction of general anaesthesia is usually defined as the state where an orotracheal tube can be placed without purposeful avoidance movements of the head (Dugdale 2010; Grimm et al. 2015). Other end-points for induction of general anesthesia have been used, such as the loss of the righting reflex, and loss of complex, purposeful movement during noxious stimulation, such as a pedal withdrawal reflex. Once induced into general anaesthesia then this state can be maintained by either administering inhalation or intravenous drugs (Ball 2007; Ball & Hofmeyr 2014).

In clinical practice with impala, general anaesthesia has been maintained by the Author using volatile anaesthetics, such as halogenated alkenes (halothane) or ethers (isoflurane or sevoflurane). Ordinarily, the volatile drug is delivered to the impala via a vaporiser and breathing system unique to the anaesthetic machine being used. The carrier gas for the volatile anaesthetic is usually 100% oxygen. Although compact and portable anaesthetic machines are commercially available, they are often not

available unless specifically purchased for field work. Often, these field ready anaesthetic machines are expensive and require oxygen cylinders or concentrators to provide a fresh gas flow source. There are a number of regulations and legal concerns that need to be met prior to transporting oxygen cylinders into the field that may make the practicality of using these machines less convenient. Oxygen concentrators, even the battery operated ones, require some source of power for operation or charging, which in turn also limit their practicality. Therefore, for most private wildlife veterinarians, it is not always feasible to maintain general anaesthesia in the field with an anaesthetic machine (Dzikiti 2013). Transporting impala to a veterinary facility where there is an anaesthetic machine is often logistically challenging and risky. The risk is especially high if wild unhabituated impala are being transported because they tend to mount a profound distress response. Despite the logistical and practical challenges of using the inhalation technique to maintain general anaesthesia it remains the Author's preferred technique because: 1) impala tend to recovery quickly once the administration of the inhalation drug has been stopped and the immobilisation drugs antagonised; 2) the inhalation drugs are easy to titrate to the desired anaesthetic depth; 3) the impala receive oxygen support during the general anaesthesia; 4) hypoventilation can be treated easily by administering intermittent positive pressure ventilation; and 5) because the airway of the impala must be secured with a cuffed endotracheal tube to deliver the gas mixture to the lungs, it minimises the risk of aspiration pneumonia (from saliva or regurgitated food) (Dugdale 2010; Ball & Hofmeyr 2014; Grimm et al. 2015).

Maintenance of general anaesthesia by administering injectable anaesthetic drugs alone or with adjuvant drugs intravenously is termed total intravenous anaesthesia (TIVA). The anaesthetic drugs can be administered as a bolus (given when the impala begins to respond to stimuli) or as a constant rate infusion, whereby the drug(s) are continuously infused intravenously at a rate that is fast enough to maintain a therapeutic plasma concentration of the drug (Dugdale 2010; Grimm et al. 2015). Target controlled infusion of hypnotic drugs (mainly propofol) is a form of TIVA that is used in human medicine. The target in question is either the plasma concentration or the effect site concentration, depending on the model (the Marsh versus the Schnider model, respectively) (Peck & Hill 2014; Grimm et al. 2015). The idea is to rapidly infuse a loading dose of the drug to reach the equilibrium concentration (maintenance infusion rate), where the rate of drug elimination (clearance) is balanced with the rate of infusion to maintain a plasma (or effect site) concentration within the therapeutic window (Peck & Hill 2014). Despite this technology being available, target controlled infusions have not yet been described

for antelope because drug-specific and species-specific pharmacokinetic values are not available for impala or other antelope. However, what is more practical and feasible is a constant rate infusion of drugs to maintain the required depth of general anaesthesia in impala. Ideally, the drugs used should be titrated to an effective clinical end-point (Grimm et al. 2015). The most common end-point in veterinary studies is the minimum infusion rate that prevents response to deep pain. The advantages of a TIVA drug protocol are: 1) that it can be used in the field easily; 2) drugs that can be fully antagonised at the recovery period of general anaesthesia can be used; 3) it can be cost effective because an expensive anaesthetic machine does not need to be purchased and maintained; and 4) it obviates the use of inhalation anaesthetics which are greenhouse gases (Dzikiti 2013). In ruminants such as impala, the use of TIVA to produce general anaesthesia does not remove concern for the possibility of aspiration of saliva or regurgitated rumen contents, or the likelihood of ruminal distension from fermentation. Thus, tracheal intubation with a cuffed endotracheal tube is recommended to guard the airway against aspiration and for providing mechanical ventilation (with an ambu bag) (Dugdale 2010; Grimm et al. 2015).

The investigation purposes, the deep pain response (clinical end-point evaluation for determining the minimum amount of drug for maintaining general anaesthesia for surgery) is elicited by a standard application of pressure (mechanical threshold) to a defined anatomical site. In domesticated ungulates (goats), this stimulus is achieved by clamping a vessuleum forceps around the coronary band of a claw for up to 60 seconds or until a purposeful withdrawal is elicited (Dzikiti et al. 2015; Ndawana et al. 2015). Other stimulating devices have also been described which maximally stimulate the patient and include: 1) pressure devices such as von Frey filaments or blunt pin actuators (such as a ProdPro device; Topcat Metrology, United Kingdom) to test the mechanical threshold; 2) thermal heating devices that rapidly heat a focal skin area up to 55°C to test the high temperature threshold; and 3) electrical stimulating devices, where a maximal current is applied over a defined time or until a withdrawal reflex response is evoked (Grimm et al. 2015). A purposeful withdrawal reflex is typically defined by the patient withdrawing the stimulated limb and attempting to move away from the pain source by moving other limbs or its head (Eger 1965; Grimm et al. 2015). However, the intravenous administration of anaesthetic drugs often alters the withdrawal response, whereby muscle relaxants and other drugs supress the movement of non-stimulated limbs and the head in response to painful stimulation (Dzikiti 2013; Dzikiti et al. 2015; Ndawana et al. 2015). Therefore, other criteria need to be considered, such

as a profound autonomic response, where the heart and respiratory rate or arterial blood pressure (mean arterial blood pressure) increases by more than 25% from pre-stimulation values in response to painful stimulation (Dzikiti et al. 2015; Ndawana et al. 2015). Total intravenous anaesthesia of impala has been described recently (Buck et al. 2017; Gerlach et al. 2017) and a description of one such protocol can be found in this thesis (Zeiler et al. 2015).

1.2.4 Drugs used during chemical capture and general anaesthesia of impala

Over the last seven decades, many drugs have been used to chemically capture and anaesthetise impala - a detailed description of all the different drugs falls outside the scope of this thesis. Therefore, only drugs that have been used in the drug trials described within this thesis will be mentioned.

The characteristics of the ideal chemical capture drug for immobilisation have been proposed and include the following (Harthoorn 1967; Haigh 1990):

- A wide safety margin

- Suitable for administering in most species

- Easily absorbed from most injection sites

- Safe to administer intravenously (or accidently via the intraosseous route)

- Allow maintenance of the righting reflex

- Retain essential body function

- Stable at high formulation concentrations to allow low volumes of administration

- Stable at a range of environmental temperatures and pressures

- Non-irritant to tissues at site of injection

- Soluble in water and miscible with other drugs

- Easy to reverse clinical effects or have antagonist (physiological or pharmacological) drugs that are safe to administer with minimal untoward effects

- Provide good muscle relaxation

- Provide analgesia

- Safe for the operator to handle

The list of ideal characteristics is extensive and to-date no single drug fulfils all of these characteristics. Therefore, in chemical capture, co-administration of drugs is often recommended, whereby drugs are used in combination at their lowest effective dose rates to minimise the side-effects of individual drugs and enhance (through additive or synergistic drug effects) the overall immobilisation effect. The practice of combining drugs at their lowest effective doses to achieve a desired clinical effect has long been used in veterinary anaesthesia and this technique is termed balanced anaesthesia (Dugdale 2010; Grimm et al. 2015). Regardless of the drugs used, the drug combination must be able to be placed into an appropriately sized dart taking the species (animal size) and distance of darting (related to velocity of the dart; projection over longer distances requires higher projection velocities) into account. Also, if the volume of the combination is too large, the rate of absorption from the injection site is decreased and could lead to unreliable onset of action and poor immobilisation quality (Bush 1992). Pharmacokinetic data of the drugs important to this thesis are scant in wildlife species and extrapolation among the species is difficult because of major differences in absorption, metabolism and excretion of drugs even in the domesticated species. Therefore, a focus on pharmacodynamic characteristics of the drugs of interest that have been used in impala will be discussed and brief mention will be made of extrapolated data (receptor interaction thought to be responsible for the analgesic, respiratory and cardiovascular effects of the drugs), where applicable. Agonist drugs are those that, when bound to a receptor, activate that receptor and initiate a full or partial response (Dugdale 2010; Peck & Hill 2014; Grimm et al. 2015). On the other hand, antagonist drugs bind to a receptor but do not have intrinsic activity and therefore do not initiate a cellular response, however, they displace or block agonist drugs from binding to the receptor. The pharmacodynamic explanations that follow in the broad discussion will focus mainly on what happens when an agonist drug is administered.

Opioid drugs

Opioid receptors are part of a large family of receptors, collectively grouped together as the G-protein coupled receptors (GPCRs). The GPCRs are made up of seven transmembrane subunits that are tethered to the inner surface of the cell plasmalemma. If an agonist binds to and activates the G-proteins, it will initiate an intracellular signalling cascade that will mediate (stimulate or inhibit) the actions of hormones and neurotransmitters (Riviera & Papich 2009). Binding of an agonist to the opioid

receptor will activate inhibitory intracellular pathways. The intracellular changes include closing of voltage gated calcium channels, promotion of potassium efflux and reduced cyclic adenosine monophosphate (cAMP) production, which all lead to decreased neuronal excitability (Riviera & Papich 2009). There are four classes of opioid receptors that are currently accepted and they include the *Mu* (MOP; μ), *Kappa* (KOP; κ), *Delta* (DOP: δ) and nociception/orphanin FQ peptide receptor (NOP) (Riviera & Papich 2009; Peck & Hill 2014). Endogenous opioid ligands bind to these receptors and together make up the endogenous opioid system. The endogenous opioid system is responsible for mediating many physiological effects that include pain, respiratory control, stress responses, appetite, locomotion, behaviour and thermoregulation (Peck & Hill 2014).

The brain and spinal cord (central nervous system) have greater opioid receptor density compared to the peripheral nervous system and other non-neural sites (Brunton et al. 2005; Grimm et al. 2015). Measurements of the density of opioid receptors in various anatomical locations has allowed more complete understanding of the physiological effects the endogenous opioid system. The endogenous opioids peptides belong to three distinct families, namely: enkephalins, dynorphins and beta-endorphin and these peptides are found throughout the central nervous system. None of the opioid peptides bind specifically to MOP, KOP or DOP receptors. The physiological roles of these peptides are not entirely understood, but appear to function as neurotransmitters, neuromodulators and neurohormones in the nervous, cardiovascular, respiratory and thermoregulatory systems (Grimm et al. 2015). Ongoing investigation to determine the role of the endogenous opioid peptides play within the body has revealed some mechanisms unique to the opioid pathways, especially in pain physiology (Grimm et al. 2015). Also, understanding the mechanics of the endogenous opioid system allows the prediction of drug effect when exogenous opioid drugs are administered to a patient. The effect that the various opioid agonist drugs have on the analgesic, respiratory and cardiovascular systems differ depending on how potent the agonist is (Grimm et al. 2015). In this thesis, the most potent agonists include etorphine and thiafentanil while the least potent "agonist" is butorphanol. Their drug effects differ substantially and thus will be described as such, where applicable.

The pathophysiology of pain is complex and a full account is outside the scope of this thesis. However, mention of the endogenous opioid system role in pain modulation is important (Dugdale 2010; Grimm et al. 2015). The peri-aqueductal grey matter, in the midbrain, has a high concentration of opioid receptors and is thought to be the origin of the descending opioid system (or tract). The descending

tracts (from the brain, down the spinal cord) are important in modulating the pain signals that ascend towards the brain by way of the ascending tracts (Dugdale 2010). The descending tracts are not well defined anatomically, unlike the various ascending tracts, and should rather be termed descending systems (Dugdale 2010). The descending systems are poorly understood. However, it appears that once the opioid receptors within the peri-aqueductal grey matter are stimulated, then the descending opioid system stimulates the nucleus raphe magnus (pons and rostral medulla) and the dorsal horns within the spinal cord (Grimm et al. 2015). The stimulation of the nucleus raphe magnus activates other descending inhibitory systems that include the serotonergic and noradrenergic systems. The descending serotonergic system interacts with the dorsal horn through the release of adenosine. The descending noradrenergic system (which includes the alpha$_2$-adrenoceptors) stimulates the cholinergic system, which interacts with the dorsal horn through the release of acetylcholine. Both the serotonergic and noradrenergic descending systems result in analgesia through the modulation of the transmission of pain signals, at the level of the dorsal horn, that are ascending towards the brain (Grimm et al. 2015). Peripheral opioid receptors are expressed in increased numbers in inflamed tissue, especially tissue surrounding synovial joints (Dugdale 2010). Administration of opioid agonist drugs at peripheral sites of injury, especially synovial joints, provides analgesia by decreasing neuronal excitability and possibly by modulating the response to inflammation (Brunton et al. 2005; Grimm et al. 2015). The analgesic effects of opioid receptor agonist drugs is critical to the initial management of acute (and chronic) pain states. However, the response to opioid receptor agonists is variable among the species and the benefit of opioid use in managing acute pain in domesticated ruminants (ovine, caprine and bovine) has had variable success in alleviating pain (Nolan et al. 1987; Nolan et al. 1988; Waterman et al. 1990; Waterman et al. 1991a, b).

The physiological control of the respiratory cycle and how the endogenous opioid system mediates this physiological process is complex. Simplistically, the respiratory system is chiefly responsible for gas exchange between the blood and ambient air (Grimm et al. 2015). The principle goal is to absorb oxygen and vent CO_2 so that the arterial blood has enough oxygen for the metabolic demand of the body and that CO_2 (a by-product of metabolism) is regulated within a physiological range (Clarke et al. 2014; Haskins 2015). Therefore, the respiratory system is responsible for moving enough gas into and out of the alveoli to ensure that oxygen and CO_2 levels remain within physiological ranges to ensure homeostasis (Grimm et al. 2015). The total movement of gases is measured as the minute volume.

Mathematically, the minute volume (measured in mL or L per minute) is the product of the tidal volume (volume [ml or L] of gas moved in and out during a normal resting breath) and respiratory rate (the breaths per minute) (Dugdale 2010). One respiratory cycle is divided into three sequential phases: inhalation, exhalation and a respiratory pause, in that order. Autonomic nervous system control ensures continuous, subconscious, rhythmic cycling through the three phases for each breath (Grimm et al. 2015). The respiratory rhythm generating area of the brain is found in the medulla and the function is strongly modulated by influences emanating from the pons. The area in the medulla has been named the pre-Bötzinger complex, which is a small area located in the ventrolateral medulla in rats (*Rattus sp.*). In humans, and possibly other large mammals, this area has not yet been identified, but appears to form a coupled oscillator with nearby structures called the retro-trapezoid and parafacial respiratory group (Pattinson 2008). Regardless of this fact, the rhythm generator will be referred to as the pre-Bötzinger complex from here onwards. The modulating influence from the pons emanates mainly from the Kölliker-Fuse nucleus, the parabrachial complex, and the locus coeruleus. Simplistically, the pre-Bötzinger complex initiates the inhalation phase of the breath and the Kölliker-Fuse nucleus is responsible for the termination of the inhalation phase and initiation of the exhalation phase of the breath during normal subconscious (autonomic) breathing (Shook et al. 1990; Pattinson 2008). The fundamental drive to breathe is modulated by central (brainstem) and peripheral (carotid and aortic bodies) chemoreceptors that monitor and sense changes in the chemical (pH, CO_2 and oxygen gas tensions) constituents of blood. Furthermore, conscious inputs may be initiated from the cortex (i.e. holding one's breathe at any stage of the cycle). Also, mechanoreceptors located in the epithelial, submucosal and mucosal layers of the respiratory tract relay mechanical and sensory information from the lungs (and airways) to the pons, which in turn influence the medulla respiratory centre (Pattinson 2008). During resting tidal breathing, the effects of the arterial partial pressure of carbon dioxide has the most influence on the respiratory system to breathe enough to maintain a steady partial pressure between 35 and 45 mmHg (Dugdale 2010; Grimm et al. 2015). The central chemoreceptors are the most important detectors of any change in arterial partial pressure of carbon dioxide. The central chemoreceptors detect a change in pH within the cerebrospinal fluid. Carbon dioxide is very soluble and easily crosses the blood brain barrier. Once within the cerebrospinal fluid, it dissolves in water to form carbonic acid, which rapidly dissociates into bicarbonate ions and hydrogen ions (Nattie & Li 2012). The hydrogen ions are responsible for the resultant pH. Therefore, if a large load of CO_2 reaches the

cerebrospinal fluid, it results in a higher concentration of hydrogen ions, which decreases pH and thus stimulates the chemoreceptors to activate the respiratory centre to increase the minute volume (Pattinson 2008; Nattie & Li 2012). The latter will increase minute volume and thus the amount of CO_2 (volatile acid) that is vented from the alveoli during exhalation (Grimm et al. 2015). Thus carbon dioxide is the most important regulator and stimulant for autonomic breathing in a healthy patient. The partial pressure of oxygen is important for activating the peripheral chemoreceptors (Grimm et al. 2015). Only in states of hypoxaemia, where the arterial partial pressure of oxygen drops below 60 mmHg, will the peripheral chemoreceptors activate to increase ventilation (Prabhakar 1985; Grimm et al. 2015). The hypoxic drive for ventilation tends to peak when the arterial partial pressure of oxygen is approximately 40 mmHg in most animals (Grimm et al. 2015). The mechanoreceptors, especially those present in the lung parenchyma, are responsible for regulating the tidal volume. The mechanoreceptors are stretch receptors, and once stretched, will increase signalling towards the pons to stop the inhalation phase of the respiratory cycle (Yu et al. 1985). Opioid receptors (mainly *mu* opioid receptors) are expressed in all of the areas known to influence the respiratory cycle, including the medulla, pons, chemoreceptors (central and peripheral) and mechanoreceptors (Shook et al. 1990; Pattinson 2008). Therefore, administration of an exogenous opioid agonist drug will affect the respiratory cycle and respiratory system. However, there are species variations in drug effect. Primates (human and non-human) are thought to be the most sensitive to the respiratory depressant effects of opioid agonist drugs, whereas domestic canines, felines, equines, bovines, ovines, caprines and porcine appear to have a smaller respiratory response to opioid agonist drugs at doses used routinely to produce analgesic (e.g., morphine, methadone, buprenorphine, butorphanol and fentanyl) (Dugdale 2010; Grimm et al. 2015). When the routine opioid agonists are administered at appropriate clinical doses, they tend to alter the ventilatory response so that a higher level of carbon dioxide is required prior to initiating a breath (Grimm et al. 2015). The overall effect on minute volume in most domesticated animals is minimal and normally of little clinical concern. However, when these drugs are administered at higher doses than those recommended for clinical use then the respiratory depression becomes more complex and profound. In this case, mechanisms other than simple hypoventilation (decreased minute volume due to overall central nervous system depression and depressed central chemoreceptor response) are involved and therefore the respiratory depression should rather be termed respiratory inefficiency (Ko et al. 2003). The pathophysiology of drug-induced respiratory inefficiency involve the respiratory and cardiovascular

system, and how these two systems integrate at the alveolar-capillary junction, discussed next. The potent opioids (etorphine, carfentanil and thiafentanil, and fentanyl at high doses) are thought to disrupt normal respiratory rhythm generation by interfering with the pre-Bötzinger complex (causing a slow inspiratory effort or struggling to initiate an inhalation effort) and Kölliker-Fuse nucleus (cannot easily transition from inhalation phase to exhalation phase) through activation of mu-receptors (Pattinson 2008). Furthermore, the opioids also decrease the central and peripheral chemoreceptor response to arterial carbon dioxide and oxygen gas tensions (Shook et al. 1990; Pattinson 2008). Another manifestation of respiratory impairment induced by the potent opioids is the "woody chest syndrome" (also termed opioid-induced chest wall rigidity). This syndrome is observed when the animal displays a rigid thoracic wall and diaphragm so that attempts to inhale do not move any air (Coruh et al. 2013; Soares et al. 2014). The mechanism responsible for the muscle rigidity and failure to initiate an inhalation effort is poorly understood and appears complex in nature. The mechanism is thought to originate due to conflicting inputs into the respiratory centre from within the brain (cortex and respiratory centre), spinal cord (descending opioid tracts modulating inputs towards the brain) and peripheral mechanoreceptors (altered pressure mechanics within the lung parenchyma induced by the rigid thorax and diaphragm) (Bowdle & Rooke 1994; Jackson 1994; Coruh et al. 2013; Soares et al. 2014).

The drug effect of the opioid agonists on the cardiovascular system are minimal when routine drugs are administered at clinically relevant doses to domesticated animals. Some of the drugs might stimulate the release of histamine (morphine and pethidine), which could cause vasodilation and decrease blood pressure (Nolan et al. 1988; Dugdale 2010). If the conventional drugs are administered at high doses then central nervous system depression and depression of the cardiovascular centre (sympatholytic effect) and stimulation of the parasympathetic system (mediated through the vagal nerve) can result in bradycardia and even hypotension. The potent opioids (etorphine and thiafentanil) have an opposite effect and, through poorly understood mechanisms, cause systemic and pulmonary hypertension and tachycardia (Lance & Kenny 2012; Meyer et al. 2015). In order for sufficient gas diffusion to take place to maintain gas tensions (CO_2 and oxygen) within their physiological ranges, the alveoli must be perfused adequately. The opioids, especially the potent opioid agonist drugs, can disrupt the cardiopulmonary integration at either the respiratory system or the cardiovascular system, or both systems could fail to meet adequate integration (discussed further in section 1.2.5).

Inter-species differences in the response to opioid drugs is likely due to differences in receptor density (Grimm et al. 2015). The ratio of the *mu* to *kappa* opioid receptors is also thought to differ among the species (Yoburn et al. 1991). Also, the opioid receptor affinity for the various drugs could differ among the species (Schattauer et al. 2012; Sirohi et al. 2016). The variable effects of the opioid agonist drugs makes extrapolating from one species to another a near impossible challenge (Schattauer et al. 2012). Furthermore, opioid drugs are not routinely administered to domesticated livestock (bovine, ovine, caprine) because of legislative restrictions and drug licencing constraints (Nolan et al. 1988; Lin & Walz 2014; Grimm et al. 2015). However, when administered mainly for analgesia, the clinical effects are not as well recognised and could be because of different receptor densities (e.g., less *mu* opioid receptors) in the CNS or because these animals are stoic in nature and we cannot accurately determine the level of pain perceived (Lin & Walz 2014; Grimm et al. 2015).

There are many different opioid drugs that have been used to capture free-ranging animals and they can be grouped into classes that describe their characteristic interaction on opioid receptors, namely: pure agonists, mixed agonist-antagonists or pure antagonists (Peck & Hill 2014; Grimm et al. 2015). The opioid drugs relevant to this thesis include etorphine, thiafentanil, butorphanol and naltrexone, and they will be discussed further.

Etorphine is a semi-synthetic oripavine derivative of the opioid alkaloid thebaine, belonging to the phenantherene chemical opioid class (Blane et al. 1967). Harthoorn and Bligh (1965) first reported the use of etorphine in domesticated ungulates, prior to use in chemical capture of free-ranging animals (Harthoorn & Bligh 1965). Etorphine is a potent pure opioid receptor agonist and binds non-specifically to the *mu*, *kappa* and *delta* opioid receptors, similar to morphine. The formulation of etorphine, in South Africa, is 9.8-10.0 mg ml^{-1} which is a high concentration considering that it is at least 1000 times more potent than morphine depending on the study model and design (Blane et al. 1967). Etorphine became a very popular drug for chemically capturing animals as small volumes are required, and can easily fit into a small volume dart (Harthoorn & Bligh 1965). The absorption from intramuscular sites of injection and onset of action is dose dependent and considered to be quick. Ungulates often progress through predictable phases during etorphine chemical capture. First, the darted animal stops running if it is fleeing from being chased, then it slows its gait pace, demonstrates signs of dissociation from its environment, and begins to wander away from its herd. The wandering is often at a slow pace and progresses into a characteristic high-stepping, stilted "Hackney gait", with or without opisthotonus

(Harthoorn & Bligh 1965). At this point, the animal becomes profoundly ataxic and often collapses or stumbles into sternal or lateral recumbency with or without attempts to stand or regain its footing. Etorphine remains the "gold standard" to which all other potent opioids are compared for chemical capture of ungulates (Lance & Kenny 2012).

Thiafentanil is a synthetic fentanyl derivative belonging to the anilidopiperidine chemical opioid class (Vardanyan & Hruby 2014). Reports on the use of thiafentanil use in ungulate chemical capture began to emerge in the early 1990s (Janssen et al. 1993), where impala were used as research model. Thiafentanil is a potent pure opioid receptor agonist and binds specifically to the *mu* opioid receptors, similar to fentanyl. The formulation of thiafentanil, in South Africa, is 10.0 mg ml^{-1} and is manufactured and bottled in South Africa (Kock & Burroughs 2012). Since the introduction of thiafentanil into clinical practice, various claims have been made, such as it being faster acting than etorphine and that it causes less respiratory depression. Furthermore, claims are made that thiafentanil has "twice the potency of etorphine" (Lance & Kenny 2012). Many of the claims are based on studies where only thiafentanil has been used to immobilise ungulates, and the study outcomes are compared to the etorphine literature. Thiafentanil is more lipid soluble than etorphine and this might facilitate a more rapid increase in effect site concentration within the brain and spinal cord, thus possibly explaining the claimed rapid onset of action (Lance & Kenny 2012). No formal study could be found where thiafentanil was compared directly with etorphine in the same animal herd, at the same dose rates, to determine if the claims were true.

Butorphanol is a semisynthetic morphine-like derivative belonging to the phenanthrene chemical opioid class. Butorphanol is traditionally classified as a mixed opioid receptor agonist-antagonist and binds to *mu* and *kappa* opioid receptors. Traditionally butorphanol was thought to have no intrinsic activity at the *mu* opioid receptor (hence it was thought to be an antagonist) and to have a stimulating action on the *kappa* opioid receptor (hence it was thought to be an agonist). However, recent pharmacology texts suggest that butorphanol has partial agonistic activity at the *mu* opioid receptor (Bush et al. 2012; Peck & Hill 2014; Grimm et al. 2015). Butorphanol is gaining popularity in wildlife chemical capture as either an immobilising drug (Bush et al. 2012) or for its *mu* antagonistic properties to reverse the respiratory depression from potent opioids (etorphine or thiafentanil) (Bush et al. 2012; Haw et al. 2016b). An important consideration is that, in mammals, butorphanol has mild, short acting analgesic activity (equine and feline) or in some animal models (canine) no analgesic activity (Dugdale 2010). Butorphanol is estimated to be 2.5 to 7 times more potent than morphine, yet the drug has a "ceiling

effect" to its pharmacodynamic properties, and especially to its analgesic properties (Muir 2009; Bush et al. 2012).

Naltrexone is a long acting opioid antagonist drug that binds to *mu*, *kappa* and *delta* opioid receptors and causes no intrinsic activity. Naltrexone is an ideal drug to antagonise the clinical drug effects of etorphine and thiafentanil (and butorphanol) because it is said to last up to 12 hours. In impala, naltrexone is administered at a ratio (mg:mg) of either 20:1 (Zeiler et al. 2015) or 40:1 (Perrin et al. 2015) to the dose of etorphine. Free-ranging impala should receive at least a 20:1 ratio, to ensure that the drug effects of etorphine are completely antagonised. Naltrexone has also been used at a ratio of 10:1 to antagonise the clinical effects of thiafentanil (Meyer et al. 2008a, b; Buck et al. 2017). Although naltrexone has no intrinsic activity when bound to the opioid receptors, it does block endogenous opioid ligands from binding. Blocking the endogenous opioid system can result in physiological changes in the respiratory and cardiovascular systems and the response to pain transmission and perception. Furthermore, side effects of naltrexone administration alone to humans have been described and include tachycardia, systemic hypertension, pulmonary oedema and cardiac dysrhythmias that include fatal ventricular fibrillation (Brunton et al. 2005; Peck & Hill 2014). In veterinary science, only empirical evidence of these side effects exists, especially in wildlife clinical practice. Administration of naloxone or naltrexone is proposed to cause activation of the cholinergic arousal systems of the brain that is independent of opioid receptor interaction (Brunton et al. 2005; Dugdale 2010; Grimm et al. 2015). The cholinergic arousal system is one of the proposed systems that is responsible for arousal and keeping mammals awake. Exogenous administration of acetylcholine (or administering acetylcholinesterase antagonists) stimulates cortical activity regardless of behavioural arousal or motor activity (Brunton et al. 2005). A number of cholinergic neuronal pathways have been described in the basal forebrain and brainstem and are thought to be the origin of cortical activation and increased arousal (Jones 2008).

Cyclohexylamine drugs

The cyclohexylamine class of drugs cause a state of anaesthesia called dissociative anaesthesia. This state is characterised by a cataleptic state (a trance-like state of diminished responsiveness and continuously maintained immobility, often with *flexibilitas cerea* [waxy rigidity of muscles]). The catalepsy is mainly caused by the suppression of thalamocortical (ascending) pathways and activation

of the limbic system (Miyasaka & Domino 1968; Massopust et al. 1973; Kästner 2007; Dugdale 2010). Therefore, many reflexes that are typically absent during general anaesthesia induced by other anaesthetic drugs, are present during cyclohexylamine-induced dissociative anaesthesia. In the dissociative state, the patient retains an open-eyed, slow, nystagmic gaze, varying random purposeful appendicular limb movements and, in some species, varying degrees of muscle hypertonia (Kohrs & Durieux 1998). An important advantage is that the cyclohexylamines are excellent hypoalgesics (Dugdale 2010; Grimm et al. 2015). Two drugs are commonly used in wildlife chemical capture and general anaesthesia, namely tiletamine (the drug formulation is combined with a benzodiazepine drug zolazepam) and ketamine (West et al. 2014). Phencyclidine, the predecessor drug to tiletamine and ketamine is no longer used commonly (Grimm et al. 2015). Ketamine is relevant to this thesis and will be discussed further.

Ketamine is a phencyclidine derivative and has a complex mechanism of action due its ability to bind to several receptors. The interaction of ketamine with neuron-excitatory *N*-methyl-*D*-aspartate (NMDA) receptors is the most studied. The NMDA receptors control non-specific cation channels of neurons and are unique in that they are both voltage-gated and ligand-gated, and both gating mechanisms must be satisfied before the channel opens (Miyasaka & Domino 1968; Massopust et al. 1973; Kohrs & Durieux 1998; Dugdale 2010). First, the neuron must undergo repeated depolarisation so that the neurolemma becomes positively charged. The positively charged neurolemma repels magnesium ions that block the ion channel. Once the magnesium ion is repelled, the ligand receptor binding sites become available (Dugdale 2010; Grimm et al. 2015). Secondly, glutamate, an excitatory neurotransmitter, (plus glycine its obligatory co-agonist) bind to the ligand receptor site opening the ion channel. The ion channel remains open for a relatively long period compared to other voltage and ligand gated ion channels (Grimm et al. 2015). Once open, the channel allows the movement of cations, especially calcium, into the neuron. Calcium influx into the neuron affects many intracellular and signalling processes which include activation of enzymes, altering gene expression and synthesis and activation of receptors (Dugdale 2010; Grimm et al. 2015). NMDA receptors have a phencyclidine binding site where ketamine binds and causes no intrinsic activity, thus antagonising the effects of the NMDA receptor. Ketamine also binds to other receptor populations and has variable activity, as follows (Miyasaka & Domino 1968; Massopust et al. 1973; Brockmeyer & Kendig 1995; Kohrs & Durieux 1998; Dugdale 2010):

- Non-NMDA glutamate receptors (α-amino-hydroxy-5-methyl-4-isoxazoleproprionoic acid [AMPA] receptor and kainite receptors) – antagonist activity

- *Mu* opioid receptor – antagonist activity; *kappa* and *delta* opioid receptor – agonist activity

- Gamma-aminobutyric-acid A receptors (GABA$_A$ receptor) – agonistic activity, but controversial

- Nicotinic and muscarinic cholinergic receptors – antagonist activity

- Monoaminergic receptors (uptake 1 and 2) of noradrenaline, serotonin (5-HT) and dopamine – antagonistic activity

- L-type voltage-gated calcium channels – antagonist activity

Furthermore, ketamine has been said to have local anaesthetic effects (proposed mechanism is due to blockage of voltage-gated sodium ion channels) and anti-inflammatory and immune-modulatory actions by supressing Nuclear factor *kappa* B (NFκB) activation and possibly the release of adenosine (Dugdale 2010; Grimm et al. 2015).

The pharmacodynamics of ketamine differs among the species where, for example, dogs, cats and horses demonstrate profound muscle hypertonia while cattle, goats and sheep do not. The same variable response could be true for wildlife species. For example, Bush et al. (2004) administered ketamine alone to impala and noted that no sedation nor adverse effects, except slight ataxia were evident at intramuscular doses of approximately 8.0 mg kg^{-1}.

The hypoalgesic effects of ketamine are profound, even when administered at sub-anaesthetic doses. The receptor interaction is complex because ketamine is thought to interact with many different receptors that are involved with pain signal transmission to the brain and pain signal modulation at the dorsal horn and brain (Ohtani et al. 1979; Kitahata et al. 1973). Also, when administered at anaesthetic dose rates, the patient is less responsive to surgical pain, such as scalpel incision. Ketamine is commonly included in treatment plans to manage domesticated animals suffering moderate to severe pain. Domesticated ruminants appear to respond better to the administration of ketamine for hypoalgesia compared to administering opioid drugs, such as butorphanol or morphine (Lin & Walz 2014; Grimm et al. 2015).

Ketamine, alone, has limited effects on the respiratory system. When ketamine is administered alone at appropriate clinical doses, it has a limited effect on the ventilator response to carbon dioxide and oxygen (Dugdale 2010). However, when administered in combination with other drugs (opioids,

benzodiazepines and alpha$_2$-adrenoceptor agonists), or at large intravenous doses, then apnoea or other changes in the respiratory breathing pattern are common (Zsigmond et al. 1980; Bidwai et al. 1975; Dugdale 2010). The most common breathing pattern that is noted in ruminant animals is an apneustic breathing pattern where animals hold their breath after the inhalation phase prior to exhaling. Often, after the apneustic breath the animal takes three to four rapid deep breaths before another apneustic breath (Evans et al. 1972; Thurmon et al. 1972; Dugdale 2010). Biot's (three to four large breaths followed by a period of apnea during the respiratory pause after exhalation between breaths) and Cheney-Stokes (a series of four to five breaths that increase and then decrease in depth followed by an apneic pause during the respiratory pause) breathing patters could also manifest during ketamine-based anaesthesia (Child et al. 1972; Evans et al. 1972; Thurmon et al. 1972). The upper airway guarding reflexes and swallowing reflexes remain intact, yet these reflexes should not be relied on to guard the airway during general anaesthesia because aspiration is still possible. Salivation (especially in ruminants) and tracheobronchial mucous gland secretion are increased (Lin & Walz 2014). Ketamine also causes bronchodilation, albeit through an unproven mechanism but thought to be due to the cholinergic receptor antagonism (Corssen et al. 1972; Huber et al. 1972; Brown & Wagner 1999).

The cardiovascular system effects of ketamine are complex and differ depending on the patient's haemodynamic status at the time of drug administration (Tweed et al. 1972; Baraka et al. 1973; Ivankovitch et al. 1974; Wong & Jenkins 1974; Salt et al. 1979; Altura et al. 1980). When ketamine is administered there is a notable increase in systemic and pulmonary arterial blood pressure, heart rate, cardiac output, cardiac workload and myocardial oxygen consumption (Grimm et al. 2015). All of these effects resemble the effects of sympathetic nervous system stimulation (Dugdale 2010; Grimm et al. 2015). Ketamine appears to transiently stimulate the release of endogenous catecholamines, such as noradrenaline, which is the neurotransmitter of the sympathetic nervous system at the neuron-effect organ synaptic junction (Grimm et al. 2015). Thus, the haemodynamic stimulatory effects of ketamine are said to be indirect in that an intact sympathetic nervous system is required to cause stimulation of the cardiovascular system. The direct drug effects of ketamine are opposite in effect and cause negative inotropy and a decrease in cardiac output and arterial blood pressure (Tweed et al. 1972). The effects of ketamine on cardiac rhythm is variable.

Alpha$_2$-adrenoceptor drugs

Alpha-adrenoceptors are a part of the receptor series of the sympathetic branch of the autonomic nervous system. The alpha-adrenoceptors are classified based on their pharmacological properties into two different types, namely alpha$_1$-adrenoceptor and alpha$_2$-adrenoceptor types (Peck & Hill 2014). The sympathetic branch of the autonomic nervous system also has beta-adrenoceptors that are divided into three different pharmacological types and are mentioned here for completeness sake but will not be considered further in this thesis. The alpha-adrenoceptors belong to the GPCRs similar to opioid receptors (Riviere & Papich 2009; Peck & Hill 2014). The alpha$_1$-adrenoceptor binds to a Gp protein and produces intracellular effects by activating the enzyme phospholipase C. The activation of phospholipase C causes hydrolysis of inositol trisphosphate (IP$_3$) and release of calcium from the intracytoplasmic stores which causes contraction of myocardial and vascular smooth muscle cells (Brunton et al. 2005; Riviere & Papich 2009). The alpha$_2$-adrenoceptors bind to Gi proteins which inhibit adenylate cyclase activity and attenuates the production of cAMP, similar to the effects of opioid drugs (Dugdale 2010; Grimm et al. 2015). However, an alternative signalling transduction mechanism is responsible for the vasoconstriction effects of alpha$_2$-adrenoceptor agonist administration. This alternative signalling is complex and important to mention here and not in the cardiovascular effects of the drugs. Within vascular smooth muscle, alpha$_2$-adrenoceptor receptors are located not only on the post-synaptic membrane but also presynaptically and extrasynaptically (Brunton et al. 2005; Riviere & Papich 2009; Grimm et al. 2015). Binding of alpha$_2$-adrenoceptor agonists to the extrasynaptic receptors activates the opening of receptor-operated calcium channels, which increases the intracellular concentration of calcium that results in vascular smooth muscle contraction (Brunton et al. 2005; Grimm et al. 2015). This extrasynaptic receptor effect complements the alpha$_1$-adrenoceptor induced vascular smooth muscle contraction. The vascular smooth muscle response to alpha$_2$-adrenoceptor agonist administration is more profound on the venous side of the systemic (and possible pulmonary) circulation compared to the arterial side (Brunton et al. 2005; Grimm et al. 2015). Therefore, the vasoconstriction induced by the endogenous alpha$_2$-adrenoceptor agonists (noradrenalin and adrenalin) could be important in mobilising blood volume from the venous capacitance vessels that augment cardiac return and output during stress situations (Grimm et al. 2015). However, the simple explanation of how alpha$_2$-adrenoceptor agonists, whether endogenous (noradrenalin or adrenalin) or exogenous (alpha$_2$-adrenoceptor agonist drugs), is complicated by the presynaptic alpha$_2$-

adrenoceptors. Therefore, the response of the vascular smooth muscles to the administration of alpha$_2$-adrenoceptors is divided into two phases, a peripheral phase and a central phase (Bousquet et al. 1999; Head 1999). The peripheral phase is because of the extrasynaptic and postsynaptic receptor activation, as described (Brunton et al. 2005; Grimm et al. 2015). The central phase is when the presynaptic receptors are activated by the alpha$_2$-adrenoceptor agonist. Normally, when sympathetic nerves are activated (action potential reaching the synaptic junction of the presynaptic nerve) noradrenaline is released as the neurotransmitter (Brunton et al. 2005; Peck & Hill 2014; Grimm et al. 2015). The noradrenaline, released from the presynaptic receptor into the synaptic cleft, binds to the alpha$_1$-adrenocetors (mainly) on the postsynaptic effect organ. As the concentration of noradrenaline rises within the synaptic cleft, the excess noradrenaline will bind to the presynaptic alpha$_2$-adrenoceptors causing a negative feedback effect, which inhibits further release of noradrenaline from the presynaptic nerve terminal (Grimm et al. 2015). Exogenous alpha$_2$-adrenoceptor agonist drugs can bind to the presynaptic receptors and therefore create a "false" negative feedback effect which decreases the amount of noradrenalin being released from the presynaptic nerve terminal, thus decreasing alpha$_1$-adreneceptor effects and decreasing the vascular smooth muscle contraction (Riviere & Papich 2009; Dugdale 2010). When the extrasynaptic receptor effects wane (peripheral phase) and the presynaptic effects are pronounced (central phase), the smooth muscle vascular tone decreases and thus alters the haemodynamic effect (Brunton et al. 2005; Grimm et al. 2015). The central phase (decreased release of noradrenalin from the nerve terminal because of the false negative feedback effect) is also responsible for the suppression of the cardiovascular centre in the brain, therefore decreasing sympathetic nervous system tone from supraspinal levels within the central nervous system (Bloor et al. 1992). The alpha$_2$-adrenoceptor drugs do not exclusively bind to the alpha$_2$-adrenoceptors, but also have intrinsic activity at the alpha$_1$-adrenoceptors (Brunton et al. 2005). The ratio of binding to the two different alpha-adrenoceptors differs among the different alpha$_2$-adrenoceptor drugs (Dugdale 2010; Peck & Hill 2014; Grimm et al. 2015). Alpha-adrenoceptors are not the only receptor types that the imidazoline-derivative alpha$_2$-adrenoceptor drugs bind to, they also bind to imidazoline receptors (Bousquet et al. 1999; Head 1999). The imidazoline receptors are not as well defined as the alpha-adrenoceptors, however, they are divided into two broad categories, I1 and I2 receptors (Dugdale 2010; Grimm et al. 2015). The I1 receptors are found in the central nervous system, especially the brain, while I2 receptors are found centrally (in the brain) and peripherally in organs such as the kidney and

pancreas (Dugdale 2010; Grimm et al. 2015). Interaction between alpha$_2$-adrenoceptors and imidazoline receptors has been described, where activation of imidazoline receptors can influence the activity of nearby alpha$_2$-adrenoceptors, however, this has not been well defined (Dugdale 2010). Activation of imidazoline receptors is thought to cause natriuresis and a decrease in sympathetic nervous system outflow from the central nervous system (Brenner & Stevens 2013). Furthermore, the alpha$_2$-adrenoceptor (and imidazoline receptor) density and location differs among the species and therefore the dose rates for the desired clinical effects (sedation, muscle relaxation and analgesia) differ significantly between domesticated species (Raptopoulos & Weaver 1984; Carol & Hartsfield 1996; Kästner 2006; Dugdale 2010; Lin & Walz 2014; Grimm et al. 2015). Bovine are believed to be the most sensitive, especially to xylazine, followed by ovine and caprine species and porcine being the least sensitive (Lin & Walz 2014; Grimm et al. 2015). This difference in the domesticated species might also be true for wild animals, whereby ruminants, especially from the *Bovidae* family might be more sensitive compared to other ungulate species (West et al. 2014).

The alpha$_2$-adrenoceptor agonist drugs are sedatives that also have muscle relaxation and analgesic properties. They are either administered alone (for sedation) or in combination with other drugs to achieve chemical immobilisation or general anaesthesia in wildlife (Kock & Burroughs 2012; West et al. 2014). The sedative effects are due to the Gi protein activation, which causes a decrease in cAMP and thus release of the neurotransmitter noradrenaline from the nerve terminal. Decrease in noradrenaline release in the *locus coeruleus* in the brain stem is thought to be primarily responsible for the sedative effects (Brunton et al. 2005; Grimm et al. 2015). Furthermore, a decrease in noradrenaline release within the *substantia gelatinosa* (Rexed lamina II found in the dorsal horn of the grey matter of the spinal cord) attenuates the transmission of signals to the reticular activating system (RAS) and thus could contribute to the overall sedation by decreasing RAS activity (Riviere & Papich 2009). Suppression of the RAS is also the mechanism for anxiolysis (Dugdale 2010). The muscle relaxant effects, which manifest as ataxia and recumbency, are due to postural and smooth muscle relaxation. The muscle relaxation cannot easily be attributed to a single receptor population, but rather it is likely due to a number of possible alpha$_2$-adrenoceptor agonist drug effects. Proposed mechanisms include reduced vigilance due to the sedative and anxiolytic effects, central action at alpha-adrenoceptor and imidazoline receptors, interaction at glycine and GABA$_A$ receptors and possibly peripheral and central local anaesthetic effects (Grimm et al. 2015).

The analgesic effects of the alpha$_2$-adrenoceptor agonist drugs are similar and synergistic to that of the opioid agonist drugs' direct effect on neurons within the spinal cord (Moens et al 2003). The GPCRs resultant action within the neuron is similar and results in a decreased neurotransmission of pain signals to the brain. The suppressed neural activity (decrease in cAMP and excitatory neurotransmitter release) augments the opioid agonist drugs' activation of the descending opioid systems that modulate the transmission of pain signals towards the brain (Grimm et al. 2015). The alpha$_2$-adrenoceptor agonist drugs, especially xylazine, have a similar chemical structure to local anaesthetic agents (lidocaine, bupivacaine or ropivacaine) and they also have mild local anaesthetic action which result in analgesia (Dugdale 2010).

The respiratory system effects of the alpha$_2$-adrenoceptor agonist drugs is generally minimal when they are administered at the lower end of the dose ranges used in domestic animals, especially in equides, canines and felines. Domestic ruminants tend to demonstrate dose-dependent respiratory depression (Lin & Walz 2014). A decrease in respiratory rate is evident, but the tidal volume increases and thus overall minute volume is maintained (Grimm et al. 2015). Maintenance of the minute volume is confirmed by a lack of major changes in the arterial blood gases after alpha$_2$-adrenoceptor agonist drug administration (Dugdale 2010). However, when the alpha$_2$-adrenoceptor agonist drugs are administered in high doses or in combination with opioids then the respiratory depression effects become more profound (Dugdale 2010; Grimm et al. 2015). Smooth muscle relaxation also relaxes the muscles surrounding the upper airway and this could lead to upper airway obstruction (Grimm et al. 2015). Ruminants tend to manifest bronchoconstriction and increased pulmonary vascular resistance unlike other species which do not (Lin & Walz 2014; Grimm et al. 2015). The two effects have been proposed as plausible mechanisms for pulmonary oedema formation and hypoxaemia. These respiratory effects are particularly evident in small stock ruminants, especially sheep (Kästner 2006).

The vascular smooth muscle effects of the alpha$_2$-adrenocetor agonist drugs have been mentioned above during discussion of the peripheral and central phases of alpha$_2$-adrenocetor agonism (Kästner 2006). The effects on the vascular smooth muscles are profound in all species and warrant further discussion. Arterial blood pressure oscillates between the systolic and diastolic pressures as the heart pumps blood into the arterial side of the systemic circulation (Grimm et al. 2015). The oscillating pressure can be described mathematically by determining the product of cardiac output and systemic vascular resistance (arterial blood pressure = cardiac output x systemic vascular resistance) (Dugdale

2010; Grimm et al. 2015). Cardiac output is the product of heart rate and stroke volume. Stroke volume is related to, in the simplest form, the inotropic effects of the heart and the ventricular-vascular coupling loading conditions known as preload and afterload (Grimm et al. 2015). Soon after an alpha$_2$-adrenoceptor agonist drug is given there is a pronounced increase in systemic vascular resistance due to the intense vascular smooth muscle contraction (postsynaptic and extrasynaptic alpha$_2$-adrenoceptor agonist drug effect) and this causes arterial blood pressure to rise significantly during the peripheral phase (hypertension is common with mean arterial blood pressures often exceeding 150 mmHg) (Grimm et al. 2015). Baroreceptors located in the aortic and carotid bodies detect the increase in blood pressure causing these receptors to increase their firing rate. The baroreceptor signals are transmitted to the cardiovascular centre in the brain where they initiate a counter response in an attempt to correct the raised blood pressure (Dugdale 2010; Grimm et al. 2015). The counter response is mediated through the vagal nerve which increases the parasympathetic tone of the atria in the heart (Kästner 2006; Dugdale 2010). The overall effect is a decrease in the heart rate; often the new heart rate can be considered as being bradycardia (Grimm et al. 2015). Furthermore, the intense increase in systemic vascular resistance causes an increase in afterload that the left ventricle must work against to pump blood into the aorta (and arterial side of the systemic circulation). The increase in afterload often results in decreased stroke volume and increased cardiac workload (Grimm et al. 2015). The bradycardia and increase in afterload also culminate in a pronounced dose-dependent decrease in cardiac output (Pypendop & Verstegen 1998). The central phase of the cardiovascular effects becomes more dominant when the peripheral drug concentration begins to wane. During this phase, presynaptic receptor binding causes the release of noradrenaline to decrease in the spinal cord and brain, especially the cardiovascular centre, and thus sympathetic tone decreases (Dugdale 2010; Grimm et al. 2015). The bradycardia likely persists due to decreased sympathetic tone, especially in the sympathetic nerve terminals in the heart (Kästner 2006). Furthermore, the independently acting parasympathetic tone is often more evident and could also contribute to a continued bradycardia (Grimm et al. 2015). The decreased sympathetic tone causes the vascular smooth muscles to return to their normal resting tone (or causes vasodilation in the case of xylazine and clonidine). Once the central phase dominates, it results in a normalisation of the blood pressure; the exception is when when xylazine or clonidine are used, they can result in a drop in arterial blood pressure (Greene & Thurmon 1988).

The alpha$_2$-adrenoceptor drugs are classified as either agonists or antagonists. The alpha$_2$-adrenoceptor agonist drugs that have been used in impala include xylazine and medetomidine (Cheney & Hattingh 1987; Bush et al. 2004). The alpha$_2$-adrenoceptor antagonist drugs that have been administered to impala include yohimbine and atipamezole (Cheney & Hattingh 1987; Bush et al. 2004). Relevant to this thesis are medetomidine and atipamezole and they will be discussed further. These drugs have been administered to impala, either alone or in various ketamine or potent opioid based combinations.

Medetomidine belongs to the imidazoline-derivative group of alpha$_2$-adrenoceptor agonist drugs and it is formulated as a 1:1 racemic mixture of levo-medetomidine and dex-medetomidine (Kuusela 2001). Medetomidine has a binding affinity ratio for alpha$_2$-adrenoceptors to alpha$_1$-adrenoceptors of 1620:1; this is the highest ratio of all alpha$_2$-adrenoceptor agonist drugs (Virtanen 1989). Medetomidine is a popular drug used in wildlife chemical capture, it has often been combined with a cyclohexylamine to achieve immobilisation, and it is especially effective in chemical capture of carnivores. Impala appear to be much less sensitive than domesticated animals to the sedative and muscle relaxation effects of medetomidine. Bush et al. (2004) administered medetomidine alone to impala at 200, 300 and 400 µg kg^{-1} by darting or hand injection and noted that the time to achieve recumbency was shorter with the higher doses. Furthermore, they noted that despite the impala being recumbent after injection, many of them stood when the research team approached, therefore physiological monitoring was not always possible. Medetomidine has also been used with success in ketamine, etorphine or thiafentanil based drug combinations (Bush et al. 2004; Meyer et al. 2008b). The clinical effects (sedation, muscle relaxation and analgesia) can be pharmacologically antagonised using alpha$_2$-adrenoceptor antagonist drugs (Virtanen 1989; Kock & Burroughs 2012).

Atipamezole is the most potent and alpha$_2$-adrenoceptor specific antagonist drug and is therefore said to be the ideal pharmacological antagonist to reverse the clinical effects of medetomidine. The alpha$_2$-adrenoceptor to alpha$_1$-adrenoceptor binding ratio is 8526:1 and it has very little activity at any other receptor classes (Virtanen 1989). Personal experience by the author suggests that opioid-medetomidine immobilised animals do not always arouse as rapidly after antagonising the opioid only, which suggests that medetomidine continues to have a sedative effect and delay full arousal. Furthermore, in wildlife species, and in particular the ruminants, clinical experience suggests that the reversal of clinical signs can be incomplete if atipamezole is administered at doses less than 5 times

the amount of medetomidine (<5:1 dose ratio). A number of theories have been proposed (personal communications), such as: 1) the duration of action of medetomidine being longer than atipamezole causes a resedation after 20 minutes, 2) the dose ratio is too low whereby the medetomidine is not adequately displaced from the presynaptic receptors to decrease the false negative feedback mechanism and therefore no noradrenalin is released, 3) atipamezole, when bound to the presynaptic receptors continues the false negative feedback mechanism, 4) the dose ratio is too low and therefore does not arouse the animal enough to encourage their own sympathetic nervous system to reactivate to a normal tone, and 5) receptor densities and locations and response to drug binding are different and could be the origin of the variable drug response among the species. Regardless of these clinical findings, Bush et al. (2004) made use of a 2:1 dose ratio and said that perhaps a 5:1 ratio is more appropriate to avoid the resedation they observed. Perrin et al. (2015) used a 3.5:1 dose ratio in ketamine-medetomidine immobilised impala and noted ataxia and failed attempts to stand. Recently, Gerlach et al (2017) made use of a 5:1 dose ratio in ketamine-medetomidine-butorphanol immobilised impala and noted that recoveries were calm with mild ataxia and no resedation. Atipamezole is not inert, and when overdosed or administered when no agonist is present, signs ranging from over-alertness to seizure-like activity, disorientation and tachycardia may be observed, therefore it is important to determine the correct dosing ratio (Grimm et al. 2015). In clinical practice, the administration of atipamezole can be titrated to clinical effect to avoid the side-effects of overdose (Zeiler 2015). Furthermore, in small domestic species (dogs and cats), the registered route of administration is intramuscular (Dugdale 2010; Grimm et al. 2015). The intravenous route is speculated to be more effective because absorption from the intramuscular route is thought to be hindered by the intense vasoconstriction induced by medetomidine, which results in a delayed onset of action and decreased bioavailability. Intravenous administration should be done slowly and titrated to clinical effect to avoid excitement, hypotension and tachycardia (Zeiler 2015).

Phenothiazine and butyrophenone derivative drugs

The phenothiazine derivatives make up a large class of drugs that are classified as tranquillisers. Common to this diverse group is the chemical structure of the drugs; they contain two benzene rings that are linked by a sulphur and a nitrogen atom (Peck & Hill 2014). The difference in the clinical effect

and duration of action of the drugs is due to different substitutions on the benzene rings (Brunton et al. 2005; Peck & Hill 2014). The drugs are broadly classified into short (<12 hours), medium (< 72 hours) and long (up to 7-16 days) acting drugs based on their duration of action (Gandini et al. 1989; Kock & Burroughs 2012). Furthermore, drugs from this class have a wide variety of actions, where most are associated with depression of parts of the central nervous system which causes tranquillisation, hypotension, hypothermia (poikilothermic effect), anti-emetic, anti-arrhythmic and anti-histaminic effects. These drugs are classified as multi-potent blockers because their effects are attributable to binding and blocking several different populations of receptors including (Riviere & Papich 2009; Dugdale 2010; Cole et al. 2015; Grimm et al. 2015):

- Dopamine (DA1 and DA2 which are GPCRs) – antagonist (tranquillisation, hypothermia, anti-emetic, anti-arrhythmic effects)
- Alpha-adrenoceptor (alpha$_1$-adrenoceptor mainly) – antagonist (hypotension, anti-arrhythmic effects due to binding to alpha-adrenoceptors in myocardium)
- Cholinergic (muscarinic receptors mainly) – antagonist (anti-spasmodic gut effect)
- Histamine (histamine-1 receptors) – antagonist (anti-emetic, anti-histamine)
- Serotonergic (5-HT receptors) – antagonism (anti-emetic)
- Ion channel (sodium channels mainly) – antagonism (local anaesthetic effect causing anti-arrhythmic effect)
- Membrane stabilising effect due to binding to phospholipid bilayer

Inter-species variation in the effects of these drugs has been described, and this variation could be due to differences between species in receptor densities and locations (Riviere & Papich 2009; Dugdale 2010; Grimm et al. 2015). Regardless, the main clinical effects are due to the anti-dopaminergic effects of this class of drugs (Cole et al. 2015; Grimm et al. 2015). Sedation is not always observed, but behaviour changes may be seen, for example, free-ranging animals that are caught and relocated to captivity can quickly become accepting to their new restricted environment, suggesting that tranquillising effects are more prominent (Riviere & Papich 2009). Dose rates for these drugs are empirical and have been established over decades of clinical use. However, overdosing is sometimes suspected in animals that are still heavily sedated after co-administered capture drugs have been reversed, or when extrapyramidal signs are seen (Riviere & Papich 2009; Dugdale 2010). In the case

of over-sedation, the drug of choice to increase arousal is a generalised central nervous system stimulant, such as doxapram (an analeptic drug commonly available to the wildlife veterinarian) or even amphetamines. The non-specific approach of using a generalised central nervous system stimulant to reversing over-sedation is because there are no specific antagonists and that the drug is blocking multiple receptors (Dugdale 2010; Grimm et al. 2015). The most common extrapyramidal effect of the phenothiazines is akathisia characterised by increased locomotor activity and uncontrollable restlessness (Riviere & Papich 2009). Excessively high doses can cause excitement, muscle tremors and rigidity, sweating, tachycardia, seizures and recumbency (Cole et al. 2015; Grimm et al. 2015). Wildlife veterinarians should also consider the interactions these drugs have during ectoparasite treatment and their role in neuroleptic malignant syndrome and central anticholinergic syndrome (Dugdale 2010; Cole et al. 2015). Ectoparasite treatment usually involves administration of a topical dip containing organophosphate compounds to treat tick, flea, or fly burdens (Riviere & Papich 2009). The phenothiazine derivatives potentiate the effects organophosphate drugs have on their reversible inhibition of acetylcholinesterase and pseudocholinesterase activities, therefore patients could demonstrate a cholinergic crisis characterised by excessive central nervous system depression and bradycardia. Neuroleptic malignant syndrome develops over 1-3 days after administering a phenothiazine derivate (or butyrophenone derivative, discussed later); animals that are dehydrated, exhausted, or suffering from pre-existing central nervous system disease are more susceptible (Dugdale 2010). The clinical signs include hyperthermia, tachycardia, altered consciousness, muscle rigidity due to extrapyramidal dysfunction and autonomic instability (fluctuating blood pressure, sweating, salivation and urinary incontinence) (Grimm et al. 2015). The author suspects that in wildlife practice, a diagnosis of neuroleptic malignant syndrome would be overshadowed by more commonly known syndromes such as capture myopathy or malignant hyperthermia (Dugdale 2010; West et al. 2014). However, it is not uncommon for wildlife veterinarians to be presented with dehydrated and exhausted animals during chemical capture from relocation, where animals will be treated with phenothiazine derivatives (or butyrophenone derivatives). Therefore, perhaps neuroleptic malignant syndrome is more prevalent than actually diagnosed. Central anticholinergic syndrome occasionally manifests after administering any drug with anticholinergic activity including the phenothiazine derivatives (Dugdale 2010; Grimm et al. 2015). The clinical signs include oscillations between depression and hyperexcitability, muscle incoordination, restlessness, anxiety, convulsions and coma.

Hallucinations are thought to be present when an animal is observed gazing into the distance with intense concentration, yet there is nothing of interest to look at, or when an animal exhibits "fly catching" behaviour (making repeated biting movements at a non-existent fly). Other clinical signs also include anticholinergic activity such as tachycardia, xerostomia, urinary retention and dry skin (Riviere & Papich 2009; Dugdale 2010). Administering phenothiazine derivatives to highly stressed animals, where adrenaline concentrations are high, could result in profound hypotension, cardiovascular collapse and syncope (Grimm et al. 2015). The mechanism of profound hypotension that can develop into cardiovascular collapse involves the receptor interaction of the catecholamines and the phenothiazine derivatives. The catecholamines, noradrenaline and adrenaline (at high concentrations) act as vasoconstrictors, especially noradrenaline, through their alpha$_1$-adrenoceptor agonist action. The vasoconstriction is more profound in the peripheral tissues (skin and skeletal muscle) and therefore the circulating blood is redirected centrally to ensure optimum perfusion of vital organs (brain, heart, lung, liver and kidney) (Grimm et al. 2015). However, adrenaline (at low and high concentrations) interacts with beta$_2$-adrenoceptors, which cause vasodilation within the central compartment of the circulatory system, therefore ensuring optimum vital organ perfusion during a fight-flight response (Dugdale 2010; Grimm et al. 2015). Also, muscle perfusion is increased by adrenaline's beta$_2$-adrenoceptor agonist interaction (Grimm et al. 2015). When phenothiazine derivatives are administered, the alpha$_1$-adrenoceptors are blocked (despite noradrenaline and adrenaline) and therefore peripheral vasodilation is the predominant sign (Dugdale 2010). The culminating peripheral (phenothiazine derivative effect) and central (adrenaline effect) vasodilation causes a profound decrease in systemic vascular resistance and therefore a decrease in blood pressure, often to pressures considered hypotensive (mean arterial blood pressure < 60 mmHg) (Grimm et al. 2015).

The phenothiazine derivatives do not have analgesic (or hypoalgesic) properties, with the exception of methotrimeprazine (Dugdale 2010; Grimm et al. 2015). However, these drugs do tend to enhance the analgesic effects of opioid receptor agonists and alpha$_2$-adrenoceptor agonist drugs (Dugdale 2010). The proposed mechanisms are related to their anti-dopaminergic, anti-adrenergic and local anaesthetic effects.

The respiratory effects of phenothiazines appear to be limited when they are administered alone (Riviere & Papich 2009; Dugdale 2010; Grimm et al. 2015). The phenothiazine derivatives reduce the respiratory centre responsiveness to the effects of carbon dioxide. Minute volume is normally

maintained, despite a slight drop in tidal volume, and the blood gases usually remain unchanged (Cole et al. 2015; Grimm et al. 2015). However, the phenothiazine derivatives can potentiate the respiratory depressant effects of other drugs such as opioid receptor agonists and alpha$_2$-adrenoceptor agonists. Smooth muscle relaxation can be pronounced and therefore upper airway obstruction is not an improbable complication, especially when administering these drugs to animals with abundant soft tissue around the oropharynx (Dugdale 2010; Cole et al. 2015; Grimm et al. 2015).

The cardiovascular effects that are most pronounced and clinically relevant, are the alpha$_1$-adrenoceptor antagonistic activity, which has already been described. It is unknown, as of yet, if there are differences between phenothiazine derivative drugs among different animal species (Riviere & Papich 2009; Dugdale 2010; Cole et al. 2015). Hypovolaemic animals are at risk of suffering profound hypotension in the presence of vasodilation (decreased systemic vascular resistance) and therefore administration of these drugs to ill or injured animals may be contraindicated (Dugdale 2010; Grimm et al. 2015). Furthermore, the overall decreased sympathetic tone could unmask an unbalanced and high parasympathetic tone, where vagal mediated bradycardia could lower arterial blood pressure or prevent the animal from mounting an effective sympathetic response to hypotension. An advantageous aspect of these drugs is the anti-arrhythmic effects, where a regular heart rate within normal species limits (if the autonomic tone between the sympathetic and parasympathetic systems is balanced) can result in optimal cardiac output and tissue perfusion (Cole et al. 2015). Healthy animals, administered an appropriate drug dose, are more likely to demonstrate the advantageous effects on the cardiovascular system.

The phenothiazine derivative drug that is relevant to this thesis is zuclopenthixol acetate and it will be discussed further. Zuclopenthixol acetate is a thioxanthene and is classed as a medium acting phenothiazine derivative and has been administered to wild caught impala that have been relocated to a boma with success (Meyer et al. 2008a; Kock & Burroughs 2012). The onset of action is prolonged and can take up to 12 hours before clinical effects are noticed. The duration of action lasts up to 72 hours in wild ungulates (Kock & Burroughs 2012; West et al. 2014). Clinical experience of administering this drug to healthy animals, especially ungulates, suggests they tend to experience a self-limiting period of anorexia.

The butyrophenone derivatives are also a large class of drugs that are classified as tranquillisers and are more commonly used as antipsychotic drugs in human medicine (Riviere & Papich 2009; Dugdale 2010; Kock & Burroughs 2012). The use of the butyrophenone derivatives has lost popularity in managing domestic animals, but remains in swine and wildlife practice (Lin & Walz 2014). The decline in popularity was related to the unpredictable response of animals to this group of drugs (Riviere & Papich 2009). These drugs are said to be less reliable compared to the phenothiazine derivatives. The main drug effects are due to antagonistic activity at the dopamine receptors (mainly DA2). Similar to the phenothiazine derivatives, the butyrophenone derivatives also have antagonist activity at the $alpha_1$-adrenoceptors, histamine receptors (H1) and cholinergic receptors (muscarinic receptors). In wildlife practice, most of the dose rates of these drugs have been derived empirically (Kock & Burroughs 2012). These drugs have no known analgesic effects and their respiratory and cardiovascular effects are thought to be similar to phenothiazine derivatives, although no physiological data on impala (or other medium-sized antelope species) treated alone with these drugs could be found.

The butyrophenone derivative drug that is relevant to this thesis is haloperidol and it will be discussed further. Haloperidol was first introduced into human medical practice in 1957 and was the first butyrophenone derivative belonging to the heterocyclic-substituted phenylbutylpiperdines (Janssen 1974). Haloperidol has been used with success in impala that are caught in the wild and relocated to a boma (Meyer et al. 2008b). The onset of the action is quick (under 30 minutes) and is estimated to last up to 12 hours (Hofmeyr 1981). Therefore, haloperidol is a useful drug to administer to "bridge" the time gap between being introduced into a boma and the delayed onset of action of the medium and long acting phenothiazine derivatives (such as zuclopenthixol acetate) (Kock & Burroughs 2012; West et al. 2015). Therefore, it is common practice to administer the shorter acting tranquilliser drugs at the same time as administering the longer acting tranquiliser drugs (which have a prolonged onset of action) in wildlife veterinary practice (Kock & Burroughs 2012).

Benzodiazepine drugs

The benzodiazepine agonist drugs belong to a large group of drugs known for their sedative, anxiolytic and anticonvulsant effects. The name of this group of drugs is derived from their chemistry in that they are comprised of a benzene ring fused to a seven-membered di-azepine ring (Peck & Hill 2014; Cole

et al. 2015). The benzodiazepine agonist drugs bind to specific sites associated with the GABA$_A$ receptor called benzodiazepine binding sites (on the α-subunit of the pentometric receptor) (Peck & Hill 2014). Once these drugs bind to the GABA$_A$ receptor, they enhance the affinity and action of endogenous gamma-aminobutyric-acid (GABA), an important inhibitory neurotransmitter. GABA$_A$ receptors, once activated, open up chloride channels, thus producing hyperpolarisation of the postsynaptic cell membrane and rendering the postsynaptic neurons more resistant to excitation (Riviere & Papich 2009; Dugdale 2010; Cole et al. 2015). Other clinical effects that are advantageous include spinally-mediated muscle relaxation and anterograde amnesia (Brunton et al. 2005; Grimm et al. 2015). The amnesic effects last longer than the sedation (Peck & Hill 2014). The benzodiazepine agonist drugs also inhibit nucleoside transporter systems and thus decrease the degradation of adenosine. Adenosine is considered an important regulator of cardiac function as it reduces cardiac oxygen demand by decreasing the heart rate, and increases myocardial oxygen delivery by dilating the coronary vessels (Peck & Hill 2014).

Benzodiazepine agonist drugs do not have analgesic (or hypoalgesic) effects (Dugdale 2010; Grimm et al. 2015). However, in clinical management of a patient suffering injuries that cause skeletal muscle spasm (spinal injuries or long bone fractures) patients benefit from the central muscle relaxant effects (Grimm et al. 2015). Furthermore, patients that are anxious tend to experience and perceive a higher degree of pain compared to calm, relaxed patients. Therefore, treating patients with a benzodiazepine agonist drug can alter the patient's processing and perception of pain (Dugdale 2010).

The respiratory system effects are limited when benzodiazepine agonist drugs are administered at clinically relevant dose rates (Lin & Walz 2014; Cole et al. 205; Grimm et al. 2015). However, the benzodiazepine agonist drugs do enhance the respiratory depression effects of other drugs, such as the opioid agonist drugs. The respiratory depression is characterised by reduced ventilatory response to carbon dioxide, and relaxation of the intercostal muscles (Grimm et al. 2015). Also, smooth muscle relaxation of the oropharynx could result in upper airway obstructions (Dugdale 2010; Grimm et al. 2015).

The cardiovascular system effects are also limited, especially when benzodiazepine agonist drugs are administered at clinically relevant dose rates (Grimm et al. 2015). At higher doses, some

benzodiazepines can cause vasodilation and negative Inotropy, which can produce a decrease in the arterial blood pressure (Brunton et al. 2005; Dugdale 2010; Grimm et al. 2015).

There are many benzodiazepine agonist drugs used in human and veterinary practice. The response to the drugs, with regards to sedation, differs tremendously among the various veterinary species (Riviere & Papich 2009; Dugdale 2010; Grimm et al. 2015). Adult domestic feline and equine patients do not seem to become sedated, but rather agitated or even become excited, unlike their neonates, which do become sedated (Cole et al. 2015). Domesticated canines demonstrate a variable sedation response and therefore these drugs cannot be considered a reliable sedative in this species (Dugdale 2010; Grimm et al. 2015). The domesticated farm animals (bovine, ovine, caprine, and porcine) do demonstrate excellent sedation, however, local drug regulation might preclude their routine use in these species (Dugdale 2010; Lin & Walz 2014). Wildlife animals may also demonstrate similar species variation to the drug effects induced by the benzodiazepine agonist drugs (Kock & Burroughs 2012). Despite the variable response to the sedative effects, the anxiolytic effects and anterograde amnesia (which are both hard to measure in animals) are thought to be the most beneficial drug effects (Grimm et al. 2015). The muscle relaxation properties are also useful and, therefore, the benzodiazepine agonist drugs could be considered for counteracting the muscle hypertonia sometimes seen with opioid agonists (West et al. 2014). The effects of the benzodiazepine agonist drugs can be pharmacologically antagonised with benzodiazepine antagonist drugs such as flumazenil (Riviere & Papich 2009; Dugdale 2010). The administration of flumazenil is not routine and this may be because of the expense, or because clinically relevant doses benzodiazepine agonist drugs do not cause enough physiological derangement to warrant antagonism (Grimm et al. 2015).

The benzodiazepine agonist drug that is relevant to this thesis is midazolam and it will be discussed further. Midazolam is unique compared to other benzodiazepine agonist drugs in that it is water soluble at acidic pH (drug formulated to a pH of 3.5), because the di-azepine ring structure is open and therefore ionised (Grimm et al. 2015). When midazolam is exposed to a physiological pH (pH of 7.4; or any pH > 4) the structure of the di-azepine ring undergoes a conformational change and the di-azepine ring structure closes, thus the molecule becomes unionised and lipid soluble (Peck & Hill 2014). This unique molecular structure that undergoes a conformation change in different pH environments is called tautomerism (Peck & Hill 2014; Grimm et al. 2015). Midazolam has been administered to impala,

especially when muscle hypertonia is profound after administration of potent opioid agonist drugs like etorphine or thiafentanil (Zeiler et al. 2015; Buck et al. 2017).

1.2.5 Physiological effects of chemical capture and general anaesthesia in impala

Reports of chemical capture of impala began to appear in the early 1960s (van Niekerk et al. 1963; Pienaar et al. 1966). The focus of many of these reports was to investigate whether it was possible to immobilise the impala, and whether profound clinical derangements or death occurred. Little emphasis was placed on reporting physiological data, but rather on reporting the "knockdown times", quality of the immobilisation and if muscle rigidity, for example, was present. Ables & Ables (1969) were the first to report simple physiological variables such as heart and respiratory rate, of chemically captured impala. Changes in body temperature during chemical immobilisation were reported by Drevemo & Karstad (1974) in the 1970s. Cheney & Hattingh (1987) were the first to report changes in haematological parameters during chemical immobilisation. In the 1990s, reports of additional physiological data began to emerge, where catecholamines, cortisol and plasma protein concentrations, among other analytes, were determined (Knox et al. 1990, Knox et al. 1992). Lastly, reporting on arterial blood gases emerged in the 2000s (Bush et al. 2004; Meyer et al. 2008a, b; Perrin et al. 2015). Despite the growing number of reports regarding the effects of chemical immobilisation and general anaesthesia on the physiological systems which are important for maintaining homeostasis (respiratory, cardiovascular, thermoregulatory and neuromuscular systems), it remains difficult to compare the various studies. This difficulty is because different drugs, used alone or in combination, were administered (using different methods, projectors and darts) to different populations of impala under different environmental conditions. Therefore, further research focusing on reporting the physiological effects of drugs used alone or in combination for chemical immobilisation and general anaesthesia is warranted.

Some physiological derangements appear to be fairly consistent in the current literature. Hypoxaemia, hypercapnia and acidaemia are the most obvious physiological derangement that are induced by the drugs commonly used to chemically capture impala (Bush et al. 2004; Meyer et al. 2008a, b; Kock and Burroughs 2012; Perrin et al. 2015). The presumption is that the drugs cause hypoventilation (decrease

in minute volume) because this could explain all three signs, hypoxaemia, hypercapnia and acidaemia. Extrapolation from the human literature suggests that opioid agonist drugs, ketamine and alpha$_2$-adrenoceptor agonists all decrease ventilation. In the veterinary literature, hypoventilation is often diagnosed when hypercapnia is evident and this assumption may be true (Dugdale 2010; Grimm et al. 205). However, CO_2 is produced by tissue metabolism and transported via the blood to the lungs to be vented via the alveoli into the atmosphere. Therefore, a rise in CO_2 might be caused by increased tissue metabolism and not simply due to hypoventilation (Grimm et al. 2015). The respiratory system, at least in ruminants, takes seconds to adjust to the increased carbon dioxide load and therefore this dynamic relationship that is constantly changing, should not be forgotten. Furthermore, carbon dioxide is an important constituent of the bicarbonate buffering system (Dugdale 2010; Muir 2015). Therefore, a rise in carbon dioxide could result in a rise in bicarbonate ions and hydrogen ions. The hydrogen ions bind to haemoglobin (or any other extracellular or intracellular buffering systems) and, thus, the circulating carbon dioxide may not necessarily increase or cross the blood brain barrier to stimulate the respiratory system (central chemoreceptors), but rather result in an increase of bicarbonate ion concentration in blood (Muir 2015). When reporting hypercapnia, many of the articles only report a respiratory rate (Perrin et al. 2015). It is very difficult to determine the significance of changes in the respiratory rate because this is only one component of minute volume (the other being tidal volume). Therefore, determining a change in minute volume made on respiratory rate is fraught with difficulty, and conclusions should not be made on the respiratory rate and presence of hypercapnia alone (Grimm et al. 2015). To date, few papers report minute volume in potent opioid treated impala (Meyer et al. 2010), and in these cases, they are within expected resting reference ranges for similar sized ungulates (Hales & Webster 1967; Bakima et al. 1988). This suggests that hypoventilation is less likely to play a significant role in explaining the hypercapnia and hypoxaemia that is evident in chemically captured impala using the potent opioids etorphine and thiafentanil (Janssen et al. 1993; Murray et al. 1981). Other proposed mechanisms of altered gas diffusion include pulmonary hypertension, right-to-left intrapulmonary shunting of blood, excessive dead space ventilation and diffusion impairments across the alveolar-capillary membrane (Dugdale 2010; Grimm et al. 2015). Thus, research focusing on describing more complete physiological changes induced by various drugs used for chemical immobilisation and general anaesthesia in healthy impala is warranted. A number of oxygenation and ventilation indices have been published in an attempt to identify the aetiology of gas diffusion derangements more accurately

(Armstrong et al. 2007). Perfect cardiopulmonary integration results in the best gas diffusion conditions, whereby CO_2 and oxygen are maintained within the physiological ranges appropriate for maintaining homeostasis. Cardiopulmonary integration relies on adequate movement of gas into and out of the lung respiratory zone (area where gas diffusion takes place) and adequate alveoli capillary perfusion (Grimm et al. 2015). The ratio of ventilation to perfusion (V/Q ratio) at the alveolar-capillary interface can be measured, but requires the accurate measurement of many variables using expensive equipment. Achieving this is not always easy in clinical practice, let alone in field research. The theoretical ideal V/Q ratio is 0.8 in a healthy mammal and is determined by dividing the animal's simultaneously measured minute volume by their cardiac output (Mills 2001; Dugdale 2010). The most often used oxygenation indices are the arterial oxygen tension to fractional inspired oxygen ratio and the alveolar to arterial oxygen tension gradient (Armstrong et al. 2007; Kathirgamanathan et al. 2009). The most often used ventilation index is the arterial to end-tidal carbon dioxide tension gradient (Armstrong et al. 2007).

The arterial oxygen tension to fractional inspired oxygen ratio is a simple mathematical calculation (Armstrong et al. 2007; Theodore et al. 2013). Arterial blood gas analysis provides arterial oxygen tension (arterial partial pressure of oxygen measured in mmHg; PaO_2). The fractional inspired oxygen concentration (FiO_2) is the fraction of inspired oxygen, where room air has a fractional oxygen concentration of 0.21 (21%). The ratio is calculated by dividing the PaO_2 by the FiO_2 to obtain the ratio. A ratio of >300 mmHg is considered normal. This ratio is used to aid diagnosis of acute lung pathology such as that seen acute respiratory distress syndrome (ARDS) in human patients (Armstrong et al. 2007). The ratio is a broad indicator of how efficient the gas exchange is at the respiratory zone and if the ratio is above 300 mmHg then the lung is said to be exchanging gases adequately, while values over 400 mmHg suggest near perfect gas exchange conditions. The focus of this ratio is only on oxygen diffusion and the efficiency of ventilation, therefore no assessment of minute volume can be made when interpreting the outcome of this ratio.

The alveolar to arterial oxygen tension gradient is another useful assessment of the lung's ability to oxygenate blood. However, the mathematics is more complex and makes use of important assumptions that must be taken into consideration when interpreting the results.

First the alveolar oxygen tension must be calculated, using the following equation (Armstrong et al. 2007):

$$PAO_2 = FiO_2(Pbar-PH_2O) - PaCO_2/RQ$$

Where: PAO_2: alveolar tension of oxygen; FiO_2: fractional inspiration of oxygen; Pbar: barometric pressure; PH_2O: tension of water vapour; $PaCO_2$: arterial carbon dioxide tension; RQ: respiratory quotient (assumed to be 1.0 for ruminants: Grimm et al. 2015; Meyer et al. 2010).

The alveolar oxygen tension equation assumes that the body temperature is 37.0 °C and, therefore, that the PH_2O is 47 mmHg (Armstrong et al. 2007). Although the water vapour tension can be corrected using mathematical adjustments, these corrections further complicate the overall interpretation. Another assumption is that the $PaCO_2$ (usually measured from an arterial blood gas analysis) is equivalent to the alveolar concentration of carbon dioxide, which may sometimes not be true (Armstrong et al. 2007). Furthermore, the respiratory quotient is estimated although, in the case of ruminants, it has been assumed to be equal 1.0 (Meyer et al. 2010; Grimm et al. 2015). Additionally, the equation assumes that the blood returning to the alveolar capillary (from the systemic circulation) via the pulmonary artery has a normal oxygen tension of >45 mmHg. Once the alveolar oxygen tension is calculated, then the arterial oxygen tension is simply subtracted from this figure to obtain the alveolar to arterial oxygen gradient. When breathing room air (FiO_2 of 0.21), then a gradient value of <20 (10-25) mmHg is generally considered normal. A gradient larger than the normal range indicates that oxygen is not adequately diffusing into the blood. The alveolar to arterial oxygen tension gradient gives information regarding the efficiency of oxygen diffusion but does not inform regarding ventilation or carbon dioxide diffusion (Armstrong et al. 2007).

The arterial to end-tidal carbon dioxide tension gradient is commonly used in anaesthetic and critical care practice to assess the efficiency of ventilation (Armstrong et al. 2007). The arterial tension of carbon dioxide (arterial partial pressure of carbon dioxide; $PaCO_2$) is measured by blood gas analysis (Dugdale 2010; Grimm et al. 2015). The end-tidal carbon dioxide is measured using capnometery (measurement of exhaled carbon dioxide pressure in real time over each breath); the value measured at the end of exhalation, just before the next inhalation starts, is the end-tidal value. Capnography (volumetric or time scalar) is a better method than capnometery of determining the end-tidal carbon dioxide level because the exhaled carbon dioxide can be visually assessed (Grimm et al. 2015). The

gradient is then calculated by subtracting the end-tidal carbon dioxide value from the arterial carbon dioxide tension (Armstrong et al. 2007). Because carbon dioxide is more soluble than oxygen, the difference between the arterial and alveolar tensions is minimal in normal animals. A gradient value of less than 15 mmHg is considered normal, but ideally the gradient should be less than 5 mmHg for animals under 100 kg in body weight (Grimm et al. 2015).

Acidaemia, which is defined as a low blood pH compared to a normal reference range, is due to an increase in H^+ concentration. The blood pH of healthy ruminants ranges from 7.37 to 7.48 and is regulated by various buffer systems and compensatory responses to be within this range (Lin & Walz 2014; Grimm et al. 2015). Acidaemia can result in clinically relevant derangements that include a decrease in cardiac output, decreased systemic vascular resistance and altered oxygen binding to haemoglobin (Mitchell et al. 1972; Crimi et al. 2012; Clarke et al. 2014). Therefore, the blood acid-base balance should be determined in chemically immobilised antelope to determine what effect the drug might have on the regulation of pH. Several different mathematical approaches have been used to explain derangements in blood pH. The two most common approaches are the Henderson-Hasselbalch and the Stewart, and these will be considered further. The Henderson-Hasselbalch approach focuses on investigating the relationship between the constituent components of the bicarbonate buffer system (Henderson 1908; Hasselbalch 1916). In the plasma, CO_2 (a metabolic by-product) dissolves in water to form carbonic acid (H_2CO_3). The carbonic acid quickly dissociates into bicarbonate ions and hydrogen ions (Muir 2015). The relationship is then represented mathematically by the following equation:

$$pH = pKa \text{ of } H_2CO_3 + \log_{10} ([HCO_3^-]/[H_2CO_3])$$

This equation has been modified for clinical application by the following equation (Constable 1999; Constable 2014):

$$pH = pK_1' + \log_{10} ([HCO_3^-]/S.PaCO_2)$$

where pK_1' is the equilibrium dissociation constant of carbonic acid = 6.105 at 37.0°C (human); S is the solubility coefficient of carbon dioxide in plasma = 0.0307 [mmol L^{-1}]/mmHg.

In general, the respiratory system controls the arterial partial pressure of carbon dioxide and the kidneys control the bicarbonate ion concentration (Muir 2015). Changes in blood pH can be viewed as being brought about by changes in the respiratory system (change in $PaCO_2$) and/or changes in the metabolic

system (change in HCO_3^-). If the pH decreases, then by examining the $PaCO_2$ and HCO_3^-, the observer can classify the acidaemia as being either a respiratory (increase in $PaCO_2$) or metabolic (decrease in HCO_3^-) derangement, respectively (Clarke et al. 2014; Muir 2015). However, under normal circumstances the blood pH is intensely regulated and therefore, considering the clinical pH equation (above), the $[HCO_3^-]:[PaCO_2]$ ratio is tightly regulated to be 20:1 (Clarke et al. 2014). The lung and kidney are generally considered to be the two organ systems where compensation takes place (Muir 2015). Compensation for a pH outside the normal range is said to take place when the compensating organ's component of the bicarbonate buffer system shifts in the same direction as the offending component. For example, if there is acidemia due to hypoventilation, then the $PaCO_2$ rises above 45 mmHg (offending component) and the compensating organ (kidney) increases the HCO_3^- concentration to return the $[HCO_3^-]:[PaCO_2]$ ratio to 20:1. This simple explanation of the bicarbonate buffer system and its relationship with blood pH gets more complicated where acute and chronic pH derangements and compensation mechanisms occur simultaneously. Differences have been described between humans and dogs in the particulars of their response to pH change, and other species differences are likely (Muir 2015). An important factor in such species differences resides in inter species dissimilarity in how haemoglobin interacts as a buffer in the blood, and how it manages CO_2 and HCO_3^- through the Bohr Effect and chloride shift in erythrocytes (Muir 2015). Extrapolating compensatory outcomes based on data from humans and dogs could be misleading therefore, in the absence of relevant species specific information, calculations of the magnitude of compensation will not be considered further in this thesis.

The Stewart approach suggests that the HCO_3^- and H^+ represent the effect rather than the cause of acid-base derangements (Stewart 1978). Furthermore, the Stewart approach is based on the dissociation of water (H_2O) to produce H^+ or hydroxide ions (OH^-) to maintain electrical neutrality within a solution (like blood) where there are independent variables (arterial partial pressure of carbon dioxide [$PaCO_2$], strong ion difference [SID], anion gap [AG], total weak acids [Atot]) and dependent variables (H^+, OH^-, HCO_3^-, CO_3^{2-}, weak acids [HA] and ions [A^-]) which influence the neutrality (Stewart 1978). A change in an independent variable will effect a change in the dependent variables to maintain electrical neutrality within the solution.

Stewart's theory has led to a revised version of the blood pH equation as follows (Constable 1999; Constable 2014):

$$pH = pK_1' + \log\{([SID^+] - Ka[Atot]/(Ka + 10^{-pH})) / S.PCO_2\}$$

where pK_1' is the equilibrium dissociation constant of carbonic acid; S is the solubility coefficient of carbon dioxide in plasma; Ka is the effective equilibrium dissociation constant of weak acids, the value is species dependent (Ka = 0.8×10^{-7} where pKa = 7.08; calves [Constable 2014]). When using the Stewart approach, the observer must measure ($PaCO_2$ using a blood gas analyser) or calculate the independent variables to help interpret the resultant blood pH (Kellum et al. 2009).

The physiological responses to drugs used during chemical capture and general anaesthesia are often serious and include hypoxaemia, hypercapnia and acidaemia (West et al. 2014). Yet, the response to the process of administering these drugs (for example hand injection or darting) is perhaps a greater concern, especially in flighty species such as impala. Regardless of the method of administration of drugs to wildlife species, there will always be some degree of a fight-flight response. However, impala which are naturally flighty species, can also suffer from a fright response, which is thought to lead to a pronounced surge in catecholamine and cortisol release (Meyer et al. 2008a). The fright response has been associated with a rapid increase in body temperature that has also been witnessed during chemical capture and is termed capture-induced hyperthermia (Meyer et al. 2008a). Therefore, impala that suffer a fright response may have different response to drugs and have different cardiovascular and respiratory physiological reserves for correcting derangements. Furthermore, the physiological response to pain and bruising caused by the dart impact should also be taken into account even though the magnitude of effect is impossible to predict accurately.

1.2.6 Capture and anaesthesia associated mortalities in impala

Antelope capture and anaesthesia, especially in anxious and flighty species such as impala, is not without risk (Knox et al. 1990; Meyer et al. 2008b). Physical capture and restraint for clinical examination can cause an overall mortality rate of up to 50% (Knox et al. 1990). Murray et al. (1981) dubbed this capture associated mortality "fatal stress" when they observed up to 30% fatalities during physical capture and restraint. Thus, when animals need to be clinically examined or worked on, chemical

capture remains the preferred immobilisation technique in impala. However, chemical capture also causes fatalities of approximately 3% (0.5 to 7.0%), according to the opinions of wildlife veterinarians (Author's unpublished data and personal communications). Mortality rates have occasionally been reported in capture-related studies, and range from 0% (Cheney & Hattingh 1987; Bush et al. 2004; Meyer et al. 2008b; Meyer et al. 2010) to 14% (Ables & Ables 1969). Conventionally, animals with poor body condition are thought to be at increased risk of capture related mortality. Visual assessment of body weight in relation to body skeletal frame size has been used to assess an animal's body condition. However, Bender (2015), using rump body condition scoring and estimates of body fat percentage, could not find an association between poor body condition and increased risk of mortality in medium sized ungulates undergoing chemical capture. No reports could be found describing mortality during general anaesthesia in impala. Thus, there is a need for information regarding the causes of mortality during capture in impala; the investigations should focus independently on the effects of the drugs used and the techniques of chemical capture. Furthermore, it is also necessary to investigate whether the effects of placing free-ranging impala into captivity contributes to the overall mortality rates.

1.2.7 Behaviour characteristics of impala

Understanding impala social behaviour in an extensive free-ranging environment will assist in detecting abnormal social behaviour in confined captive impala. Social structure and behaviour has been well documented by Jarman and Jarman in the early 1970s, where they described free-ranging impala behaviour in various conservancies in sub-Saharan Africa (Jarman 1970; Jarman & Jarman 1973). Three social groupings are commonly described: lone adult males, bachelor herds composed of juvenile and adult males, and lastly, breeding herds comprising females of all ages and un-weaned juvenile males. There are two major factors that determine the overall social behaviour, the first being their response to external factors such as food abundance, predator load and other environmental factors like terrain (Skinner 1971; Jarman & Jarman 1973; Murray 1981). The second major factor is the dynamic within the social group because of differences among its members and their reaction to one another. In areas where rainfall and food abundance fluctuate through the seasons, two types of social herd structures can be defined: non-territoriality and territoriality structures. When the rainfall is high and the availability of food is abundant, impala tend to congregate, with little to no conflict between adult

males is observed and thus can be considered as a non-territoriality structure. In these herd structures, none of the impala are marginalised and individuals freely forage within the area and move about within the herd unhindered (Jarman & Jarman 1973). During the drier months, food and water becomes a more scarce resource and, thus, the overall herd structure becomes focused on territoriality. Adult males separate and spread out to demarcate a territory that they defend for as long as possible (Bramley & Neaves 1972). The breeding herds, consisting of female impala and very young juveniles that are not weaned, roam freely through the mosaic of breeding adult male territories and have free access to food and water (Young 1972; Jarman & Jarman 1973). Females in oestrus mate the adult male who is guarding the territory they find themselves in. The adult males spend most of their time mating and warding off other adult males; they do not spend much time resting, eating or ruminating. A male's reign of his territory will last as long as he has energy and stamina to remain the dominant male; this usually lasts around 85 days (Jarman & Jarman 1973). Once an older or stronger male enters his territory, he will likely be chased away and then he will return to a bachelor herd to rebuild his strength. The bachelor herd does not socialise with the roaming breeding herd, but tends to be marginalised and only forages on the perimeter of abundant food areas (Young 1972; Jarman & Jarman 1973). Dominant males chase juvenile males away from the breeding herd, even as early as 4-6 months old, thus forcing early weaning. These juvenile males enter a bachelor herd at the lowest level of an age-based ranking system. Only when the juvenile male matures into an adult, will he shift up in the herd dominance ranking and be eligible to contest and maintain a territory as a lone breeding adult male (Jarman & Jarman 1973). Rutting is the term used to describe how a male impala defends his territory and chases away other breeding males. Rutting can be very violent and can result in horning injuries that might be catastrophic, such as intra-abdominal or intra-thoracic puncturing of vital organs or long bone fractures of the limbs (Skinner 1971). Nevertheless, impala work cohesively in that, despite territoriality, and the other behaviours described above, all impala have access to food and water (Young 1972; Dunham 1982).

Impala herds, regardless of the territoriality of the structure, demonstrate attachment to a home area. Attachment to home areas was first described in 1970, after observing various individual impala (male and female) that were recognisable over a study period of two years (Jarman 1970). Impala tended to roam out of their normal home area in search of food, especially during the dry months (Jarman 1970; Young 1972; Dunham 1982). But during the wetter months the impala tended to move back to their

home area, regardless of the abundance of food and water. This social behaviour of returning to a home area is not well understood, but could be related to a familiarity with the terrain and predator load in the area (Jarman 1970). Translocation of impala away from their home area increases the risk of mortality due to predation (Matson et al. 2004). When translocating impala, it is recommended to move at least 15 individuals from the same herd to ensure a positive herd growth curve in their new environment (Matson et al. 2004). Vigilance is an important survival behaviour trait for impala but the time spent on being vigilant differs according to gender and herd size (Mooring & Hart 1995). Males, especially breeding adult males, spend more time being vigilant compared to females (Jarman 1970; Skinner 1971). Also, impala herds of six or fewer impala spend more time being vigilant and less time foraging or breeding than do larger herds (Matson et al. 2004).

The social behaviour of impala within a zoological garden is not well published. Food and water are usually provided in abundance and are therefore not a limiting resource thus the overall herd social behaviour results from interactions among members of the herd (Jarman & Jarman 1973). The size of the impala enclosure in relation to the herd size also contributes to overall herd social behaviour (Bramley & Neaves 1972). Less conflict is seen in herds with more female impala. Also, female impala do not have horns and, therefore, conflict is limited to ramming, head-butting of flanks, and chasing. As already mentioned, male impala conflict is inherently more dangerous because it occurs frequently during the antagonistic social behaviour of rutting, and because it can involve catastrophic horning injuries (Bramley & Neaves 1972).

For research purposes, free-ranging impala are often caught in the wild and relocated to the confines of a boma. Researchers have made use of impala as a research model, but have only reported basic husbandry practices (Knox et al. 1990; Bush et al. 2004; Meyer et al. 2008a, b). The behaviour of such animals has yet to be described.

1.2.8 Impala research models

Impala that have been enrolled into studies are either free-ranging or wild caught and kept in captivity for short or long-term periods (Ables & Ables 1969; Jarman & Jarman 1973; Knox et al. 1990; Bush et al. 2004; Meyer et al. 2008a, b). The frequency and type of human-impala interactions vary and depend on the objectives of the study, the study design and scientific methodology employed during the study.

The type of human-impala interaction ranges from the least invasive where they are observed without physical contact (presence of humans or man-made devices within impala eye sight) to the most where physical capture and restraint are carried out without sedation or tranquilisation. Chemical capture for immobilisation and general anaesthesia supresses the impala's perception and level of conscious awareness (obtunded to unconscious) during human-impala interaction (Bush et al. 2004; Meyer et al. 2008a, b; Perrin et al. 2015). Therefore, human-impala interactions during chemical immobilisation and general anaesthesia can be considered an intermediate type of invasiveness. However, if the impala undergo surgical procedures while immobilised or under general anaesthesia, then this type of invasiveness could escalate to a higher level, especially if hypoalgesic (non-steroidal-anti-inflammatory drugs, opioids) or analgesic (local anaesthetics) interventions are inadequate.

Observation studies focused on determining herd dynamics, social organisational activity, and movement of impala, and have been conducted in large conservancies of defined geographical area (Jarman 1970; Jarman & Jarman 1973; Matson et al. 2004; Shorrocks & Cokayne 2005). Such observational studies have very limited or no human-impala interactions. These observational studies raise no major welfare or ethical concerns for the impala. Therefore, the animals are at no (or minimal) risk of increased morbidity and mortality from the study procedures.

The other extreme example of using impala as a research model employs physical capture and restraint for periods longer than 10 minutes as part of the experimental procedures (Murray et al. 1981; Knox et al. 1992). In this case human-impala interaction is physical and resulted in deaths (Murray et al. 1981; Knox et al. 1992). These particular studies aimed to assess the acute stress response to this type of capture technique and subsequent physical restraint. The studies were well thought out and provided enough evidence that we no longer need to re-examine or validate their findings (Knox et al. 1992). The physiological effects of physical capture of impala and the resultant mortalities have been eluded to already (points 1.2.5 and 1.2.6 above). In those studies, the impala were either free-ranging or confined to a boma under captive conditions. Regardless of the state of captivity, impala do not adapt well to repeated physical capturing (Pienaar 1973; Murray et al. 1981). The data collected to date is sufficient such that further proposed studies of this type should undergo rigorous ethical examination before they can be approved.

In-between the two extremes of human-impala interaction noted above are a range of studies that include research designs which investigate parasites, reaction to tranquilisation, immobilisation, and general anaesthesia (Hattingh et al. 1988; Gandini et al. 1989; Knox et al. 1990; Meyer et al. 2008a, b; Perrin et al. 2015; Zeiler et al. 2015). Although not well defined in the literature, impala can adapt to captive living in a boma. However, deaths resulting from mal-adaption have been described in naïve free-ranging impala. Few of these types of studies have reported on the management of captive impala because the literature emanating from these studies focuses on study design, methods and results specific to the particular aims and objectives of the study. Studies of this nature, where the human-impala interaction is in-between the extremes, will continue. Therefore, the need to better understand what the welfare and ethical implications are, is important to avoid unnecessary morbidities and mortalities in future studies (Sikes et al. 2016). In order to appreciate these welfare and ethical implications in context, the current standard practice of captive management of impala during short and long-term confinements should be understood.

1.2.9 Short and long-term captive management of impala

Impala that are kept under captive conditions could originate from the wild or have been born in captivity. Any impala which is not free-ranging in an extensive environment should be considered as being confined in captivity. The confinement could be 1) temporary, such as being transported in a purpose designed trailer or crate, or 2) short-term, where impala are placed in a boma for a period of time before being released back into an extensive free-ranging environment, or 3) long-term captive confinement, where impala are confined to small camps such as ones that may be seen in intensive breeding farms or zoological gardens.

Impala are inherently nervous and anxious, regardless of their state of captivity, and therefore management techniques have been described to ensure a successful transition between different environments (captive to free-ranging or free-ranging to captivity) or different locations within one type of environment (translocation from one extensive conservancy to another, or from one intensive camp to another) (Bothma & van Rooyen 2005; Kock & Burroughs 2012). The transfer from one area to another will often involve temporary confinement, which is very stressful for impala. To minimise the stress, rapid acting tranquillisers are often administered to alter the behavioural response to acute

stress (Gandini et al. 1989; Hattingh et al. 1990; Kock & Burroughs 2012; West et al. 2014). This can be accomplished by physically capturing the impala using drive netting and quickly hand injecting a rapidly acting tranquiliser prior to loading into the transport trailer or crate (Knox et al. 1990). To prevent aggressive behaviour adult male impala should ordinarily be loaded alone, while female and juvenile impala can be loaded together. That said, adult male impala can be successfully loaded together if they are from the same bachelor herd. The separation of genders in transport trailers minimises horning injuries, and additional stress and anxiety experienced by subordinate impala in the presence of a dominate impala (Kock & Burroughs 2012). The advantage of the drive net capture is that there is minimal to no risk of respiratory depression and cardiovascular collapse due to the drug effects of chemical capture. However, the physical capture and loading time must be kept under 10 minutes to avoid excessive mortalities (Murray et al. 1981). If a veterinary examination or procedures (e.g., ear tagging) is required, chemical capture is arguable the best management methodology. Here the impala's perception and conscious awareness is obtunded to such as extent that they do not mount an excessive stress response to handling. However, caution is advised when darting an already stressed impala because the fright response could lead to capture-induced hyperthermia (Meyer et al. 2008a). Once darted and immobilised, the veterinarian is advised to administer a rapidly acting tranquiliser before reversing the drugs used for chemical capture. The rapidly acting tranquilisers are clinically effective for 2-12 hours, depending on the drug used (described in Chapter 6). Therefore, it is common practice to administer a medium or long acting tranquilisers at the same time, to keep the impala calm when introduced to their new environment (Kock & Burroughs 2012; West et al. 2014).

Impala have been kept in captivity for many decades and therefore there are some recommendations for the conditions in the confined housing structures such as bomas, intensive breeding camps or zoological enclosures to ensure a high standard of welfare is maintained.

1.2.10 Welfare and ethics related to captive management of impala in research

Despite the use of impala as a research model over a number of decades there is limited information describing the methods that improve or attend to their welfare needs during research studies. However, recommendations for captive housing in zoological enclosures do exist (Zoolex 2017). The number of capture-related research studies in impala has grown in the last two decades. This increase in research

has encouraged consideration of the welfare and ethics issues raised by using impala as a research model. This is particularly important because impala, despite outwardly appearing to adapt to boma confinement, remain anxious and flighty during such studies (Knox et al. 1990; Meyer et al. 2008b). Furthermore, as already mentioned, mortalities of impala enrolled in such studies have been reported. These known issues raise welfare and ethical concerns that need clarification.

Guidelines of the America Society of Mammalogists for the use of wild mammals in research and education have recently been updated (Sikes et al. 2016). These guidelines, which are also applicable to captive research studies, broadly discuss how free-ranging mammals should be captured and confined to ensure five welfare freedoms: Freedom: 1) from hunger and thirst; 2) from discomfort; 3) from pain, injury or disease; 4) to express normal behaviour; 5) from fear and anxiety. These guidelines are used to help inform members of animal ethics committees who are unfamiliar with the management of free-ranging mammals. The guidelines make very broad recommendations on housing conditions, feeding and providing water and how and when to implement environmental enrichment. Furthermore, the guidelines indicate that the caregivers of the animals placed in captivity must provide appropriate bedding, day-light cycling, access to shelter from environmental elements (precipitation, sun and wind), and temperature and humidity conditions specific for the species being housed. They also stipulate that noise levels and olfactory stimulation should be kept within reasonable limits (e.g., providing familiar scents from familiar plants, and not housing predators close to prey species). The maintenance of the enclosure, such as cleaning out bedding and excess food should be done frequently, in some cases as often as twice a week and in enclosures housing species from arid environments every two weeks (or longer). Implementation of environmental enrichment in enclosures housing impala has not been well described. It may be speculated that in modern zoological gardens, species originating from a similar geographical location are housed in the same large display enclosure, and impala are often included in African enclosures. The mixing of species, large enclosures with an appropriate amount of vegetation will meet the environmental enrichment conditions of impala. In intensive breeding camps, there appears to be minimal environmental enrichment yet the impala breed successfully (Bothma & van Rooyen 2005). This raises the question as to whether environmental enrichment is necessary and, if so, what can be done to improve environmental enrichment in enclosures housing impala? The guidelines of the America Society of Mammalogists serve as an excellent starting point to aid in the planning and building of a research boma to house impala. Specific requirements, such as the floor size

of the enclosure, the stocking density, the number of feeding and watering points, the height and opacity of the perimeter fence, the location and proximity to other species housed close by, and the activity level around the boma should be considered (Bothma & van Rooyen 2005; West et al. 2014).

Boma sizes have been reported in studies where impala have been used as a research model, and they range from 285 m^2 to 800 m^2 total surface area, giving a surface area per impala of 8.6 to 25 m^2/impala (Heard et al. 1990; Knox et al. 1991; Knox et al. 1992; Gamble et al. 1994; Bush et al. 2004). The recommended minimum area for permanent housing of impala in zoo enclosures is 500 m^2 for up to 10 impala, with an additional 40 m^2 for each additional impala (Zoolex 2017). The southern Africa "Norms and Standards" recommend boma to be 2 m^2/50 kg live weight for temporary housing of antelope (Bothma & van Rooyen 2005), in other words, approximately 0.63 m^2/impala if each impala weighs ±40 kg. There are no specific requirements or recommendations made for the number of feeding and water points for impala. However, common sense dictates that if the social structure of the herd is territorial in nature (unlikely in captive conditions) then a few feeding and water points located at different sites within the enclosure should be provided (Bothma & van Rooyen 2005). Under captive breeding conditions, food and water are not a limited resource and therefore the herd is more likely to adopt a non-territoriality herd structure. Thus, feeding and water points should be enough to ensure that the welfare of the animals is maintained in that they are free from hunger and thirst. Impala are skilled jumpers and will easily leap over walls that are not high enough. Although no formal recommendation for boma or enclosure wall height could be found, the boma wall height reported in various studies range from 2.7 to 3.0 meters in height (Meyer 2008a, b). These studies reported that the boma wall was either constructed from wooden poles (Meyer et al. 2008a, b) or wire diamond-mesh covered with shade cloth. The advantage of providing a solid wall is that activity from outside the boma will not overstimulate the impala. Also, if the boma wall is high and solid in appearance then this will discourage impala from attempting to escape through jumping (Bothma & van Rooyen 2005). Impala tend to remain anxious and therefore the boma should be built in a quiet area where there is minimal activity and noise emanating from outside the boma, thus preventing startling and minimising the number of escape attempts through jumping. The time dedicated to vigilant behaviour is lowest in female herds of 10-15 impala and highest in small adult male herds (Mooring & Hart 1995; Blanchard et al. 2008). Bachelor herds dedicate an intermediate period of time to being vigilant. The more time dedicated to being vigilant, the less time is available to spend on other activities like resting, eating and ruminating (Matson

et al. 2004). Herds of at least 10 impala should be considered preferable, because this shares the responsibility of herd protection and provides "extra eyes" for vigilance, which allows for more time for each individual to dedicate to other essential behaviours (Shorrocks & Cokayne 2005). Regardless of these specific requirements, impala must be monitored frequently to ensure that they are adapting to captivity and that their welfare state is appropriate to avoid unintentional animal abuse and cruelty (Sikes et al. 2016).

The "ideal" welfare state of animals used for research can be assessed by monitoring indicators of their physical state (body weight or condition, state of coat, posture, lameness and excessive attention to surgical sites), physiological or biochemical state (heart and respiratory rate, temperature, level of stress hormones) and psychological state (changes in behaviour: aggression to herd mates; withdrawal; stereotypies and changes in use of enrichments) (Hawkins et al. 2011; Koknaroglu & Akunal 2013). All animals are different, therefore a databank of what is considered normal physical, physiological or biochemical and psychological state indicators are often used to benchmark a given study population. There is little data available on such indicators to assess welfare in free-ranging impala that have been confined to a boma. However, there is data on these indicators from free-ranging caught and released impala that could be used for comparison (Cheney & Hattingh 1987; Sleenman 1993; Karesh et al. 1997). Monitoring and assessing these indicators will assist in refining the use of impala as a research model for capture and anaesthesia in antelope. Furthermore, study refinement does not only imply addressing welfare related issues but it also involves such things as using appropriate statistics (e.g., using sample size calculation), validating experimental procedures, and improving study design (Smaje et al. 1998; Hawkins et al. 2011).

The refinement of scientific methods will also help address the ethical use of impala as a research model. It is already established is that physically restraining impala for periods longer than 10 minutes produced an unacceptably high mortality rate of up to 30% and therefore proposed future studies where the investigating team wish to physically restrain impala for longer than 10 minutes should not be accepted as ethical (Murray et al. 1981; Knox et al. 1992). Allowing a period of time for the impala to adapt to the boma before data collection is an important methodical refinement. This permits individuals that are not adapting to the boma be identified, and allows the population be standardised somewhat (Knox et al. 1992). Several adaption times ranging from 2 to 6 weeks have been published (Meyer et al. 2008a, b). Another ethical consideration is the fate of the animals after the study. Studies

investigating drug protocols usually last only a few weeks, thus wild caught impala can be released back into the wild after the study. Because adult male impala demonstrate territorial behaviour they seem more stressed in the boma than do females; this suggests that females are a better choice for boma based research (Jarman 1970; Skinner 1971). Any study that might hinder any of the five welfare freedoms should be scrutinised to ensure that the scientific gain is worth the proposed risk to the animals enrolled into the study (Sikes et al. 2016). Studies that investigate different drug combinations for chemical immobilisation and general anaesthesia have inherent risk for causing stress and anxiety, temporary discomfort and pain from darting, and even injury or death. Deaths from capture myopathy, horning injuries, and fractures have been reported in impala that were enrolled in capture related studies (Murray et al. 1981; Knox et al. 1992; Meyer et al. 2008a). However, deaths in impala are also reported by wildlife veterinarians in private practice during capture and transport, thus there is a need to investigate what the causes are and how they can be mitigated to prevent further suffering. To date, there are no studies that have investigated the welfare and ethical considerations for captive impala enrolled in research, despite seven decades of capture-related literature.

1.3   Scope of the thesis

1.3.1 Problem statement

Due to the importance of antelope in conservation and their significant contribution to local and international economies through zoological collections and game farming industries, improvement of management and veterinary practices, like chemical immobilisation and capture, is important. It is impractical to investigate how to improve these practices in all species, therefore, an appropriate research model is essential. To date, impala have often been used as that model, but despite this there is a deficiency in the literature regarding 1) the physiological effects of the drugs used for immobilisation and general anaesthesia and 2) the welfare and ethics of using impala. Impala have an innate nervous disposition and do not always adapt to confined housing, therefore the mortality and morbidity rates are expected to be higher compared to other calmer antelope species and more adaptable domesticated medium-sized ungulates (goats and sheep). Therefore, investigation into their feasibility as a research model is warranted, especially in intensive immobilisation and general anaesthesia studies because they are subjected to risks beyond merely adapting to captivity.

1.3.2 Aims of the thesis

Research focused on welfare and the ethical use of wildlife animals as a research model for studies investigating capture (physical or chemical) and general anaesthesia is needed. Impala have been used as a research model for many decades, yet the practicality of using impala as a research model has never been investigated. This thesis will focus on investigating 1) the extensive physiological effects of the drugs used for immobilisation and general anaesthesia and 2) the welfare and ethics of using impala, to better understand the risks impala are subjected to during intensive study conditions. Below is an outline of how each subsequent chapter furthers our understanding of the practicality of using impala as a research model. Chapters 2 to 4 address deficiencies in the literature by describing the extensive physiological effects of the drugs used for immobilisation and general anaesthesia in impala. Chapters 5 and 6 address the deficiencies in the literature regarding the ethics and welfare of using impala, and advance our understanding of the risks we subject impala to during intensive study conditions and routine chemical capture.

Chapter 2: Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (Aepyceros melampus)

Chemical capture of impala using the potent opioids etorphine and thiafentanil is common practice in South Africa (Kock & Burroughs 2012). Thiafentanil is arguably the drug of choice due to its rapid onset of action and few reported deaths (Burroughs et al. 2012). However, when used alone both of these drugs cause hypoxaemia (Janssen et al. 1993; Meyer et al. 2010). The practice of co-administering two or more drugs that have additive or synergistic interactions, allows lower doses of each to be used. In principle this minimises the adverse effects of each of the drugs and is termed balanced anaesthesia (Dugdale 2010). Similarly, the potent opioids are often administered in combination with a synergistic drug such as a sedative, tranquiliser, or ketamine for chemical immobilisation. Medetomidine has been proposed as a synergistic drug to improve the speed, quality and safety of immobilisation. Medetomidine has been used alone (Bush et al. 2004) or in combination with ketamine (Bush et al. 2004; Perrin et al. 2015), etorphine or thiafentanil (Meyer et al. 2008b) with conflicting reports on its ability to sedate impala and improve the speed and quality of immobilisation. Therefore, in this study, impala were immobilised with combinations of thiafentanil-medetomidine and etorphine-medetomidine

and various time and physiological parameters collected to compare the speed, quality and safety of these combinations. To determine the speed of action of the drug combination, the time from dart placement until the impala became recumbent without attempting to stand (time to recumbency) was recorded. To determine the quality of the immobilisation, the amount of muscle rigidity and the ability to keep the impala immobilised during the study data collection period were subjectively assessed. To determine the safety of the drug combination, the number of episodes of apnoea (no visible inspiratory effort over 60 seconds), and the number of rescue interventions for apnoea (butorphanol:thifentanil or etorphine at 1:1 (mg:mg)) were recorded; the arterial partial pressure of oxygen and carbon dioxide, the end-tidal carbon dioxide, the minute volume and the arterial blood pressure were also measured. To investigate possible aetiologies of hypoxaemia and hypercapnia, various oxygenation and ventilation indices were calculated and these values in conjunction with the measured physiological pulmonary and cardiovascular parameters were interpreted.

Chapter 3: Etorphine-ketamine-medetomidine total intravenous anaesthesia in wild impala (Aepyceros melampus) of 120 minute duration

To date, no reports describing the maintenance of general anaesthesia and the collection of various clinical parameters to evaluate the cardiopulmonary stability of the impala have been found. In clinical practice, I have anaesthetised many impala that underwent invasive surgical repair of bone fractures. As per routine domestic small stock and wild antelope recommendations for general anaesthesia lasting longer than 60 minutes, I have used volatile inhalation agents to maintain surgical anaesthesia (Ball 2007; Ball & Hofmeyr 2014). However, the use of bulky anaesthetic machines is not always practical in the field (Dzikiti 2013), therefore a novel total intravenous anaesthetic infusion of etorphine-ketamine-medetomidine that was administered for 120 minute to determine if this is suitable for field surgical anaesthesia. To determine the practicality and feasibility, the cardiopulmonary stability, quality of maintenance and recovery characteristics were investigated. To achieve general anaesthesia the ketamine and medetomidine were combined into a single infusion bag and infused at a constant rate. The etorphine was added to a separate infusion bag and started at a set infusion dose and titrated to clinical effect. To determine the cardiopulmonary stability, the impala were instrumented with devices to measure arterial blood pressure, end-tidal carbon dioxide, tidal volume, respiratory rate, inhaled and

exhaled respiratory gases (oxygen and carbon dioxide) and peripheral oxygen haemoglobin saturation. Additional parameters that were measured included serial arterial blood gas analysis, serial deep-pain response (to titrate the etorphine infusion to the lowest dose rate), and counts of apnoea. Additional data was collected to calculate various oxygenation and ventilation indices to assist in interpreting the measured cardiopulmonary values. To determine the quality of the general anaesthesia, the ease of tracheal intubation, necessity for administering additional drugs to deepen the plane of general anaesthesia, muscle rigidity and depth of anaesthesia were monitored. To determine the recovery characteristics, the recovery time (times to standing after administering antagonists) were measured and the quality of recovery were subjectively scored by noting the level of ataxia and how soon the impala returned to the herd.

Chapter 4: Blood acid-base status in impala (Aepyceros melampus) immobilised and maintained under total intravenous anaesthesia using two different drug protocols

Enzymes are important for metabolism and regulation of organ function and are labile to extreme acid-base changes, but operate efficiently within a narrow regulated pH range (Mitchell et al. 1972; Castilli et al. 2000; Crimi et al. 2012). Research in patients demonstrating pH shifts outside the narrow regulated range show decreased cardiac output and systemic vascular resistance and altered the oxygen binding to haemoglobin (Mitchell et al. 1972; Crimi et al. 2012; Clarke et al. 2014; Muir 2015). There is a paucity in the literature detailing the blood pH response to immobilisation and general anaesthesia in wild antelope. Therefore, the blood pH status is in impala immobilised and anaesthetised using two different drug protocols over time within and between the protocols were investigated. To determine the blood pH status of the impala during immobilisation and general anaesthesia, the arterial pH and a number of analytes (sodium, potassium, chloride, bicarbonate, phosphorus, albumin, globulin and lactate) in order to evaluate the pH status using the Henderson-Hasselbalch and Stewart approaches were measured. The samples were obtained after immobilisation (prior to starting the total intravenous anaesthetic infusion) and at the end of the maintenance phase of the study (at the end of the total intravenous anaesthetic infusion), using two different drug protocols. To evaluate the blood pH, the pH over time within and between the two drug protocols were compared. The Henderson-Hasselbalch approach evaluated the components of the bicarbonate buffer system (bicarbonate ion

and arterial partial pressure of carbon dioxide) responsible for altering the hydrogen ion concentration. The Stewart approach evaluated the independent variables (arterial partial pressure of carbon dioxide, strong ion difference, anion gap and total weak acid concentration) responsible for the change in bicarbonate and hydrogen ion concentrations.

Chapter 5: Captive management of wild impala (Aepyceros melampus) during intensive immobilisation and general anaesthesia trials

Studies investigating various physical and chemical capture methods in wild medium-sized ungulates, like impala, often require temporary captive management during the trials (Knox et al. 1990; Knox et al. 1991; Knox et al. 1992; Janssen et al. 1993; Bush et al. 2004; Meyer et al. 2008a, b; Meyer et al. 2010). Furthermore, deaths have been reported (Knox et al. 1992; Meyer et al. 2008a). There is a paucity in the literature investigating the overall management of captive impala enrolled in intensive studies investigating drug combinations for immobilisation and general anaesthesia. Therefore, our captive management protocols of impala were investigated by scrutinising our scientific and welfare methodology to highlight areas of concern where we placed the impala at risk. Our scientific methods were scrutinised by evaluating our experimental procedures (Koknaroglu & Akunal 2013). Our animal welfare was scrutinised by monitoring and evaluating various indicators such as: 1) the physical state (body weight, body condition scoring, coat condition, posture and lameness, evidence of diarrhoea); 2) the physiological state (biochemistry and haematology); and 3) the psychological state (changes in behaviour) of the animals (Hawkins et al. 2011). To determine if our scientific methodology compromised the impala, the morbidity and mortality rates of the impala were measured and the aetiology of injury and death were identified to detect areas of risk. To determine the welfare of the impala, the monitored indicators of welfare over time during the 16-week long stay in captivity were compared.

Chapter 6: Chemical capture of impala (Aepyceros melampus): a review of factors contributing to morbidity and mortality, and their mitigation

Mortalities not related to darting injuries have been described in the literature (Knox et al. 1992; Meyer et al. 2008a). In clinical practice, some impala die during chemical capture but this is not well documented in the literature. Using data from the series of studies that are presented in this thesis, it was determined that chemical capture, especially by means of darting, caused the highest morbidity and mortality rates in captive managed impala compared to brief physical capture and release techniques. Therefore, a comprehensive review of the literature was conducted to identify the factors contributing to morbidity and mortality in impala undergoing chemical capture and describe their mitigation. To conduct the review, various databases (Google Scholar, PubMed, Science Direct, Onderstepoort Veterinary Academic Hospital records) and textbooks for information related to impala chemical capture and morbidity and mortality rates were searched. Keywords (*Aepyceros melampus*, capture, immobilisation, morbidity, mortality) were used alone and in various phrases to systematically query each database. The data was accumulated, information interpreted, and a narrative review was compiled to highlighting the factors contributing to morbidity and mortality. Also, interventions to help mitigate these catastrophic outcomes of chemical capture in impala were described.

The series of studies presented in this thesis were approved by the Faculty of Veterinary Science's Research Committee and Animal Ethics Committee prior to commencement of the investigation (V099/13 & V012/16).

CHAPTER 2

Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (*Aepyceros melampus*)

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2.1 Abstract

Impalas (*Aepyceros melampus*) are increasingly valuable in the South African wildlife industry and there is a greater need to chemically immobilise them, ideally with minimal risk. This study aimed to compare the times to recumbency and physiological effects of thiafentanil-medetomidine versus etorphine-medetomidine immobilisation. A combination of thiafentanil (2 mg) + medetomidine (2.2 mg) and etorphine (2 mg) + medetomidine (2.2 mg) was administered (to nine impalas; crossover design) via a dart. After darting, a stopwatch was started to record times to recumbency (time from darting until recumbent without attempts to stand). If apnoea was present the impalas received one or more boluses of butorphanol (1:1 potent opioid dose). Data collection included arterial blood gas analysis and number of butorphanol boluses. Two-sample t-tests were used to compare differences between combinations. The time to recumbency for thiafentanil-medetomidine was 12.2 (±6.8) minutes and no different from 14.5 (±5.2) minutes for etorphine-medetomidine (p =0.426). The thiafentanil-medetomidine combination required more butorphanol boluses (median: 2; interquartile range: 2-3) compared to etorphine-medetomidine (median: 0; interquartile range: 0-1) (p = 0.001). Despite butorphanol treatment and resolution of apnoea, all impalas suffered hypoxaemia (PaO2 ≈ 44.0 mmHg). Thiafentanil-medetomidine did not immobilise impalas more rapidly than etorphine-medetomidine, and resulted in more apnoea that required rescue butorphanol boluses. Marked hypoxaemia resulted from both combinations, mainly due to right-to-left intrapulmonary shunting and not due to hypoventilation. Butorphanol and oxygen supplementation should be considered as essential rescue interventions for all impalas immobilised with these potent opioid combinations.

Keywords: *Aepyceros melampus*, etorphine, impala, medetomidine, thiafentanil

2.2 Introduction

Impalas (*Aepyceros melampus*) have been commonly used as a research animal model for antelope immobilisation in recent years (Meyer et al. 2008b; Zeiler et al. 2015). This increased interest is due to impalas and other antelopes being increasingly valuable in the South African wildlife industry; thus, they are commonly immobilised for translocation and owners are more prepared to consent to invasive surgical interventions and other veterinary treatments in injured animals. In order to achieve these goals, antelopes need to be immobilised safely. Due to an inherent flight response and nervous disposition, impalas make an excellent animal model to study immobilisation protocols in antelopes (Knox et al. 1990).

Thiafentanil and etorphine are potent opioids commonly used to immobilise antelope due to their rapid, predictable and reversible effects (Pienaar et al. 1966). Thiafentanil is a fentanyl analogue (of the 4-anilidopiperidine opioid class) and like fentanyl has an exclusive affinity for the mu-opioid receptor (Vardanyan & Hruby 2014) and is the next generation of opioid immobilisation drugs, which is claimed to induce more rapid immobilisation and shorter duration of action compared to etorphine (Lance & Kenny 2012). Also, claims that thiafentanil produces less respiratory and cardiac depression, when compared with other potent opioids such as fentanyl, carfentanil, and etorphine, exist (Lance & Kenny 2012). Furthermore, thiafentanil is claimed to be the opioid drug of choice in impala immobilisation (Burroughs et al. 2012). Whereas etorphine is a semi-synthetic derivative of the opioid alkaloid thebaine (belonging to the phenanthrene chemical opioid class), which is a non-specific agonist at the mu-, kappa- and delta opioid receptors (Blane et al. 1967; Yaksh & Wallace 2011), medetomidine is a specific alpha$_2$-adrenoceptor agonist used as a synergist with potent opioids in immobilisation drug combinations. It is used for its reliable sedative, good muscle relaxation and analgesic properties (Kästner 2006).

This study aimed to evaluate and compare the times to recumbency and physiological effects of thiafentanil-medetomidine versus etorphine-medetomidine immobilisation in impalas.

2.3 Materials and Methods

The study reported here was a part of a larger collaborative study evaluating different total intravenous anaesthetic (TIVA) maintenance protocols in immobilised impalas, reported elsewhere (Buck et al. 2017; Gerlach et al. 2017; Zeiler et al. 2015). The data reported in this study compare two immobilisation protocols used in the collaborative study, whereas the TIVA studies focus on reporting the

pharmacodynamic characteristics of the maintenance protocols alone, without comparisons between the two immobilisation protocols described herein.

Animals and study area

Nine adult female impalas were first habituated (6 weeks prior to drug trials) to captivity in a boma (outdoor antelope holding facility) and then enrolled into the prospective crossover study. The boma was made up of two adjoining partitions, a smaller feeding area (150 m^2) and a larger home area (300 m^2). The partitions were divided by an internal shade cloth-covered wire fence (same construct as the boma perimeter fencing) with inter-leading swing-gates at both ends of the partition wall (Zeiler & Meyer 2017c; Chapter 5). Thirty minutes prior to chemical capture, the impalas were confined to the feeding area and allowed to rest.

Procedures

The impalas were immobilised with thiafentanil-medetomidine and then four weeks later with etorphine-medetomidine. The doses of the drugs used in the immobilisation were calculated for a 40 kg impala, as follows:

> Thiafentanil-medetomidine: thiafentanil (2.0 mg; Thianil 10 mg ml^{-1}; Wildlife Pharmaceuticals; Karino, South Africa) + medetomidine (2.2 mg; Medetomidine 10 mg ml^{-1}; Kyron Prescriptions; Benrose, South Africa);

> Etorphine-medetomidine: etorphine (2.0 mg; Captivon 9.8 mg/ml; Wildlife Pharmaceuticals) + medetomidine (2.2 mg).

The drugs were administered into suitable muscles of the pelvic girdle using a 3 ml dart (Dan-Inject 3 ml, 25 mm plain needle; S300 Syringe Dart; Dan-inject International SA; Skukuza, South Africa) projected over a 10-15 metre distance from a carbon dioxide-powered dart rifle (Model JM; Dan-inject International SA). The drugs were added to the dart and then the drug chamber was filled to 3 ml by adding sterile water for injection (Kyron Laboratories Ltd., Benrose, South Africa). Once the dart was placed and discharged, a stopwatch was started to record the time of events. Time to recumbency was defined as the time from dart placement until the impalas became recumbent (sternal or lateral) without attempts to stand. Once recumbent, the remaining herd was shepherded into and confined to the home area of the boma. The immobilised impala was blindfolded and cotton wool swabs were placed into the

ear canals to minimise external visual and auditory stimulation. One of the cephalic veins was aseptically cannulated using an over-the-needle catheter (20 Gauge; Jelco; Smiths Medical; Lancashire, UK). A basic field clinical examination (temperature, heart and respiratory rates) was conducted. The impala was loaded onto the back of a pickup truck and driven to the procedure room 650 metres away. During this time period the impala's respiratory rate and effort were monitored and if apnoea (no visible breath attempt for 60 seconds) or dyspnoea or cyanosis were detected then a single bolus of butorphanol (2 mg; Butorphanol 10 mg ml^{-1}; V-Tech Pharmacy; Pretoria, South Africa) was administered intravenously. Additional butorphanol boluses were administered if the initial intended response (improved respiratory rate without signs of arousal) was unsatisfactory. The total number of butorphanol boluses required to achieve the intended response was recorded.

Once in the procedure room the temperature, heart and respiratory rates were measured again. The auricular artery was aseptically cannulated using an over-the-needle catheter (22 Gauge; Jelco). Arterial blood was collected anaerobically in a pre-heparinised syringe and analysed immediately using a patient-side gas analyser (EPOC Reader Blood Analysis and EPOC BGEM smart cards; Epocal; Kyron Laboratories). Information from the blood gas analysis important to this study included the arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) tensions, lactate concentration and barometric pressure. The arterial blood pressure was measured using an electronic strain gauge (BD DTX; Bacton and Dickson Medical; NY, USA) zeroed to atmospheric air pressure at the level of the right atrium) coupled to a multiparameter monitoring machine (Datex-Ohmeda S/5 Anesthesia Monitor; GE Healthcare; Finland). The trachea of the impala was intubated with a cuffed endotracheal tube (size 8) and the animal was allowed to breathe spontaneously. A pitot-tube and side-stream respiratory gas analyser (200 ml minute^{-1} sampling rate) was coupled to the end of the endotracheal tube and connected to the multiparameter monitoring machine to measure minute volume and end-tidal carbon dioxide pressure ($P_{ET}CO_2$). A venous blood sample was collected from the lateral saphenous vein and stored in a serum tube (BD Vacutainer tube; BD Diagnostics; New Jersey, USA). The time from darting until completion of all data collection for the impala was recorded (time to data collection completion). The venous blood samples were allowed to clot (60 minutes; room temperature 18-23°C) prior to centrifugation (5 000 revolutions per minute for 10 minutes) to separate the cellular and fluid components of the blood. The serum was carefully pipetted into cryovials and stored in a -80°C freezer until serum cortisol

concentration determination. Total serum cortisol concentration was determined (Immulite 1000; Siemens; Isando, South Africa) using a chemiluminescent enzyme immunoassay.

The impala was kept under general anaesthesia for 120 minutes. After the general anaesthesia, the impala were returned to the feeding area of the boma for an uneventful recovery (Buck et al. 2017; Zeiler et al. 2015).

Ethical consideration

The University of Pretoria's research and animal ethics committees approved the study prior to commencement (V099/13 and V012/16). The wildlife and veterinary community will benefit from the results of this study by better understanding the physiological effects of using different potent opioid drug combinations during chemical capture. Fifteen healthy adult female impalas were purchased from an extensive game farm operation. They were captured and translocated to the study boma. A six-week pre-trial boma adaption period was observed. Experienced research and wildlife veterinarians conducted the study procedures. On completion of the trials, the impalas were recaptured and released onto an extensive game farm.

Data analysis

The two data sets (obtained in the boma and procedure room) for temperature, heart and respiratory rates were averaged for each impala prior to analysis. The following equation was used to calculate the alveolar oxygen tension: $PAO_2 = FiO_2 (Patm - PH_2O) - PaCO_2/RQ$; where: FiO_2 is the fraction of inspired oxygen (21% for room air); Patm is the barometric pressure (measured during the study); PH_2O is the water vapour tension in the alveoli (47 mmHg at 37°C, uncorrected for body temperature); and RQ is the respiratory quotient (1.0 for ruminants). The alveolar-to-arterial oxygen tension ($P(A-a)O_2$) gradient was calculated by subtracting the measured arterial oxygen tension (PaO_2) from the calculated alveolar oxygen tension (PAO_2). The arterial-to-end-tidal carbon dioxide tension ($PaCO_2$-$P_{ET}CO_2$) gradient was calculated by subtracting the measured end-tidal carbon dioxide tension ($P_{ET}CO_2$) from the measured arterial carbon dioxide tension ($PaCO_2$).

Data were assessed for normality by evaluating descriptive statistics, plotting of histograms and performing the Anderson-Darling test for normality. Parametric quantitative data (heart rate, respiratory rate, mean arterial blood pressure, cortisol concentration, temperature, PaO_2, $PaCO_2$, $PaCO_2$-$P_{ET}CO_2$

and P(A-a)O$_2$ gradients) were compared between groups using two-sample t-tests. Non-parametric data (number of butorphanol boluses, lactate concentration) were compared using Moods Median Test. Data were reported as mean (±SD: standard deviation) unless otherwise stated. Scatter plots and general linear regression were used to compare cortisol concentrations to times to recumbency within and between the two drug protocols. Correlations between variables of interest (times to recumbency, cortisol concentrations) were determined using the Pearson correlation test. Data were analysed using commercially available software (MiniTab 17.1.0; MiniTab Incorporated; State College, Pennsylvania, USA) and results interpreted at the 5% level of significance.

2.4 Results

The study was conducted at an altitude of 1252 metres above sea level, where the average barometric pressure was 665 mmHg (88.6 kPa). The weight of the impalas averaged 38.6 (±4.3) kg and 40.4 (±4.2) kg during the thiafentanil-medetomidine and etorphine-medetomidine data collection sessions, respectively (p = 0.371). Thus the mean (range) total dose for thiafentanil and medetomidine was 0.052 (0.047 to 0.058) mg kg^{-1} and 0.057 (0.051 to 0.064) mg kg^{-1}, respectively; and for etorphine and medetomidine was 0.050 (0.045 to 0.055) mg kg^{-1} and 0.054 (0.049 to 0.061) mg kg^{-1}, respectively. All impalas were successfully immobilised with both drug combinations.

Figure 2.1 Scatter plots with general linear modelling demonstrating the significant difference (p = 0.023) of cortisol concentration (nmol L^{-1}) of impala (*Aepyceros melampus*) within five minutes after immobilisation versus the time to recumbency (minutes) between thiafentanil-medetomidine compared to etorphine-medetomidine.

The overall time to recumbency for thiafentanil-medetomidine was 12.2 (±6.8) minutes, which was no different from 14.5 (±5.2) minutes for the etorphine-medetomidine combination (p = 0.426). However, the association between cortisol concentrations and times to recumbency differed significantly between the immobilisation combinations (p = 0.023; slopes and intercepts on linear regression graphs; **Figure 2.1**). The cortisol concentrations versus times to

recumbency demonstrated a strong negative correlation ($p = 0.036$; $r = -0.699$) when the thiafentanil-medetomidine drug combination was used compared to no correlation ($p = 0.238$; $r = 0.438$) for the etorphine-medetomidine combination. Nonetheless, the overall cortisol concentrations did not differ between the combinations (**Table 2.1**). The overall times to data collection completion were 35.5 (±8.4) and 32.2 (±8.3) minutes for thiafentantil-medetomidine and etorphine-medetomidine, respectively ($p = 0.424$).

The thiafentanil-medetomidine combination required more frequent butorphanol rescue boluses (median: 2; interquartile range: 2-3) during the data collection period (time period between recumbency and data collection completion) compared to the etorphine-medetomidine (median: 0; interquartile range: 0-1) to rescue the impala from bouts of apnoea and/or respiratory distress ($p = 0.001$). Most butorphanol boluses (11 out of 23; n = 8 for thiafentanil-medetomidine and n = 3 for etorphine-medetomidine) were administered within 2 minutes after recumbency. Follow-up butorphanol boluses

Table 2.1 Physiological variables obtained from impala (*Aepyceros melampus*) immobilised with thiafentanil-medetomidine and etorphine-medetomidine combinations.

Parameter	Unit	Thiafentanil-medetomidine			Etorphine-medetomidine			p-value
		Mean	±SD	Range	Mean	±SD	Range	t-tests
Clinical examination measurements								
Temperature	°C	38.9	±1.1	37.6-40.8	39.3	±0.7	38.2-40.6	0.341
Heart Rate	Beats min^{-1}	122	±40	71-197	79	±37	45-150	0.032
Respiratory Rate	Breaths min^{-1}	9	±5	2-15	9	±2	2-13	0.818
Minute volume	L min^{-1}	8.9	±4.7	2.4-14.3	8.0	±3.2	3.5-14.0	0.659
MAP	mmHg	126	±14	108-152	117	±18	94-133	0.226
Clinical pathology measurements and calculations								
PaO_2	mmHg	47	±8	38-62	41	±12	24-55	0.193
	kPa	6.3	±1.1	5.1-8.3	5.5	±1.6	3.2-7.3	
$PaCO_2$	mmHg	51	±9	38-68	51	±7	45-63	0.952
	kPa	6.8	±1.2	5.1-9.1	6.8	±0.9	6.0-8.4	
$P_{ET}CO_2$	mmHg	41	±7	29-52	51	±7	41-64	0.019
	kPa	5.5	±0.9	3.9-6.9	6.8	±0.9	5.5-8.5	
$PaCO_2$-$P_{ET}CO_2$	mmHg	10	±9	(-4)-23	0	±8	(-11)-12	0.028
$P(A-a)O_2$	mmHg	31	±7	19-40	38	±8	29-48	0.078
Lactate	mmol L^{-1}	2.2*	(1.8-4.9)*	1.4-5.4	2.2*	(1.9-4.2)*	1.2-5.3	0.637†
Cortisol	nmol L^{-1}	84.5	±41.9	35.6-173.0	80.0	±37.7	21.8-136.0	0.817

SD: standard deviation; °C: degree Celsius; min: minute; MAP: mean arterial blood pressure; mmHg: millimetres mercury; kPa: kilopascals; PaO_2: arterial oxygen tension; $PaCO_2$: arterial carbon dioxide tension; $P_{ET}CO_2$: end-tidal carbon dioxide tension; $PaCO_2$-$P_{ET}CO_2$: arterial-to-end-tidal carbon dioxide tension gradient; $P(A-a)O_2$: alveolar-to-arterial oxygen tension gradient; mmol: millimoles; nmol: nanomoles; †: p-value for Moods Median Test; *: median and (interquartile range)

were only required in the thiafentanil-medetomidine combination and were administered at approximately 6 (7 out of 23) 10 (4 out of 23) and 15 (1 out of 23) minutes after recumbency. Despite the required rescue interventions, the respiratory rates (obtained in the boma and procedure room, between bouts of apnoea), and measured minute volumes (which were obtained after all apnoea events) were similar between the combinations (**Table 2.1**). It was noticed that the impalas that suffered apnoea were also much more rigid (hypertonic appendicular, thoracic and abdominal muscles) compared to those that did not suffer apnoea. Furthermore, the impalas appeared more relaxed soon after the butorphanol bolus. Even after successful apnoea treatment all impalas suffered from hypoxaemia, where mean PaO_2 tensions were 47 mmHg (6.3 kPa) and 41 mmHg (5.5 kPa) for thiafentanil-medetomidine and etorphine-medetomidine, respectively (p = 0.193). Heart rates were higher when thiafentanil-medetomidine was used (p = 0.032) compared to etorphine-medetomidine, but the mean arterial blood pressures did not differ between the combinations (p = 0.226).

2.5 Discussion

A number of important clinical findings need highlighting. The first novel finding is that the time to recumbency was no different when the thiafentanil-medetomidine combination was used compared to the etorphine-medetomidine combination. The second is that the more rigid the impala was (more so with thiafentanil-medetomidine) due to generalised muscle hypertonia, the more apnoea was noticed. This apnoea was successfully treated with one to three low doses of butorphanol. These doses did not cause arousal but improved breathing and reduced muscle rigidity. The third and final observation is that despite apparently normal temperature, heart rate and respiratory variables (rate and minute volume) once apnoea was resolved, all impalas suffered clinically significant hypoxaemia.

Thiafentanil has been advocated as the preferred potent opioid for immobilisation of free-ranging impalas because it causes rapid time to recumbency and less fatalities compared to when fentanyl and etorphine are used (Burroughs et al. 2012). Janssen et al. (1993) reported that thiafentanil (0.050 mg kg^{-1}) alone, at a similar dose to our study, induces immobilisation rapidly, with times to recumbency of 2.7 (±0.6) minutes. Etorphine, when used alone, appears to demonstrate a dose-dependent decrease in times to recumbency, where a low dose (0.020 mg kg^{-1}) and high dose (0.070 mg kg^{-1}) induce recumbency in 12.9 (±4.9) and 3.7 (±1.4) minutes, respectively (Cheney & Hattingh 1987; Meyer et al.

2010). Furthermore, combinations of thiafentanil (0.032 ±0.0028 mg kg^{-1})-medetomidine (0.053 ±0.0047 mg kg^{-1}) and etorphine (0.041 ±0.0035 mg kg^{-1})-medetomidine (0.053 ±0.0047 mg kg^{-1}) cause recumbency in 4.9 (±3.1) and 9.2 (±3.8) minutes, respectively (Meyer et al. 2008b). All current evidence indicates that thiafentanil alone, or in combination with sedatives or tranquillisers, is the potent opioid that achieves more rapid time to recumbency in impalas. However, in contrast we found that thiafentanil-medetomidine did not produce a more rapid time to recumbency compared to etorphine-medetomidine, despite using higher drug doses compared to the other studies. We assumed complete intramuscular injection of the drugs when dart placement was good and they fully discharged. However, we cannot rule out the possibility that the drugs could have been deposited into a fascial plane (between muscles and subcutis), which could explain poor drug absorption and therefore delayed onset of action. We used 3 ml volume darts, in order to keep the dart size consistent between all the protocols used in the entire drug trial study (Buck et al. 2017; Gerlach et al. 2017; Zeiler et al. 2015). One component of the entire drug trial study was to evaluate the effects of an etorphine-medetomidine-ketamine versus a butorphanol-medetomidine-ketamine immobilisation combination (reported elsewhere), which required up to 3.0 ml volume, therefore 3 ml darts were used during the entire drug trial study (Gerlach et al. 2017). However, the drug combinations reported in this study (total volume = 0.42 mL of drugs; 14% volume of the dart) were 7 times diluted, with sterile water for injection, to increase the volume to 3 ml. The change in drug concentration could have altered the pharmacokinetics of the drugs mainly by reducing the rate of absorption from the intramuscular site, thus prolonging the time to recumbency, despite administering a total drug dose higher than those previously reported (Meyer et al. 2008b). Unfortunately, *in vitro* and *in vivo* physicochemical interactions of the mix of the potent opioids, medetomidine and injectable sterile water were not determined or ruled out as possible causes of a slower clinical effect. However, similar mixtures, used at higher concentrations, did not appear to interact, or have reduced efficacy (Meyer et al. 2008a). Our findings highlight that further studies are warranted to determine what the volume-concentration relationship effects are on speed of absorption and efficacy of potent opioids, and whether physicochemical interactions are present when mixing drugs in a dart.

Rapid times to recumbency are desirable when capturing impalas, or any other antelopes, because this is believed to translate into a decrease in capture-related morbidities, such as capture-induced hyperthermia and myopathies, which often develop into mortalities (Meyer et al. 2008a). Etorphine (1.5

mg) and thiafentanil (1.2 mg) were used in combination with either azaperone (40 mg) or medetomidine (2 mg) (Meyer et al. 2008a). The mean (±SD) time to recumbency and cortisol concentrations were 6.5 (±3.6) minutes and 66.0 (±48.6) nmol L^{-1}, respectively, averaged over the four drug combinations. Surprisingly, the higher dose of the potent opioids used in our study, compared to the Meyer et al. (2008a) study, resulted in longer times to recumbency (13.1 ±6.0 minutes) and higher cortisol concentrations (76.7 ±36.5 nmol L^{-1}), overall. Furthermore, in contrast to Meyer et al. (2008a), in our study we found that the shorter the induction time the higher the cortisol concentration when the thiafentanil-medetomidine protocol was used, and did not find an association between these variables when the etorphine-medetomidine protocol was used. Meyer et al. (2008a) made use of powder charged darts (Pneu-Darts) with barbed needles, which ensured rapid injection speeds and stability of the dart depth during injection. The darts (Dan-Inject darts, charged with compressed air, with plain needles) used in this study most likely injected the drugs more slowly and at a variable depth. The difference in the injection characteristic of the darts used could have also contributed to the prolonged induction times reported in our study. These contradictory findings highlight that further research is required to determine the effects of a stress response on times to recumbency, but they also highlight the limitations of using a single serum cortisol concentration as an indicator of the magnitude of a stress response (Hart 2012).

The overall appendicular (King & Klingel 1965; Pienaar 1969; Seal et al. 1985), thoracic (Benthuysen et al. 1986; Haigh 1977; Pearce & Kock 1989; Weisner et al. 1984) and abdominal rigidity is a frequently reported observation when potent opioids are used during chemical capture. Opioid-induced muscular rigidity remains a serious clinical challenge in many animals, including humans treated with potent opioids, especially when fentanyl and its analogues are used (Bowdle 1998). We observed that impalas that were more rigid tended to suffer more bouts of apnoea. Moreover, thiafentanil-medetomidine caused more profound rigidity compared to etorphine-medetomidine. The increased appendicular and abdominal muscle rigidity is thought to be mediated through the mu-opioid receptor type (Woolf et al. 1973). The rigidity of the respiratory muscles could be due to the potent opioids disrupting usual respiratory rhythm generation by interfering with the pre-Bötzinger complex (causing slow inspiratory effort or struggling to initiate an inspiratory effort) and Kölliker-Fuse nucleus (transition from inspiration to expiration) through activation of mu-receptors (Pattinson 2008). The fact that thiafentanil specifically interacts with the mu-receptor (Yaksh & Wallace 2011) compared to etorphine could explain our

observation that thiafentanil-medetomidine caused more profound muscle rigidity. Also, thiafentanil is likely to be more lipid soluble than etorphine (as fentanyl is compared to morphine) (Yaksh & Wallace 2011), which could have translated into higher central concentration of the drug interacting with the central mu-receptors in a shorter period of time, resulting in more profound effects on the respiratory system. Also, our study made use of equal total doses (2 mg) of thiafentanil and etorphine. It is claimed that thiafentanil has "twice the potency of etorphine" (Lance & Kenny 2012), but there is no scientific evidence whether these drugs are equipotent or not. Therefore, it is plausible that the more profound muscle rigidity and respiratory depression in the thiafentanil-medetomidine group could have been as a result of administering this more potent opioid at the same dose of etorphine. The muscle rigidity caused by the opioids can be remedied by administering drugs with muscle relaxant properties (benzodiazepines and alpha$_2$-adrenoceptor agonists) or drug with mu-antagonist properties (butorphanol). However, medetomidine did not appear to reduce this rigidity as would be expected from its prominent central muscle relaxant effects (Kästner 2006). Furthermore, butorphanol, a mixed mu-antagonist and kappa-agonist (at 1:1 potent opioid dose), tended to reduce muscle rigidity and resolved etorphine-induced apnoea more reliably than thiafentanil-induced apnoea. However, etorphine-induced apnoea (n = 3) was not as frequent in our study as thiafentanil-induced apnoea (n = 8). Also, multiple butorphanol doses were required in the thiafentanil-medetomidine immobilised impalas to completely resolve apnoea. Similar to findings reported in etorphine-immobilised goats (Haw et al. 2016b), butorphanol, at the doses used, did not induce arousal from immobilisation, but caused muscle relaxation in the goats and improved their breathing rate and rhythm.

Overall, the impalas in our study demonstrated respiratory rates (between the bouts of apnoea) that were within normal limits (7 to 15 breaths per minute) for antelopes immobilised with potent opioids (Harthoorn 1967). Also, our overall mean ±SD minute volumes were 8.5 ±4.0 L minute^{-1} and were similar to etorphine-immobilised impalas (10.9 L minute^{-1}; mean body weight 36 kg; Meyer et al. 2010) and those of small domestic ruminants (6.3 to 10.4 L minute^{-1}: Bakima et al. 1988; Hales & Webster 1967). Despite the adequate respiratory rates and minute volume, hypoxaemia and hypercapnia were observed. The marginally widened $PaCO_2$-$P_{ET}CO_2$ (normal < 10 mmHg) and wide $P(A-a)O_2$ (normal < 20 mmHg) gradients suggest that the origin of the hypoxaemia was more likely due to impedance of oxygen diffusion and physiological right-to-left intrapulmonary shunting of blood rather than dead-space ventilation. Etorphine, when administered to goats, caused pulmonary hypertension (mean pulmonary

artery pressure 23 ±6 mmHg), which is a plausible cause of oxygen diffusion deficits and right-to-left intrapulmonary shunting (Meyer et al. 2015; Vodoz et al. 2009). Alpha$_2$-adrenoceptor agonists, in small stock, are also believed to cause pulmonary hypertension, or a rapid transit time of blood flow across the pulmonary capillary bed decreasing time for oxygen diffusion (Kästner 2006). Furthermore, medetomidine causes intense peripheral vasoconstriction mostly through its direct activation of alpha$_2$-adrenoceptors in the endothelium. This vasoconstriction is thought to cause a delay in blood flow through peripheral tissues resulting in increased oxygen extraction (Kästner 2006). Thus, blood returning to the pulmonary capillary has a lower oxygen content, which then could translate into a wider than anticipated P(A-a)O$_2$ gradient (Zeiler et al. 2015).

Thiafentanil, the latest potent opioid used for free-ranging ungulate capture, was introduced in the early 1990s (Janssen et al. 1993). Compared to etorphine, claims exist that thiafentanil immobilises animals more rapidly, is safer and causes less respiratory depression (Burroughs et al. 2012; Lance & Kenny 2012). However, our findings are contradictory and therefore more investigation, using well designed studies, are required to ensure a more complete understanding of the pharmacodynamic effects of thiafentanil and etorphine and their role in free-ranging ungulate capture.

Limitations of the study

Unfortunately, serial samples of blood for serum cortisol and catecholamine (adrenalin and noradrenalin) concentration measurements were not obtained. This serial sampling would have allowed a more precise understanding of the magnitude of contribution that acute stress and increased sympathetic tone have on the overall cardiopulmonary effects of immobilisation and times to recumbency. The trials were not randomised as impalas were first immobilised with thiafentanil-medetomidine and then four weeks later with etorphine-medetomidine. Therefore, we cannot rule out the influence of the effects of time in the boma and data collection experience gained by the handlers on the results of the study. However, there were no statistical differences in the times when data was collected and the washout period was long enough to rule out residual drug effects (minimum washout period is 6 days). Therefore, we believe that this limitation had little to no influence on the outcomes of the study. Administering drugs by dart is prone to a number of errors, especially in flighty species such as impala. The operator can never be confident that all of the drugs were administered intramuscularly, therefore, the limitations of darting should be recognised when interpreting data from any immobilisation

study. In light of these limitations it would have been invaluable to have hand-injected the impala and compare these findings to the data from darted impala (Smith et al. 1993). Furthermore, comparison of our findings to other studies was attempted in the discussion, but the reader should be aware of the limitations of comparing these studies due to differences in: the animals themselves, population dynamics of the herd, conditions under which animals were immobilised, the degree of stress the animals experienced prior to dart placement, different dart types and rifles being used and operator experience in placing darts.

2.6 Conclusions

The thiafentanil-medetomidine combination induced recumbency in times that were no different to those induced by etorphine-medetomidine, and both these times were longer than those seen in other studies and field captures. These unexpected prolonged times to recumbency were possibly due to the administration of diluted drugs (7 x dilution with sterile water for injection to fill a 3 ml dart) that caused a delay in intramuscular absorption due to a decreased absorption surface area to volume ratio. The effects of different stress responses may have also played a role. However, further research to clarify what primarily influences recumbency times is needed. The clinical implications of immobilising impalas using a potent opioid (especially thiafentanil) and medetomidine combination) is that apnoea and profound muscle rigidity can be expected and that butorphanol (1:1 the potent opioid dose) is an effective rescue intervention that does not cause arousal. In addition, clinically relevant hypoxaemia and hypercapnia were present despite seemingly normal heart and respiratory rates and ventilation. The hypoxaemia and hypercapnia are suspected to be primarily due to pulmonary hypertension, causing gas diffusion deficits, and an increase in right-to-left intrapulmonary shunting of blood. Therefore, butorphanol and oxygen insufflation should be considered as essential rescue interventions for all impalas immobilised with these potent opioid combinations.

CHAPTER 3

Etorphine-ketamine-medetomidine total intravenous anaesthesia in wild impala (*Aepyceros melampus*) of 120 minute duration

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3.1 Abstract

The need for performing long-term anaesthesia in wildlife, especially antelope, is becoming a growing necessity. The costs and logistics of transporting wildlife to veterinary practices make surgical intervention a high-stakes operation. Thus, there is a need for a field-ready total intravenous anaesthesia (TIVA) infusion to maintain anaesthesia in antelope. This study explored the feasibility of an etorphine-ketamine-medetomidine TIVA for field anaesthesia. Ten wild-caught, adult impala (*Aepyceros melampus*) were enrolled in the study. Impala were immobilised with a standardised combination of etorphine (2 mg) and medetomidine (2.2 mg), which equated to a median (interquartile range; IQR) etorphine and medetomidine dose of 50.1 (46.2-50.3) and 55.1 (50.8-55.4) µg kg^{-1}, respectively. Recumbency was attained in a median (IQR) time of 13.9 (12.0-16.5) minutes. Respiratory gas tensions, spirometry and arterial blood gas were analysed over a 120 minute infusion. Once instrumented the TIVA was infused as follows: etorphine at a variable rate initiated at 40 µg kg^{-1} hour^{-1} (adjusted according to intermittent deep-pain testing); ketamine and medetomidine at a fixed rate of 1.5 mg kg^{-1} hour^{-1} and 5 µg kg^{-1} hour^{-1}, respectively. The etorphine had an erratic titration to clinical effect in four impala. Arterial blood pressure, respiratory and heart rates were all within normal physiological ranges. However, arterial blood gas analysis revealed severe hypoxaemia, hypercapnia and acidosis. Oxygenation and ventilation indices were calculated and highlighted possible co-aetiologies to the suspected etorphine-induced respiratory depression as the cause of the blood gas derangements. Impala recovered in the boma post atipamezole (13 mg) and naltrexone (42 mg) antagonism of medetomidine and etorphine, respectively. The etorphine-ketamine-medetomidine TIVA protocol for impala may be sufficient for field procedures of up to 120 minute duration. However, hypoxaemia and hypercapnia are of paramount concern and thus oxygen supplementation should be considered mandatory. Other TIVA combinations may be superior and warrant further investigation.

Keywords: *Aepyceros melampus*, etorphine, impala, ketamine, medetomidine, TIVA

3.2 Introduction

Etorphine immobilisation in impala (*Aepyceros melampus*) has been studied since the 1960s (Pienaar et al. 1966; Ables & Ables 1969). Despite decades of use in impala, etorphine immobilisation has only been described and no reports are available mentioning its use for the long-term maintenance of surgical anaesthesia. The lack of its incorporation into anaesthetic maintenance protocols is perhaps due to pronounced dose-related respiratory depression (Buss & Meltzer 2001; Meyer et al. 2008b; Meyer et al. 2010). In order to decrease the dose of etorphine and thus its undesirable characteristics, it can be used in a combination with other drugs with known analgesic and anaesthetic sparing effects, such as a low dose of ketamine (a cyclohexylamine dissociative anaesthetic) or medetomidine (an alpha$_2$-adrenoceptor agonist) to achieve surgical anaesthesia (Kästner 2007).

In South Africa, due to the importance of antelope for conservation and the value of these animals in zoo and private wildlife collections, veterinary intervention is often required (Bezuidenhout 2013; McGroarty 2014). These animals, in particular impala, are prone to injury and are often presented to veterinarians for surgical intervention. Long duration transport to veterinary facilities, field equipment, man power limitations and high cost make surgery on wildlife a challenge. A reliable field anaesthetic protocol that does not require the logistical challenges of inhalation agents, may make surgical interventions more accessible to antelope situated in remote areas. The present study was designed to determine the efficacy and safety of an etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion for field surgical anaesthesia of 120 minute duration in a wild antelope species.

3.3 Materials and Methods

The study was approved by the University of Pretoria's animal ethics committee (V099/13) prior to investigation. The study was the final of four different anaesthesia studies conducted on fifteen wild-caught, adult female impala over a three month period with a fourteen day washout period between the different studies. A six-week boma-habituation period was completed prior to the studies. The impala where housed in a partitioned 2.7 meter high walled boma (outdoor enclosed paddock; 20 m x 15 m). The smaller of the partitions (10 m x 15 m) was used for daily *ad libitum* feeding (*Medicago sativa, Eragrostis teff*) and commercially available herbivore pellets according to body condition. The impala had free access to water throughout the day. Ten out of fifteen impala were randomly selected to participate in the study.

<u>Immobilisation</u>

All impala were temporarily confined in the smaller partition of the boma and darted with a standardised combination of etorphine (2 mg; Captivon 0.98%; Wildlife Pharmaceuticals; South Africa) and medetomidine (2.2 mg; 1% medetomidine compounded; Kyron Prescriptions; South Africa) delivered by via a full (sterile water for injection used to fill dart; Kyron Laboratories; South Africa) 3 ml air-pressurised dart (Dan-Inject) projected into the muscles of the pelvic girdle (approximately 10 to 15 meters range) using a carbon dioxide powered projector (set to 5 bar pressure; Dan-Inject; Model JM; Denmark). A time period of 15 minutes was allowed for the impala to become recumbent. Re-darting using either a half-strength or full-strength dart, depending on the level of ataxia and sedation demonstrated, was performed when recumbency did not occur during the specified time. Time to achieve recumbency and a quality score of immobilisation (**Table 3.1**) were recorded. Once recumbent, the remaining impala in the group were allowed to move to the large partition of the boma. The immobilised impala was blindfolded and the ears were plugged so as to limit external stimuli.

Table 3.1 Simple descriptive scoring systems used to determine the quality of immobilisation, endotracheal intubation and recovery in impala (*Aepyceros melampus*) undergoing an etorphine-ketamine-medetomidine total intravenous anesthesia (TIVA) infusion of 120 minute duration.

Score	Description
	Immobilisation quality
1	Calm transition, no excitement, remains down once recumbent
2	Mild excitement before becoming recumbent, maintains recumbency
3	Pronounced excitement, attempts to stand after becoming recumbent
4	Profound excitement, does not become recumbent
	Intubation quality
1	Orotracheal intubation easy at first attempt
2	Orotracheal intubation achieved on subsequent attempts
3	Midazolam bolus required before orotracheal intubation successful
4	Orotracheal intubation unsuccessful
	Recovery quality
	Early recovery (from reversal agent administration to first attempt to stand)
1	Calm transition to alertness
2	Generally calm, but startles easily
3	Uncoordinated whole body movements, paddling, startled
4	Emergence delirium, thrashing
	Late recovery (from attempt to stand to reuniting with herd)
1	Two or less efforts to stand, minimal ataxia, normal gait
2	Two or less efforts to stand, noticeable ataxia, gait mostly normal
3	More than two efforts to stand, considerable ataxia, unsteady gait
4	Unable to stand more than 30 minutes post reversal agents

A cursory field clinical examination was conducted noting peripheral oxygen-haemoglobin saturation (SpO$_2$, probe placed on vulva; Nonin PureSat 2500 A; Nonin Medical Inc.; MN, USA), rectal temperature, pulse and respiratory rate. The left cephalic vein was aseptically cannulated (20 Gauge; Jelco; Smiths Medical; Lancashire, UK) in the boma. The impala was transported via vehicle to the procedure room approximately 650 meters away. Apnoea, recorded as no visible inspiratory effort for longer than 60 seconds, was treated with a single dose of intravenous butorphanol (at a ratio of 1:1 to the immobilisation etorphine dose; Butorphanol 1%; V-Tech Pharmacy; South Africa).

Instrumentation

Once in the procedure room, the impala was weighed and then placed on a preheated (circulating warm-water blanket set to 38°C) table top in right lateral recumbency. A forced air warming blanket was placed over the recumbent impala. An auricular artery was aseptically cannulated (22 Gauge) and an initial arterial blood sample was drawn for immediate blood gas analysis (EPOC Reader Blood Analysis Analyser and EPOC BGEM smart cards; Epocal; Canada) using a sodium-heparinised syringe. Immediately after arterial blood sample collection, the trachea was intubated with a cuffed polyvinyl-chloride endotracheal tube (ET-tube; Size 8). A single intravenous bolus of midazolam (0.2 mg kg^{-1}; Dormicum 0.5%; Roche Products; South Africa) was administered if there was a failure to intubate due to inadequate jaw muscle relaxation. A pitot-tube spirometer with a gas sampling port was attached to the end of the ET-tube for monitoring of spirometry and respiratory gases, respectively. Supplemental oxygen (2 L minute^{-1}) was administered via a nasogastric feeding tube (8 French Gauge; Avacare feeding tube; Sunray Medical; Shanghai, China) inserted into the trachea via the ET-tube. Measured parameters included electrocardiogram, end-tidal carbon dioxide (P$_E$´CO$_2$), fractional expiration of oxygen (F$_E$´O$_2$), expiratory tidal volume (Vt), invasive arterial blood pressure (MAP; auricular artery; electronic pressure transducer [BD DTX; Bacton and Dickson Medical; NY, USA] zeroed to atmospheric air pressure at the level of the sternum), rectal temperature (Temp), heart and respiratory rate; which were all obtained from a previously calibrated multi-parameter monitoring machine (Datex-Ohmeda S/5 Anesthesia Monitor; GE Healthcare; Finland). A transmission pulse oximeter probe (Nonin PureSat 2500 A) was placed on the vulva for continuous SpO$_2$ monitoring. Physiological parameters were recorded at five minute intervals from instrumentation until 120 minutes after commencement of the TIVA infusions. Arterial blood gas samples were drawn at the beginning of the infusion (time 0) and then at 30 minute intervals.

Maintenance of surgical anaesthesia

Two additional peripheral veins were aseptically cannulated (lateral saphenous and right cephalic vein). Three separate intravenous infusions were delivered via the three venous cannulae using separate infusion pumps (Infusomat Space; B Braun; Germany) in order to control the infusion rates independently. Infusion one was isotonic crystalloid maintenance solution (Sodium Chloride Fresenius 0.9%; Intramed; South Africa) administered at a fixed rate of 5 ml kg^{-1} hour^{-1} via the lateral saphenous vein. Infusion two was a combination of ketamine (1.5 mg kg^{-1} hour^{-1}; Ketamine Fresenius 10%; Intramed) and medetomidine (5 μg kg^{-1} hour^{-1}; Domitor 0.1%; Zoetis; South Africa) diluted in a 0.9% saline bag with a final fluid weight of 1000 grams (1000 ml volume; final dilution: ketamine 0.75 mg ml^{-1}; medetomidine 2.5 μg ml^{-1}); administered at a fixed rate of 2 ml kg^{-1} hour^{-1} administered via the left cephalic cannula. A single loading bolus of ketamine (1 mg kg^{-1}) was administered intravenously prior to commencement of the ketamine-medetomidine infusion. The third infusion was etorphine (40 μg kg^{-1} hour^{-1}) diluted in a 0.9% saline bag with a final fluid weight of 1000 grams (1000 ml volume; final dilution: etorphine 40 μg ml^{-1}); administered at an initial rate of 1 ml kg^{-1} hour^{-1} administered via the right cephalic cannula. A deep-pain response was assessed by sequentially clamping Vulsellum forceps (first ratchet) just below the coronary band of one of the four claws of the non-dependent thoracic and pelvic limbs for 60 seconds, or until purposeful movement occurred, in an alternating clock-wise fashion every 15 minutes so that each claw was clamped twice at a 60 minute interval. A response was considered positive when there was both a clear increase in autonomic tone (increase in arterial blood pressure, heart rate, and respiratory rate) and purposeful limb withdrawal. The infusion rate of etorphine was increased or decreased by a fixed 20% of the initial infusion rate in the event of a positive or negative response, respectively. A maximum reduction of 80% of the initial etorphine dose rate was allowed. If apnoea (no visible inspiratory effort for longer than 60 seconds) was detected during the maintenance of surgical anaesthesia, a single intravenous bolus of butorphanol (at a ratio of 1:1 to the etorphine immobilisation dose) was administered.

Recovery

All infusions and oxygen supplementation were stopped at 120 minutes. All multiparameter monitoring machine leads were removed. All, but the left cephalic venous cannula, were removed. A single subcutaneous dose of meloxicam (0.5 mg kg^{-1}; Metacam 2%; Ingelheim Pharmaceuticals; South Africa) was administered. The impala was transported back to the small partition of the boma via a vehicle. Once in the boma, the impala was placed in sternal recumbency with their head supported by a pile of hay. Ten minutes after the end of the etorphine-ketamine-medetomidine infusion a single dose of atipamezole (ratio of 5:1 of the medetomidine total dose; Atipamezole 5%; V-Tech Pharmacy; South Africa) was administered into the gluteal muscle. Five minutes after atipamezole administration, a single intravenous bolus of naltrexone (ratio of 20:1 of the etorphine total dose; Trexonil 5%; Wildlife Pharmaceuticals; South Africa) was administered via the cephalic venous cannula, which was subsequently removed post injection. The ET-tube was removed at first swallowing attempt. At that time, the blindfold and ear plugs were removed and the impala left to recover alone. Recovery time and quality score (**Table 3.1**) were recorded. Once ambulatory, impala were allowed to reunite with the herd. Impala were visually monitored for 24 hours post recovery.

Statistics

Daily barometric pressure readings were obtained from the portable blood gas analyser. These measurements, as well as those obtained from the arterial blood - and respiratory gas analysis, were used in calculating various oxygenation - and ventilation indices (**Table 3.2**).

Data were assessed for normality by calculating descriptive statistics, plotting histograms, and performing the Anderson-Darling test for normality. Quantitative data were described using the median and interquartile range and analysed using nonparametric statistical methods if one or more outcomes violated the normality assumption. Changes over time in quantitative outcomes were assessed using Friedman tests followed by pairwise Wilcoxon signed rank tests using Bonferroni correction of p values for multiple post hoc comparisons. Correlation between variables was assessed using Spearman's rho. Data were analysed in commercially available software (MINITAB Statistical Software, Release 13.32, Minilab Inc, State College, Pennsylvania, USA and IBM SPSS Statistics Version 22, International Businesses Machines Corp., Armonk, NY, USA) and significance determined at $p < 0.05$.

Table 3.2 Equations used to calculate the oxygenation and ventilation indices in impala (*Aepyceros melampus*) undergoing an etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion of 120 minute duration.

Name	Equation	Normal value range
	Oxygenation indices	
P:F ratio; or arterial oxygen tension to fractional inspired oxygen ratio	Ratio = PaO_2/FiO_2[a]	>300 mmHg
Respiratory index	Index = $P(A-a)O_2/PaO_2$	<1.0
Alveolar-arterial oxygen tension gradient	$P(A-a)O_2 = PAO_2 - PaO_2$ where $PAO_2 = FiO_2(Pbar-PH_2O) - PaCO_2/RQ$[a]	10-25 mmHg (room air)
Arterial-alveolar oxygen tension ratio	Ratio = PaO_2/PAO_2	0.8-0.9
	Ventilation indices	
Dead-space to tidal volume ratio	$Vd/Vt = (P_E´CO_2 - PaCO_2)/PaCO2$	20-40%
Arterial to end-tidal carbon dioxide tension gradient	$P(a-et)CO_2 = PaCO_2 - P_E´CO_2$	<15 mmHg

[a]The fractional inspired oxygen (FiO_2) was calculated as follows: the fractional expired oxygen + 5% (normal average pulmonary uptake of oxygen in a resting mammal) (Jacobs et al. 1999).
PaO_2: arterial tension of oxygen; PAO_2: alveolar tension of oxygen; FiO_2: fractional inspiration of oxygen; $P(A-a)O_2$: alveolar to arterial oxygen tension gradient; Pbar: barometric pressure; PH_2O: tension of water vapour; $PaCO_2$: arterial carbon dioxide tension; RQ: respiratory quotient (used a value of 0.8); Vd/Vt: dead-space to tidal volume ratio; $P_E´CO_2$: end-tidal carbon dioxide tension; $PaCO_2$: arterial carbon dioxide tension; $P(a-et)CO_2$: arterial to end-tidal carbon dioxide tension gradient

3.4 Results

The University is situated approximately 1252 meters above sea level; thus the normal barometric pressure ranged from 659 to 673 mmHg (87.9-89.7 kPa). The etorphine-medetomidine immobilisation was characterised by ataxia, increased locomotive activity and eventual recumbency (**Table 3.3**).

Of the ten impala, nine completed the study, while the tenth sustained a comminuted femur fracture darting injury and was humanely euthanised. Three of the remaining nine impala demonstrated severe signs of ataxia and increased locomotive activity that required an additional half-strength dart to achieve recumbency (immobilisation score 2); one of the three demonstrated pronounced excitement and once recumbent made several attempts to stand (immobilisation score 3). Six impala demonstrated no - to mild excitement before becoming recumbent, but once recumbent they did not attempt to stand (immobilisation score 1). Four impala required a single dose of midazolam to relax the jaw enough to allow orotracheal intubation (intubation score 3). The remaining impala could be intubated on either the first (n = 2; intubation score 1) or subsequent attempt (n = 3; intubation score 2). All of the impala experienced increased limb rigidity, stiff abdomen and jerky thoracic cage movement during

spontaneous ventilation efforts within the first hour of the etorphine-ketamine-medetomidine infusion. The initial breathing pattern was generally characterised by prolonged 25 second inspiratory holds followed by three to four large respiratory efforts (apneustic respiration). Three impala required a butorphanol bolus prior to infusion, which resulted in relaxation of the limbs and abdomen as well as improved chest coordination during respiratory efforts. The butorphanol bolus at a 1:1 ratio to etorphine did not provoke arousal.

Table 3.3 Time interval and drug dosage related data reported as median (interquartile range; IQR) for nine impala (*Aepyceros melampus*) that were immobilised with etorphine-medetomidine and underwent an etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion of 120 minute duration.

Parameter	Unit	Median	IQR
Immobilisation			
Time to onset of ataxia	Minutes	4.5	(3.1 – 5.9)
Time to recumbency	Minutes	13.9	(12.0 – 16.5)
Etorphine	mg	2	(2 – 3)
Medetomidine	mg	2.2	(2.2 – 3.3)
Rescue drugs			
Midazolam	mg kg^{-1}	0	(0 – 0.2)
Butorphanol	mg	0	(0 – 2)
Maintenance			
Darting to start of infusion	Minutes	31	(27 – 36)
Ketamine bolus	mg kg^{-1}	1	(1 – 1)
Recovery			
Atipamezole	mg	13	(13 – 13)
Naltrexone	mg	42	(42 – 42)
Time period from atipamezole injection to standing	Minutes	8	(7 – 10)

mg: milligram; mg kg^{-1}: milligram per kilogram; IQR: interquartile range

The etorphine-ketamine-medetomidine total intravenous infusion reliably kept the impala immobile throughout the entire procedure. The impala appeared to remain either unconscious or in a profoundly obtunded state (sluggish to absent palpebral reflexes and absent menace reflex) mostly unresponsive to external stimuli. There was a stepwise downward titration of etorphine in response to deep pain testing in five of the nine impala. One impala had a single deep pain response at the start of the infusion; while the other three impala required multiple stepwise upward titrations to obliterate the deep-pain response (**Figure 3.1**). The four impala that required an increase in titration were not animals that required a second dart. The induction quality did not influence the quality of the TIVA or drug dose

required. The mean arterial blood pressure (> 75 mmHg), heart (between 60 and 120 beats per minute) and respiratory rate (between 8 and 15 breaths per minute) were maintained within clinically acceptable limits, despite the statistically significant correlation of these variables with the etorphine infusion rate.

The heart rate demonstrated a weak positive correlation (p = 0.01; correlation coefficient 0.345) with the etorphine infusion rate used in this study, while the mean arterial blood pressure (p = 0.01; correlation coefficient -0.559) and respiratory rate (p = 0.01; correlation coefficient -0.641) demonstrated a moderate and strong negative correlation, respectively (**Figure 3.2**).

The initial blood gas results (0 minutes) confirmed inadequate cardiopulmonary function manifesting as pronounced hypoxaemia, hypercapnia and mild hyperlactatemia. These results were obtained despite an apparent normal respiratory rate of 10 (8 - 12) breaths minute^{-1}, expiratory tidal volume of 16.4 (15.6
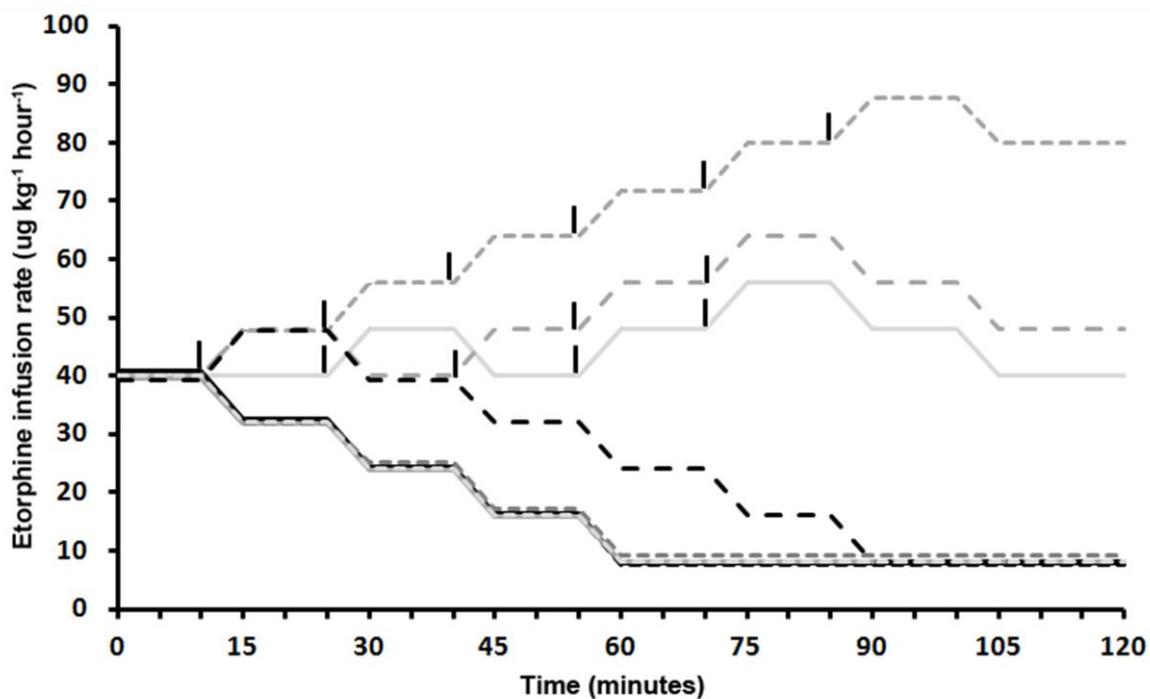
Figure 3.1 Spaghetti plots of the etorphine infusion rate (µg kg^{-1} hour^{-1}) over time in nine impala (*Aepyceros melampus*) undergoing an etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion of 120 minute duration. Infusion rate was either increased or decreased (a fixed 20% change from the initial 40 µg kg^{-1} hour^{-1} infusion rate) in a stepwise manner in accordance to a positive or negative deep-pain response test done at 15 minute intervals, respectively. Solid black vertical bars indicate a positive deep-pain response.

– 21.3) ml kg^{-1}, and cardiovascular function (mean arterial blood pressure and heart rate) for an anesthetised animal. The SpO$_2$ correlated strongly with the arterial oxygen tension (PaO$_2$; p = 0.01; correlation coefficient 0.688). The inefficiency of cardiopulmonary function was highlighted by the profoundly abnormal calculated oxygenation indices and negative value ventilation indices (**Table 3.4**). Once oxygen supplementation was provided, the oxygenation indices significantly worsened over time from the initial critical values, except the arterial oxygen tension to fractional inspired oxygen ratio (p = 0.406) which remained profoundly abnormal. However, the arterial oxygen tension did improve steadily over time compared to initial values (p < 0.001). The impala became progressively acidaemic over time (p < 0.001). Measured electrolytes (sodium, potassium and chloride) were within normal ruminant limits throughout the monitored time period.

Figure 3.2 Respiratory rate (breaths minute^{-1}), heart rate (beats minute^{-1}) and mean arterial blood pressure (mmHg) plotted against the adjusted etorphine infusion rate (µg kg^{-1} hour^{-1}; solid black line) over time. All values reported as pooled median (IQR) at 5 minute intervals for the entire 120 minute etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion in nine impala (*Aepyceros melampus*).

Table 3.4 Physiological and calculated parameters of nine impala (*Aepyceros melampus*) after etorphine-medetomidine immobilisation followed by an etorphine-ketamine-medetomidine total intravenous anaesthesia (TIVA) infusion of 120 minute duration. Parameters reported as median (IQR) at 30 minute intervals for the entire 120 minute duration of infusion.

Parameter	Unit	0 minutes (Start of TIVA)		30 minutes		60 minutes		90 minutes		120 minutes		P value†
Partial pressure of respiratory gases and spirometery												
F_iO_2		0.21	(0.21-0.21)	0.44*	(0.41-0.59)	0.45*	(0.43-0.53)	0.46*	(0.43-0.52)	0.47*	(0.43-0.51)	<0.001
$P_E′CO_2$	mmHg	48.8	(46.8-54.2)	48.2	(44.8-52.8)	48.2	(44.8-53.5)	51.5	(46.1-54.2)	48.1	(45.5-54.8)	0.514
	kPa	6.5	(6.2-7.2)	6.4	(6.0-7.0)	6.4	(6.0-7.1)	6.9	(6.1-7.2)	6.4	(6.1-7.3)	
Vt	ml kg^{-1}	21.3	(18.8-24.5)	21.5	(19.9-24.5)	16.4	(14.3-19.4)	15.1	(14.5-16.9)	15.6	(14.5-17.6)	0.005
Arterial blood gas												
PaO_2	mmHg	41.9	(34.2-52.0)	79.8*	(78.7-88.9)	88.6*	(66.4-105.1)	87.1*	(84.2-99.5)	93.0*	(89.0-106.4)	<0.001
	kPa	5.6	(4.6-6.9)	10.6	(10.5-11.9)	11.8	(8.9-14.0)	11.6	(11.2-13.3)	12.4	(11.9-14.2)	
$PaCO_2$	mmHg	47.9	(47.5-55.3)	64.9*	(53.1-66.5)	65.1*	(63.8-76.5)	69.7	(64.3-84.7)	74.0*	(70.3-78.4)	<0.001
	kPa	6.4	(6.3-7.4)	8.7	(7.1-8.9)	8.7	(8.5-10.2)	9.3	(8.6-11.3)	9.9	(9.4-10.5)	
pH		7.371	(7.355-7.382)	7.352	(7.312-7.394)	7.315*	(7.285-7.352)	7.298*	(7.253-7.338)	7.290*	(7.264-7.335)	<0.001
HCO_3^-	mmol L^{-1}	29.5	(28.0-32.0)	32.8	(31.6-36.4)	34.9*	(33.4-36.2)	36.4*	(33.2-37.0)	35.6*	(35.2-37.1)	0.005
SaO_2	%	75	(63-84)	95*	(90-96)	96*	(91-98)	95*	(94-97)	96*	(95-97)	0.001
Miscellaneous physiological parameters												
SpO_2	%	89	(85-90)	92*	(91-96)	94*	(93-96)	95*	(92-96)	95*	(95-98)	<0.001
Temp	°C	39.2	(38.9-39.8)	38.3*	(38.2-39.0)	37.8*	(37.8-38.1)	37.5*	(37.1-37.7)	37.1*	(36.7-37.3)	<0.001
Lactate	mmol L^{-1}	2.16	(1.90-3.96)	0.62*	(0.58-1.13)	0.40*	(0.33-0.48)	0.30*	(0.30-0.30)	0.3*	(0.3-0.3)	<0.001
Oxygenation and ventilation indices												
PaO_2: F_iO_2	mmHg	**200**	(163-248)	**188**	(167-196)	**176**	(155-201)	**180**	(168-193)	**195**	(174-208)	<0.406
$P(A-a)O_2/PaO_2$		0.6	(0.4-1.1)	**1.4***	(1.2-1.7)	**1.6***	(1.1-2.0)	**1.3***	(1.1-1.7)	**1.2***	(1.0-1.6)	0.002
$P(A-a)O_2$	mmHg	26	(19-28)	**108***	(97-194)	**127***	(98-161)	**121***	(93-149)	**100***	(88-140)	<0.001
PaO_2/PAO_2		**0.63**	(0.48-0.73)	**0.41***	(0.36-0.46)	**0.38***	(0.34-0.47)	**0.43***	(0.37-0.48)	**0.46***	(0.39-0.51)	0.002
Vd/Vt ratio	%	**-2**	(-7-12)	**17**	(13-21)	27*	(24-28)	33*	(26-33)	34*	(32-34)	<0.001
$PaCO_2$- $P_E′CO_2$	mmHg	**-1.1**	(-3.3-5.8)	10.6	(8.2-13.7)	**16.9***	(16.1-19.2)	**22.7***	(18-22.9)	**24.2***	(21.1-27.0)	<0.001
	kPa	**-0.2**	(-0.4-0.8)	1.4	(1.1-1.8)	**2.3**	(2.1-2.6)	**3.0**	(2.4-3.1)	**3.2**	(2.8-3.6)	

†Based on Friedman tests; Medians donated with * are significantly different from time 0 based on Wilcoxon signed rank tests with Bonferroni adjustment of P value multiple post hoc comparisons; **Bold**: profoundly abnormal indices; F_iO_2: fractional inspired oxygen; $P_E′CO_2$: end-tidal carbon dioxide tension; Vt: tidal volume; PaO_2: arterial tension of oxygen; $PaCO_2$: arterial tension of carbon dioxide; pH: arterial hydrogen ion concentration negative logarithm; HCO_3^-: arterial bicarbonate ion concentration; SaO_2: calculated arterial oxygen haemoglobin saturation; SpO_2: peripheral oxygen haemoglobin saturation; Temp: rectal temperature; PaO_2:FiO_2: arterial oxygen tension to fractional inspired oxygen ratio; $P(A-a)O_2/PaCO_2$: respiratory index; $P(A-a)O_2$: alveolar to arterial oxygen tension gradient; PaO_2/PAO_2: arterial to alveolar oxygen tension ratio; Vd/Vt: dead-space to tidal volume ratio; $PaCO_2$- $P_E′CO_2$: arterial to end-tidal carbon dioxide tension gradient; mmHg: millimeters Mercury; kPa: kilopascal pressure; ml kg^{-1}: milliliters per kilogram; mmol L^{-1}: millimoles per liter; µmol L^{-1}: micromoles per liter

3.5 Discussion

The etorphine-medetomidine combination induced an adequate immobilisation in impala. The etorphine-ketamine-medetomidine infusion provided a total intravenous anaesthesia that was adequate for field procedures if the infusion rate is titrated to clinical effect. Despite apparent normal cardiopulmonary function determined by basic monitoring techniques (heart rate, respiratory rate and SpO_2), more invasive monitoring highlighted critical physiological derangements (hypoxaemia, hypercapnia and acidosis) that warrant precaution when using this protocol in the field.

The transition between a profound obtunded state and overt unconsciousness is difficult, or even impossible, to scientifically determine (Alkire et al. 2008). This dilemma occurred in the impala, where the loss of consciousness was considered to be the point whereby there was a lack of conscious limb withdrawal reflex in response to deep-pain stimuli. The data suggest that the impala were, at the least, in a profoundly obtunded (immobile and presumed unconscious) cataleptic state, mostly unresponsive to deep-pain stimulation (analgesia) which was considered surgical anaesthesia adequate for moderately painful procedures in the field. The unpredictable nature of either the response to noxious stimulation or to the pharmacodynamic response to etorphine made calculating a minimum infusion rate a challenge. Thus, the etorphine infusion should be set to an initial rate of 40 µg kg^{-1} hour^{-1} and titrated to clinical effect. Etorphine was not infused alone; ketamine and medetomidine were co-administered at sub-anaesthetic analgesic dose rates (Kästner 2007). Ketamine likely contributed to the obtunded state. However, its inclusion was to improve the somatic analgesia properties of the infused combination (Dzikiti 2013). Prolonged ketamine infusions should be administered with caution as its hepatic biotransformation results in active metabolites (norketamine) that may contribute to an inferior recovery quality including prolonged recovery, increase ataxia, myoclonus and seizure-like activity (Kästner 2007; Dzikiti 2013). However, the low-dose ketamine infusion over 120 minutes in the impala did not cause any undesirable recovery characteristics. Medetomidine is a reliable sedative, central acting muscle relaxant and potent analgesic (Kästner 2006). It was co-administered to improve the sedation quality, provide muscle relaxation and additional analgesia (Kästner 2006; Kästner 2007). However, the medetomidine dose administered in the dart and during infusion did not provide adequate muscle relaxation for the initial 60 minutes of the infusion. The initial muscle rigidity was attributed mainly to the effects of etorphine used in the dart combination. The ketamine-medetomidine infusion was administered in an attempt to decrease the etorphine infusion rate. However, the erratic titration of the

etorphine infusion rate in four of the impala indicates that perhaps the ketamine-medetomidine infusion was not reliably etorphine sparing. Or perhaps, some of the impala are less affected by etorphine or developed either an increased hepatic biotransformation capacity or opioid tolerance due to frequent exposure to potent opioids during the previous studies, despite an apparently adequate washout period (Haigh 1990).

The advantages of the etorphine-ketamine-medetomidine protocol used include: reliable immobility; an easy infusion can be achieved by mixing the drugs into an isotonic crystalloid infusion bag via a 60 drop administration set; infusion can be titrated to clinical effect; etorphine and medetomidine could be antagonised (naltrexone and atipamezole, respectively) resulting in a rapid and predictable recovery. Basic monitoring techniques, including SpO_2, heart and respiratory rate, revealed apparent normal cardiopulmonary function during anaesthesia. However, more invasive monitoring highlighted critical physiological derangements (hypoxaemia, hypercapnia, hyperlactatemia) that warrant precaution when using this protocol in the field. Other disadvantages of this protocol include: limb rigidity and stiff abdominal and jerky thoracic respiratory movements; mandatory oxygen support; and possible rescue butorphanol administration which may result in arousal if incorrect ratios are administered (Meyer 2008b). These findings concur with reports detailing long-term intramuscular etorphine-based immobilisation in other ungulate species (Wolf et al. 1973; Haigh 1990; Ancrenaz et al. 1996).

Hypoxaemia, hypercapnia, acidosis and hyperlactatemia have been described in etorphine immobilised ungulates (Portas et al. 2003; Meyer et al. 2008b; Risling et al. 2011; Evans et al. 2012). The pathophysiology of the blood gas derangements has been attributed to opioid-induced respiratory depression (hypoventilation). Opioid-induced respiratory depression is an inconsistently defined and ambiguous term used in the literature (Ko et al. 2003). Hypoxaemia and hypercapnia may develop under various other conditions not directly related to hypoventilation. Hypoxaemia can result from ventilation-perfusion mismatch, a right-to-left shunt, diffusion impairment and increased oxygen consumption. Hypercapnia may result from an increased production of carbon dioxide due to increased metabolism, increased dead-space ventilation, or large right-to-left shunting (Mills 2001; Magnusson & Spahn 2003; Armstrong et al. 2007; Ng & Swanevelder 2010; Ray et al. 2014). Therefore, the ambiguous term opioid-induced respiratory depression does not explain the pathophysiological processes leading to hypoxaemia and hypercapnia other than hypoventilation.

Contrary to the current evidence in the literature, impala in the present study had an apparently adequate minute volume described as a normal respiratory rate and expiratory tidal volume for an anesthetised animal (Haigh 1990; Heard et al. 1990; Ancrenaz et al. 1996; Buss & Meltzer 2001; Meyer et al. 2008b; Risling et al. 2011; Evans et al. 2012). However, impala had drug-induced hypoxaemia, hypercapnia, acidosis and hyperlactatemia. Invasive monitoring techniques are required to decisively quantify and qualify the ventilation-perfusion mismatch. However, based on less invasive oxygenation- and ventilation indices the findings tend to challenge current thoughts on the mechanism of drug-induced hypoxaemia and hypercapnia (Whiteley et al. 2002; Tang et al. 2005; Kathirgamanathan et al. 2009). The calculated P:F ratio (<200 mmHg), arterial-alveolar oxygen tension ratio ($PaO_2/PAO_2 < 0.75$) and alveolar-arterial oxygen tension gradient ($P(A-a)O_2 > 25$ mmHg) were profoundly abnormal and all suggest a right-to-left shunt fraction of larger than 20% (Theodore et al. 2013). The wide arterial to end-tidal carbon dioxide tension gradient ($PaCO_2-PE´CO_2$) are typical of either dead-space ventilation or severe right-to-left shunting (Yamauchi et al. 2011).

The right-to-left shunt fraction, which is a plausible co-aetiology explaining the hypoxaemia and hypercapnia, could be caused by three mechanisms in the impala: severe atelectasis (intrapulmonary right-to-left shunting) (Magnusson & Spahn 2003); pulmonary hypertension (Vodoz et al. 2009); and intracardiac right-to-left shunting (Devendra et al. 2012). Atelectasis is a common phenomenon in patients under heavy sedation or general anaesthesia. Bronchial obstruction may have been caused by either a mucous plug (increased mucous production by ketamine) or saliva (etorphine and ketamine effect and ruminants salivate continuously) deposits prior to tracheal intubation. These obstructions cause progressive distal airway collapse (atelectasis) (Duggan & Kavanagh 2005). Compressive non-obstructive atelectasis is also a likely cause of the intrapulmonary right-to-left shunt. Under etorphine immobilisation the impala experienced pronounced abdominal and thoracic cage stiffness likely from etorphine-induced catatonia. The stiff abdomen (no to mild bloat detected; abdominal muscles were very tense) and the displacement of the abdominal organs from recumbency likely increased intra-abdominal pressure pushing the diaphragm cranial, decreasing the functional residual capacity of the lung field (an independent change without affecting the tidal volume), and perhaps even approaching the lung closing volume (a fixed volume where the small airways begin to collapse) (Mills 2001). The suspected decrease in functional residual capacity is a major contributor to the intrapulmonary right-to-left shunt. Oxygen insufflation was provided, which increased the inspired oxygen to approximately

40%. Increased oxygen concentration in the alveoli causes them to either partially or completely collapse (absorption atelectasis) (Mills 2001; Duggan & Kavanagh 2005; Lin et al. 2014). Pulmonary hypertension is a documented sequela of etorphine and medetomidine administration (Kästner 2006; Meyer et al. 2015). The hypertension is thought to be partly due to a drug-induced increase in pulmonary vascular resistance. The mechanism of non-intracardiac right-to-left shunting in healthy lungs suffering precapillary hypertension is largely unknown (Vodoz et al. 2009). The only slight increase in oxygenation in response to oxygen supplementation support a large right-to-left shunt as a co-etiology.

The hypoxaemia may be further explained by other co-aetiologies such as an increase in oxygen consumption, drug-induced diffusion impairment and delayed administration of oxygen support. Oxygen consumption determined by an increase in oxygen extraction ratio, although not measured in this study, has been reported to be a sequel of medetomidine administration (Kästner 2006). The mechanism is thought to be due to the decrease in cardiac output (due to the initial increase in systemic vascular resistance and bradycardia) which decreases oxygen delivery and increases the blood circulating time. Thus there is more time for oxygen to be extracted (more than the normal 30%) (Kästner 2006). This effect widens the alveolar capillary arterial-venous oxygen tension gradient, thus increasing the time required for oxygen to diffuse across the membrane and reach optimal oxygen-haemoglobin saturation. Drug induced diffusion (medetomidine and etorphine) impairment may be caused by an increase in speed of blood moving through the alveolar capillaries due to drug-induced pulmonary vasoconstriction (Meyer et al. 2010; Meyer et al. 2015). The rapid movement of blood decreased the time for gas diffusion and equilibration in the alveolar capillaries, oxygen being less soluble than carbon dioxide is thus affected first. The impairment could also be due to an increase in the diffusion distance between alveolar air and capillary blood. Medetomidine (Kästner 2006) and etorphine (Meyer et al. 2015), has through various proposed mechanisms (e.g., pulmonary hypertension or alveolar macrophage degranulation), increased the diffusion distance by fluid shift into the alveolar space, diluting surfactant and promoting atelectasis in ruminants. Another plausible reason for hypoxaemia is the delay between the impala becoming recumbent and the initiation of oxygen support (average of 20 minutes). The stiff abdomen and thorax may have profoundly decreased the functional residual capacity of the lung field thus hasting the depletion of oxygen reserve (Sirian & Wills 2009). The increased locomotive activity prior to recumbency may have increase oxygen consumption and created an oxygen debt.

The hypercapnia in the impala could be explained by the well documented opioid-induced respiratory depression (hypoventilation) (Buss & Meltzer 2001; Meyer et al. 2010). However, measures of the tidal volume and respiratory rate indicate that these animals' ventilation was adequate. The increased muscle activity could have caused an increase in metabolism and therefore increased the production of carbon dioxide. Of interest, the wide arterial to end-tidal carbon dioxide tension gradient may have also been due to the increased fraction of inspired oxygen (Yamauchi et al. 2011).

The initial negative ventilation indices at time 0 are of interest and may be explained by a high cardiac output and decreased functional residual capacity state, as reported in pregnant women and malignant hyperthermia (Kwetny & Finucane 2006; Lin et al. 2014).

These findings highlight the need to look beyond the well-defined respiratory centre depressive effects of the potent opioids and explore other drug-induced cardiopulmonary co-aetiologies that may contribute and even worsen the proverbial opioid-induced respiratory depression. In healthy impala these co-aetiologies appeared not to adversely affect the animal's welfare and low flow oxygen supplementation improved the arterial oxygen tension. However, in compromised animals these co-aetiologies may cause adverse effects and caution is advised.

3.6 Conclusions

Etorphine-ketamine-medetomidine, following etorphine-medetomidine immobilisation provided a total intravenous anaesthesia infusion protocol for impala under field conditions of up to 120 minutes duration. However, hypoxaemia is of paramount concern and thus oxygen supplementation should be considered mandatory. Hypercapnia and acidosis also occurred and although ventilation may correct these, other pharmacological interventions which not only stimulate ventilation but also correct other cardiopulmonary co-aetiologies should be considered. Thus further research is warranted to develop safer field anaesthetic protocols that may include other TIVA drug combinations or techniques using novel delivery of volatile inhalation anaesthetic agents. Antagonism of etorphine and medetomidine using naltrexone and atipamezole, respectively, induced a rapid, calm recovery.

CHAPTER 4

Blood acid-base status in impala (*Aepyceros melampus*) immobilised and maintained under total intravenous anaesthesia using two different drug protocols

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4.1 Abstract

In mammals, homeostasis and survival are dependent on effective trans-membrane movement of ions and enzyme function, which are labile to extreme acid-base changes, but operate efficiently within a narrow regulated pH range. Research in patients demonstrating a pH shifts outside the narrow regulated range decreased the cardiac output and systemic vascular resistance and altered the oxygen binding to haemoglobin. These cardiopulmonary observations may be applicable to the risks associated with anaesthesia and performance of wildlife ungulates on game farms. The aim of this study was to compare blood pH changes over time in impala immobilised and anaesthetised with two different drug protocols (P-TMP - immobilisation: thiafentanil-medetomidine; maintenance: propofol-ketamine-medetomidine; P-EME – immobilisation: etorphine-medetomidine; maintenance: etorphine-ketamine-medetomidine). Additionally, we discuss the resultant blood pH using both the Henderson-Hasselbalch and the Stewart approaches. Two data collection time points were defined, Time 1 before maintenance of general anaesthesia and Time 2 at end of maintenance of general anaesthesia. We hypothesise that blood pH would not be different between drug protocols and would not change over time. Significant differences were detected over time but not between the two drug protocols. Overall, the blood pH decreased over time from 7.37 ±0.04 to 7.31 ±0.05 (p = 0.001). Overall, over time arterial partial pressure of carbon dioxide changed from 51.3 ±7.5 mmHg to 72.6 ±12.4 mmHg (p < 0.001); strong ion difference from 44.6 ±2.4 mEq L^{-1} to 46.9 ±3.1 mEq L^{-1} (p < 0.001); anion gap from 15.0 ±3.1 mEq L^{-1} to 10.9 ±2.2 mEq L^{-1} (p < 0.001); and total weak acids from 16.1 ±1.2 mmol L^{-1} to 14.0 ±1.1 mmol L^{-1} (p < 0.001). The bicarbonate changed from 29.6 ±2.7 mEq L^{-1} to 36.0 ±4.1 mEq L^{-1} (p < 0.001); and lactate changed from 2.9 ±1.5 mEq L^{-1} to 0.3 ±0.03 mEq L^{-1} (p < 0.001) over time.

The profound increase in the partial pressure of carbon dioxide that worsened during the total intravenous anaesthesia in both protocols initiated a substantial metabolic compensatory response to prevent severe acidaemia. This compensation resulted in a clinically acceptable mild acidaemic state, which worsened over time but not between the protocols, in healthy impala. However, these important compensatory mechanisms require normal physiological function and therefore when immobilising ill or anorexic wild ungulates their acid-base status should be carefully assessed.

Keywords: Blood pH, impala, *Aepyceros melampus*, immobilisation, general anaesthesia, Henderson-Hasselbalch, Stewart approach

4.2 Introduction

Enzymes are important for metabolism and regulation of organ function and are labile to extreme acid-base changes, but operate efficiently within a narrow regulated pH range (Mitchell et al. 1972; Castilli et al. 2000; Crimi et al. 2012). Research in patients demonstrating a pH shifts outside the narrow regulated range show decreased cardiac output and systemic vascular resistance and altered the oxygen binding to haemoglobin (Mitchell et al. 1972; Crimi et al. 2012; Clarke et al. 2014). These cardiopulmonary observations may be applicable to the risks and success of anaesthesia and production performance of wildlife ungulates on game farms.

Blood pH regulation is complex and involves various buffering systems and compensatory responses that keep the resultant pH within an optimal range for the species of animal (Kellum & Elbers 2009; Dugdale 2010; Hickish & Farmery 2012; Clarke et al. 2014; Muir 2015). Changes in pH are due to a change in the hydrogen ion (H^+) concentration (Kelum & Elbers 2009; Hickish & Farmery 2012).

The traditional Henderson-Hasselbalch approach and the Stewart physicochemical quantitative approach are used to interpret blood pH. The Henderson-Hasselbalch approach relates the blood pH to the constituents of the bicarbonate (HCO_3^-) buffering system ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$) using the following equation (Henderson 1908; Hasselbalch 1916):

$$pH = pKa \text{ of } H_2CO_3 + \log_{10} ([HCO_3^-]/[H_2CO_3])$$

Which has been adapted for clinical application by the following equation (Constable 1999; Constable 2014):

$$pH = pK_1' + \log_{10} ([HCO_3^-]/S.PCO_2)$$

where pK_1' is the equilibrium dissociation constant of carbonic acid = 6.105 at 37.0°C (human); S is the solubility coefficient of carbon dioxide in plasma = 0.0307 [mmol L^{-1}]/mmHg.

The Stewart approach suggests that the HCO_3^- and H^+ represent the effect rather than the cause of acid-base derangements. Furthermore, the Stewart approach is based on the dissociation of water (H_2O) to produce H^+ or hydroxide ions (OH^-) to maintain electrical neutrality within a solution (like blood) where there are independent variables (arterial partial pressure of carbon dioxide [$PaCO_2$], strong ion difference [SID], anion gap [AG], total weak acids [Atot]) and dependent variables (H^+, OH^-, HCO_3^-, CO_3^{2-}, weak acids [HA] and ions [A^-]) which influence the neutrality (Stewart 1978). Any change in the

independent variable will effect a change in the dependent variables to maintain electrical neutrality within the solution. Stewart's theory has led to a revised version of the blood pH equation as follows (Constable 1999; Constable 2014):

$$pH = pK_1' + \log\{([SID^+] - Ka[Atot]/(Ka + 10^{-pH})) / S.PCO_2\}$$

where pK_1' is the equilibrium dissociation constant of carbonic acid; S is the solubility coefficient of carbon dioxide in plasma; Ka is the effective equilibrium dissociation constant of weak acids, the value is species dependent (Ka = 0.8×10^{-7} where pKa = 7.08; calves [Constable 2014]). When using the Stewart approach the veterinarian must measure ($PaCO_2$ using a blood gas analyser) or calculate the independent variables to help interpret the resultant blood pH. Strong ion differences, anion gaps and total weak acid concentrations may be calculated using frequently published equations in the veterinary literature (**Table 4.1**).

There is a growing body of literature that provide reference ranges for the independent variables in domesticated production ungulates, in healthy (Stevens et al. 2009) and diseased states (Berchtold et al. 2005; Constable et al. 2005; Gonzalez et al 2012; Muller et al. 2012; Trefz et al. 2013; Tharwat & Al-Sobayil 2014; Terfz et al. 2015). However, there is a paucity of information regarding ranges of these variables in wildlife ungulates. Furthermore, the effect of various immobilisation and total intravenous anaesthesia protocols on blood pH balance have undoubtedly not been explored. Field ready drug protocols to maintain surgical anaesthesia in wild ungulates is becoming increasingly important, due to the increased demand of completing invasive surgical procedures such as bone fracture repair (Buck et al. 2017; Zeiler et al. 2015). The drug protocol should be made up of commonly available drugs and be easy to administer. Furthermore, the combination should maintain the animal's organ physiology within clinically acceptable ranges to minimise compromising vital organ function (Clarke et al. 2014; Muir 2015; Zeiler et al. 2015).

The aims of this study were to measure and report the blood pH change over time in healthy adult female impala undergoing immobilisation and general anaesthesia using two different drug protocols. We hypothesise that blood pH would not be different between drug protocols and would not change over time. In addition, we aim to discuss the measured blood pH by describing the change in variables described by the Henderson-Hasselbalch and Stewart approaches of interpreting blood pH.

Table 4.1 Calculations used to calculate variables of interest to explain the acid-base balance in healthy impala (*Aepyceros melampus*) undergoing immobilisation and general anaesthesia using two different drug protocols.

Variable	Equation used in study	Equation references	Unit	Ruminant values	Value references
SIDa	$= ([Na^+]+[K^+]+[Ca^{++}])-([Cl^-]+[Lactate])$	(Corey 2003; Clarke et al. 2014; Muir 2015)	$mEq\ L^{-1}$	Calf: 39.3±4.5 Calf: 40.0±2.0	(Muller et al. 2012) (Constable et al. 2005)
SIDe	$= 2.46 \times 10^{pH-8} \times PaCO_2 +$ albumin (g dL^{-1}) $\times (0.123 \times pH - 0.631) +$ phosphate (mEq L^{-1}) $\times (0.309 \times pH - 0.469)$	(Kellum et al. 2009; Clarke et al. 2014; Muir 2015)	$mEq\ L^{-1}$	Calf: 34.8±4.8 Calf: 40.0±2.0	(Muller et al. 2012) (Constable et al. 2005)
SIG	$=$ SIDa-SIDe	(Kellum et al. 2009; Muir 2015)	$mEq\ L^{-1}$	Calf: 0.0±3.0	(Constable et al. 2005)
AG	$= ([Na^+]+[K^+])-([Cl^-]+[HCO_3^-])$	(Stevens et al. 2009; Dugdale 2010; Muller et al. 2012; Clarke et al. 2014)	$mEq\ L^{-1}$	Goat: 20.02±0.5 Goat: 12.62±1.7 Goat: 20.0±3 Goat: 17.1±3.9 Calf: 20.29±4.5	(Castilli et al. 2000) (González et al. 2012) (Tharwat & Al-Sobayil 2014) (Stevens et al. 2009) (Muller et al. 2012)
Atot	$= 2.25 \times$ albumin (g dL^{-1}) $+ 1.4 \times$ globulin (g dL^{-1}) $+ 0.59 \times$ Phosphate (mg dL^{-1})	(Clarke et al. 2014; Muir 2015)	$mmol\ L^{-1}$	Calf: 18.2±2.6 Calf: 19.2±6.1	(Muller et al. 2012) (Constable et al. 2005)

SIDa: apparent strong ion difference; SIDe: effective strong ion difference; SIG: strong ion gap; AG: anion gap; Atot: total weak acids in plasma; Na^+: sodium ion; K^+: potassium ion; Ca^{++}: calcium ion; Cl^-: chloride ion; HCO_3^-: bicarbonate ion; g dL^{-1}: grams per decilitre; mEq L^{-1}: milliequivilent per litre; mg dL^{-1}: milligrams per decilitre; mmol L^{-1}: millimoles per litre.

4.3 Materials and Methods

This study was a part of a larger series of studies exploring the feasibility and cardiorespiratory effects of two different immobilisation and total intravenous anaesthetic protocols (drug protocols) administered for 120 minutes. All studies were approved by the animal ethics and research committees of the University of Pretoria prior to data collection (V099-13 & V012-16). The feasibility and cardiorespiratory outcomes of the two drug protocols are reported elsewhere and their findings are independent of those reported here (Buck et al. 2017; Zeiler et al. 2015). The present study reports on the acid-base status of the impala undergoing the two drug protocols.

Ten adult non-pregnant female impala aged between 12 and 36 months old were enrolled in this prospective cross-over study. The impala were captured from a nearby game farm and transported to the Faculty six weeks prior to the drug trials. They were housed in a purpose built 2.7 meter high walled outdoor enclosure (boma) for the duration of the study. The boma was divided, by an internal wall with swing gates at either end, into a small area used for daily feeding and a larger home area. A six week pre-trial period was used to allow the impala to familiarise themselves with the boma and daily husbandry routine (Zeiler & Meyer 2017c; Chapter 5). The impala received hay (*Erogrostis curvula*), lucerne (*Medecargo sative*) and water *ad libitum*; commercially available antelope pellets (Alzu antelope pellets; Alzu; South Africa; approximately 100g animal^{-1} day^{-1}) were supplemented based on observed body condition.

All impala received two drug protocols (P-TMP & P-EME) on two occasions separated by four weeks:

- P-TMP – Immobilisation: thiafentanil (0.05 mg kg^{-1}; Thianil 1%; Wildlife Pharmaceuticals; South Africa) and medetomidine (0.055 mg kg^{-1}; Medetomidine 1%; Kyron Prescriptions; South Africa); Maintenance: propofol (12 mg kg^{-1} hour^{-1}; Propoven 1%; Intramed, South Africa), ketamine (1.5 mg kg^{-1} hour^{-1}; Ketamine Fresenius 10%; Intramed) and medetomidine (0.005 mg kg^{-1} hour^{-1}; Domitor 0.1%; Zoetis; South Africa) (Buck et al. 2017).

- P-EME – Immobilisation: etorphine (0.05 mg kg^{-1}; Captivon 0.98%; Wildlife Pharmaceuticals) and medetomidine (0.055 mg kg^{-1}); Maintenance: etorphine (0.04 mg kg^{-1} hour^{-1}), ketamine (1.5 mg kg^{-1} hour^{-1}) and medetomidine (0.005 mg kg^{-1} hour^{-1}) (Zeiler et al. 2015).

The impala were immobilised in the same order, on the same day of the week (two impala per day), at approximately the same time of the day, as randomised in the first week of data collection.

All impala were enclosed in the smaller feeding partition of the boma prior to darting. The impala were remotely injected using a filled dart (3 mL air pressurised dart; Dan-Inject; South Africa) containing the immobilisation combination, projected into the muscles of the pelvic girdle via a carbon dioxide powered rifle (set to 5 bar pressure, 12-15 meters darting distance; Dan-Inject; Model JM). Once the dart was placed and fully discharged, a stopwatch was started to record the times to sampling. When the impala was immobilised into a recumbent position without attempts to stand the remaining impala were released into the larger home area of the boma and the immobilised impala was approached. An initial field clinical examination was completed and a cannula was aseptically placed into one of the cephalic veins prior to vehicle transport to the procedure room approximately 650 meters away. Once in the procedure room, the impala was instrumented with a number of monitoring devices to measure cardiorespiratory and temperature parameters throughout the 120 minute total intravenous anaesthesia (Buck et al. 2017; Zeiler et al. 2015). Simultaneously, while placing the monitoring devices, an auricular artery was aseptically cannulated for serial arterial blood sampling and direct arterial blood pressure monitoring. The impala were left to breathe spontaneously throughout the study. If apnoea (no attempt to breathe over a 60 second period) was detected at any time during the procedures, then butorphanol (1:1 potent opioid dose) was administered intravenously (Buck et al. 2017; Zeiler et al. 2015). All impala tracheas were intubated (size 8.0 polyvinyl chloride cuffed endotracheal tube) and received oxygen insufflation (fixed rate of 2 L minute^{-1}) via a nasogastric feeding tube (8 French Gauge; Avacare feeding tube; Sunray Medical; China) placed approximately to the level of the fourth intercostal space. Physiological saline (Sodium Chloride Fresenius 0.9%; Intramed; South Africa) was administered at a fixed maintenance rate of 5 mL kg^{-1} hour^{-1} for the entire 120 minute anaesthesia period.

Data collection of importance to the present study consisted of venous (lateral saphenous; needle and syringe technique; stored in serum tube) and arterial (aspirated from the auricular artery cannula using a pre-heparinised syringe and needle) blood sampling at two distinct time points. Time 1 was immediately prior to the start of the total intravenous anaesthesia infusion and oxygen supplementation, and Time 2 was one minute prior to cessation of total intravenous anaesthesia infusion, and before transporting the impala back to the boma for recovery. The times to sampling (from dart placement until sampling) for the two distinct times were recorded.

The venous sample was allowed to clot prior to centrifugation to separate the serum from the cellular components. The serum was carefully pipetted and stored in cryovials in a -80°C freezer until analysis.

Serum phosphorus, albumin and globulin from the venous sample was analysed using a calibrated bench top serum analyser (Cobas, Integra 400 Plus; Roche Products (Pty) Ltd.; South Africa).

The arterial blood sample was collected and analysed immediately using a calibrated patient side blood gas analyser (EPOC Reader Blood Analysis Analyzer and EPOC BGEM smart cards; Epocal; USA). The blood gas analyser measured the following variables of interest: pH, $PaCO_2$, sodium, potassium, calcium, chloride and lactate, haematocrit and haemoglobin concentration. The base excess (BE) and bicarbonate (HCO_3^-) was calculated based on the analyser's internal algorithm setting for "other" species. All results were interpreted at a fixed body temperature of 37°C (alpha-stat analysis). Rectal temperature (Physitemp Model BAT-12; Physitemp Instruments; USA) was continuously monitored and recorded at the time of blood sampling.

The impala were recaptured and transported back to their source on completion of the series of studies.

Data analysis

Data were assessed for normality by plotting histograms, calculating descriptive statistics and performing the Anderson-Darling test for normality. Variables of interest (electrolytes, arterial carbon dioxide tension, base excess, bicarbonate, lactate, strong ion differences, anion gaps, total weak acids, proteins, haematocrit, haemoglobin concentration and temperature) were compared between protocols and time (both fixed effects) where impala were modelled as a random effect using a general linear mixed model analysis. Independent variables that cause the change in pH over time (partial pressure of carbon dioxide, apparent strong ion difference, anion gap and total weak acids) are presented graphically using box plots and whiskers (Stewart 1978). Correlation between blood pH and variables of interest (bicarbonate ion, partial pressure of carbon dioxide, apparent strong ion difference, anion gap and total weak acids) were assessed using Persons correlation. The times to sampling for the first and second sampling points were compared between protocols using the two-sample t-test. Results reported as mean ± standard deviation (SD). Overall values were reported as mean ± standard deviation of the combined data from both protocols at the two time points. Data were analysed using commercially available statistical software (MiniTab 17.1.0; MiniTab Incorporated; USA) and results interpreted at the 5% level of significance. The main null hypothesis tested was that there would be no difference in blood pH between protocols and over time within a protocol.

4.4 Results

The impala were weighed and the drug doses used for the immobilisation were recalculated on a per kilogram bases. In P-TMP, thiafentanil and medetomidine were dosed at 0.052 ±0.007 and 0.057 ±0.006 mg kg^{-1}, respectively. In P-EME, etorphine and medetomidine were dosed at 0.050 ±0.012 and 0.054 ±0.013 mg kg^{-1}, respectively. Both drug protocols immobilised the impala adequately. Within the first 15 minutes of recumbency butorphanol boluses were administered to eight impala in P-TMP and to three impala in P-EME that developed apnoea. Repeated butorphanol boluses were necessary in most impala receiving P-TMP. All impala were breathing regularly and spontaneously prior to Time 1 and no more butorphanol boluses were required.

One impala receiving P-EME sustained an inoperable comminuted fracture to a femur due to a darting injury and was humanly euthanised. Data collected from this impala were excluded from analysis.

The blood pH significantly decreased over time within both drug protocols (**Table 4.2**; $p = 0.001$), however, there was no significant difference between the two protocols at both the time points ($p = 0.974$; interaction: protocol x time). Overall, the pH changed from 7.37 ±0.04 to 7.31 ±0.05 at Time 1 to Time 2, respectively.

According to the Stewart approach, evaluation of the independent variables responsible for shifts of the hydrogen ion concentration, and thus blood pH, demonstrated statistically significant shifts over time that were of clinical interest (**Figure 4.1** and **Table 4.2**). The $PaCO_2$ ($p < 0.001$) and SIDa ($p < 0.001$) increased, while the AG ($p < 0.001$) and Atot ($p < 0.001$) decreased over time. Yet, there was no significant difference between the two drug protocols for $PaCO_2$ ($p = 0.754$), SIDa ($p = 0.552$), AG ($p = 0.963$) and Atot ($p = 0.860$). Overall, the independent variables changed, as follows: $PaCO_2$ from 51.3 ±7.5 mmHg to 72.6 ±12.4 mmHg; SIDa from 44.6 ±2.4 mEq L^{-1} to 46.9 ±3.1 mEq L^{-1}; AG from 15.0 ±3.1 mEq L^{-1} to 10.9 ±2.2 mEq L^{-1}; and Atot from 16.1 ±1.2 mmol L^{-1} to 14.0 ±1.1 mmol L^{-1} at time 1 to Time 2, respectively.

According to the Henderson-Hasselbalch approach, the $PaCO_2$ (already described above) and serum bicarbonate are the variables of interest. Serum bicarbonate increased ($p < 0.001$) and serum lactate decreased ($p < 0.001$) over time, without significant differences between the two protocols at the time points (serum bicarbonate $p = 0.676$; serum lactate $p = 0.782$). Overall, the serum bicarbonate changed from 29.6 ±2.7 mEq L^{-1} to 36.0 ±4.1 mEq L^{-1}; and serum lactate changed from 2.9 ±1.5 mEq L^{-1} to 0.3 ±0.03 mEq L^{-1} at Time 1 to Time 2, respectively.

Figure 4.1 Box plots and whiskers of the independent variables in healthy impala (*Aepyceros melampus*) thought responsible for the change in hydrogen ion concentration (pH) in the plasma.
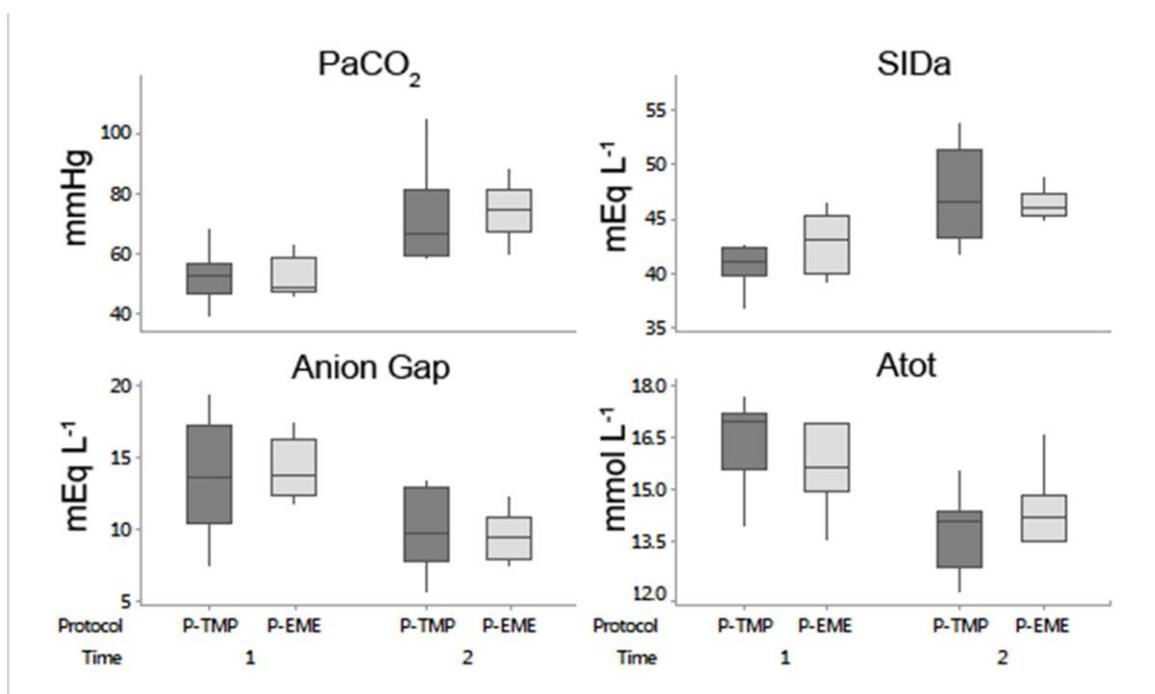
Footnote: Time 1 was sampling after immobilisation with either thiafentanil-medetomidine (P-TMP) or etorphine-medetomidine (P-EME). Time 2 was sampling at the end of either a propofol-ketamine-medetomidine (P-TMP) or an etorphine-ketamine-medetomidine (P-EME) total intravenous infusion. Where: $PaCO_2$ is the arterial partial pressure of carbon dioxide; SIDa is the apparent strong ion difference; Atot is the total weak acid concentration in plasma; mmHg: millimetres mercury; mEq L^{-1}: milliequivence per litre and mmol L^{-1}: millimoles per litre.

The blood pH demonstrated a strong negative correlation to the $PaCO_2$ ($r = -0.824$; $p < 0.001$) and a moderate negative correlation to serum bicarbonate ($r = -0.385$; $p = 0.020$). The blood pH did not correlate to the SIDa ($r = -0.316$; $p = 0.060$) and Atot ($r = 0.164$; $p = 0.341$), respectively, but it did correlate moderately and positively to the AG ($r = 0.413$; $p = 0.012$).

The electrolytes did not change over time, with the exception of potassium ($p < 0.001$) and chloride ($p = 0.046$) which both decreased without a significant difference between the two protocols (potassium p

= 0.192; chloride p = 0.398). Overall, potassium changed from 4.1 ±0.3 mEq L^{-1} to 3.6 ±0.3 mEq L^{-1}; and chloride change from 107.0 ±2.9 mEq L^{-1} to 105.1 ±2.7 mEq L^{-1} at Time 1 to Time 2, respectively.

The haematocrit ($p < 0.001$) and haemoglobin concentration decreased ($p < 0.001$) over time, without significant differences between the two protocols at the time points (haematocrit p = 0.856; haemoglobin concentration p = 0.944). Overall, the haematocrit changed from 0.29 ±0.03 L L^{-1} to 0.19 ±0.02 L L^{-1}; and haemoglobin concentration changed from 9.7 ±1.1 g dL^{-1} to 6.4 ±0.8 g dL^{-1} at Time 1 to Time 2, respectively.

The serum proteins, albumin (p = <0.001) and globulin (p = 0.003) significantly decreased over time with a significant differences between the two protocols for albumin (p = 0.036; interaction: protocol x time), yet, not for globulin (p = 0.092; interaction: protocol x time). Overall, the albumin changed from 4.3 ±0.3 g dL^{-1} to 3.6 ±0.2 g dL^{-1}; and globulin changed from 1.7 ±0.3 g dL^{-1} to 1.5 ±0.2 g dL^{-1} at Time 1 to Time 2, respectively.

Table 4.2 Measured and calculated values obtained from healthy impala (*Aepyceros melampus*) undergoing immobilisation and general anaesthesia using two different drug protocols.

Variable	Unit	Time 1				Time 2				P value
		P-TMP		P-EME		P-TMP		P-EME		
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Times to sampling (from dart placement until sampling)										
Times to sampling	min	16.8	±7.0	19.2	±5.6	150.4	±5.7	151.8	±6.3	
T-test P value			P = 0.442				P = 0.613			
Basic clinical parameters at time of sampling										
Heart rate	Beats min^{-1}	122	±40	79	±37	57	±9	59	±11	<0.001
Resp rate	Breaths min^{-1}	9	±5	9	±2	10	±2	10	±2	0.316
MAP	mmHg	126	±14	117	±18	102	±12	90	±19	0.001
Temperature	°C	38.9	±0.4	39.3	±0.2	37.0	±0.2	37.0	±0.2	<0.001
Arterial blood acid base analysis										
pH	N/A	7.36	±0.04	7.38	±0.04	7.31	±0.02	7.30	±0.05	0.001
HCO_3^-	mEq L^{-1}	29.0	±2.7	30.1	±2.9	36.0	±5.9	35.9	±1.5	<0.001
	mmol L^{-1}	29.0	±2.7	30.1	±2.9	36.0	±5.9	35.9	±1.5	
BE	mEq L^{-1}	3.6	±2.4	4.9	±3.1	9.8	±6.1	9.5	±1.5	<0.001
	mmol L^{-1}	3.6	±2.4	4.9	±3.1	9.8	±6.1	9.5	±1.5	
Lactate	mEq L^{-1}	3.0	±1.6	2.9	±1.4	0.3	±0.0	0.3	±0.0	<0.001
	mmol L^{-1}	3.0	±1.6	2.9	±1.4	0.3	±0.0	0.3	±0.0	
Electrolytes										
Na^+	mEq L^{-1}	145.8	±1.3	146.9	±2.3	146.4	±2.4	148.2	±2.4	0.170
	mmol L^{-1}	145.8	±1.3	146.9	±2.3	146.4	±2.4	148.2	±2.4	
K^+	mEq L^{-1}	4.2	±0.2	4.1	±0.4	3.6	±0.1	3.6	±0.3	<0.001
	mmol L^{-1}	4.2	±0.2	4.1	±0.4	3.6	±0.1	3.6	±0.3	
Ca^{++}	mEq L^{-1}	1.1	±0.1	1.1	±0.1	1.1	±0.1	1.1	±0.1	0.307
	mmol L^{-1}	0.55	±0.05	0.55	±0.05	0.55	±0.05	0.55	±0.05	
Cl^-	mEq L^{-1}	107.4	±3.1	106.6	±2.8	103.9	±2.6	106.3	±2.4	0.046
	mmol L^{-1}	107.4	±3.1	106.6	±2.8	103.9	±2.6	106.3	±2.4	
P^-	mEq L^{-1}	2.2	±0.4	2.2	±0.5	2.0	±0.5	2.2	±0.5	0.606
	mmol L^{-1}	2.2	±0.4	2.2	±0.5	2.0	±0.5	2.2	±0.5	

Table 4.2 (continued) Measured and calculated values obtained from healthy impala (*Aepyceros melampus*) undergoing immobilisation and general anaesthesia using two different drug protocols.

Variable	Unit	Time 1				Time 2				P value
		P-TMP		P-EME		P-TMP		P-EME		
		Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
		Proteins								
Albumin	g dL^{-1}	4.4	±0.3	4.2	±0.3	3.6	±0.2	3.7	±0.1	<0.001
Globulin	g dL^{-1}	1.8	±0.3	1.6	±0.3	1.5	±0.3	1.4	±0.2	0.003
Haematocrit	L L^{-1}	0.29	±0.03	0.28	±0.03	0.18	±0.02	0.20	±0.03	<0.001
Haemoglobin	g dL^{-1}	9.98	±0.86	9.42	±1.22	6.12	±0.72	6.72	±0.88	<0.001
		Independent variables affecting pH								
PaCO$_2$	mmHg	51.5	±8.6	51.2	±6.7	71.4	±15.6	73.8	±9.1	<0.001
SIDa	mEq L^{-1}	40.7	±1.9	42.7	±2.7	47.0	±4.3	46.2	±1.4	<0.001
	mmol L^{-1}	40.1	±1.9	42.1	±2.7	46.4	±4.3	45.7	±1.6	
SIDe	mEq L^{-1}	34.1	±3.0	35.1	±3.6	40.4	±6.0	40.8	±2.0	<0.001
	mmol L^{-1}	34.1	±3.0	35.1	±3.6	40.4	±6.0	40.8	±2.0	
SIG	mEq L^{-1}	6.6	±3.3	7.5	±1.8	6.5	±3.2	5.5	±2.2	0.243
	mmol L^{-1}	6.0	±3.2	7.0	±1.4	6.0	±3.2	5.0	±2.2	
AG	mEq L^{-1}	13.5	±4.0	14.3	±2.1	10.2	±2.8	9.5	±1.7	<0.001
	mmol L^{-1}	13.5	±4.0	14.3	±2.1	10.2	±2.8	9.5	±1.7	
Atot	mmol L^{-1}	16.4	±1.2	15.7	±1.2	13.8	±1.1	14.3	±1.0	<0.001

Time1: sampling prior to maintenance of general anaesthesia; Time 2: sampling one minute prior to ending general anaesthesia; P-TMP: protocol using thiafentanil-medetomidine immobilisation and propofol-ketamine-medetomidine infusion for general anaesthesia maintenance; P-EME: protocol using etorphine-medetomidine immobilisation and etorphine-ketamine-medetomidine infusion for general anaesthesia; P value: level of significance estimated over time; min: minute; Resp rate: respiratory rate; MAP: direct mean arterial blood pressure; HCO$_3^-$: bicarbonate ion; BE: base excess; Na$^+$: sodium ion; K$^+$: potassium ion; Ca^{++}: calcium ion; Cl$^-$: chloride ion; P$^-$: phosphorus ion; PaCO$_2$: arterial partial pressure of carbon dioxide; SIDa: apparent strong ion difference; SIDe: effective strong ion difference; SIG: strong ion gap; AG: anion gap; Atot: total weak acids in plasma; mmHg: millimetres mercury; g dL^{-1}: grams per decilitre; mEq L^{-1}: milliequivilent per litre; mg dL^{-1}: milligrams per decilitre; mmol L^{-1}: millimoles per litre.

4.5 Discussion

The initial blood pH, after immobilisation, indicated a mild acidaemia (normal ruminant arterial blood pH reference range 7.37 to 7.48 [McDonell & Kerr 2015]) due to the elevated $PaCO_2$ causing a respiratory acidosis. Thereafter, the $PaCO_2$ increased further and blood pH of the impala significantly decreased over time, regardless of the immobilisation and anaesthetic protocol used. The Henderson-Hasselbalch approach and the quantitative physicochemical Stewart approach were used to interpret the acid-base status of the impala. The profoundly elevated $PaCO_2$ at the end of the anaesthesia would cause a respiratory acidosis, while the rising apparent strong ion difference (SIDa) and waning total weak acids (Atot) contributed to a simultaneous occurring metabolic alkalosis. Furthermore, the progressive elevation of the calculated serum bicarbonate (HCO_3^-) and base excess (BE) both indicate an emerging compensatory metabolic response. Therefore the resultant pH values at the end of the anaesthesia are because of a pronounced metabolic compensatory response to the severe respiratory acidosis that resulted in an overall mild acidaemia. Because there are no published reference ranges for acid-base variables in resting impala we used ranges from closely related species (healthy awake goats and calves; **Table 4.1**) to interpret our findings.

Unfortunately, more advanced techniques used to calculate compensation, like expected compensatory changes in $PaCO_2$ or bicarbonate ion concentrations, would be difficult to use for interpretation due to the paucity in referenced normal ranges for small wild ungulates. Although the impala were habituated to the boma, they were not tame enough for us to obtain awake control or reference samples. Gaining such samples from an awake wild animal can only be achieved by using remote sampling devices (Hattingh et al. 1988). Such devices are not readily available and need to be custom made per species (Cook et al. 2000).

We expected respiratory acidosis to be pronounced, especially at the end of the anaesthesia, as $PaCO_2$ was greatly elevated compared to the normal awake range of 35-45 mmHg in mammals (Muir 2015). The $PaCO_2$ at the end of the anaesthesia was substantially higher compared to just after induction into immobilisation in our impala, and compared to values measured in other immobilised impala ($PaCO_2$ of 39.1 ±3.4 to 41.3 ±5.0 mmHg) (Bush et al. 2004). One of the stimuli to take a breath in a healthy awake animal is brought about by the rising $PaCO_2$ level reaching a threshold. The drugs used in this study, especially the potent opioids, either alone or in combination with the other anaesthetic and sedative drugs, are known to cause respiratory-neuronal depression (Buss & Meltzer 2001; Meyer et al. 2015)

which shifts the carbon dioxide respiratory response curve to the right (Ko et al. 2003), whereby a higher threshold level of $PaCO_2$ is required to stimulate the respiratory centre to initiate a breath. Therefore these drugs ultimately result in hypoventilation (decreased alveolar minute ventilation) which causes the increase in $PaCO_2$. Ventilation is challenging to assess when only subjectively monitoring the respiratory system by counting the respiratory rate and estimating the tidal volume. Often an animal will appear to be ventilating normally, as in the case of these impala that had a normal respiratory rate and tidal volume at Time 1, after dosing with butorphanol (data reported elsewhere) (Buck et al. 2017; Zeiler et al. 2015), but on closer examination this may not be the case. Thus, more invasive monitoring tools, such as arterial blood gas analysis or capnography, may be required to detect shifts in blood pH that are due to alterations in ventilation (Haskins 2015). Furthermore, other co-aetiologies should always be considered when there is an obvious respiratory acidosis without overt evidence of hypoventilation (reduced minute volume), such as severe right-to-left pulmonary shunting, large dead-space ventilation or ventilation-perfusion mismatch (Meyer et al. 2015; Muir 2015; Zeiler et al. 2015). Haemoglobin is an important intracellular buffer that will bind reversibly to either carbon dioxide or to the hydrogen ion formed by the bicarbonate buffer system, to transport them from the metabolising tissues to the lungs (Whiteley et al. 2002; Dugdale 2010; Yamauchi et al. 2011; Clarke et al. 2014; Muir 2015). With oxygen supplementation an increase in the PaO_2 will increase the force for oxygen to bind to haemoglobin as opposed to carbon dioxide or hydrogen ions (Haldane Effect; high PaO_2 levels decrease the buffering effects of haemoglobin, therefore hydrogen ions are unbound from haemoglobin to preferentially transport oxygen). Therefore, during anaesthesia, the $PaCO_2$ in the impala most likely increased due to the oxygen supplementation (Whiteley et al. 2002; Dugdale 2010; Yamauchi et al. 2011; Clarke et al. 2014; Muir 2015). Furthermore, the haemoglobin concentration (and haematocrit) dropped during general anaesthesia (Boscan et al. 2005), a known phenomenon in patients under general anaesthesia, especially if alpha$_2$-adrenoceptor agonists like medetomidine are used (Kästner 2006), therefore decreasing an important plasma buffering system which could have also contributed to the increase in $PaCO_2$ and hydrogen ion concentration. The emergency treatment of apnoea with butorphanol did result in a regular spontaneous breathing pattern in the impala immobilised with both protocols (Buck et al. 2017; Zeiler 2015). A limitation to this study is that we did not take arterial blood samples immediately after recumbency, while apnoeic episodes occurred, especially in P-TMP which required more frequent butorphanol boluses compared to P-EME. Therefore, the effects of butorphanol on the

blood pH could not be determined. However, in another etorphine immobilized ungulate, the goat, butorphanol corrected hypoxaemia but not hypercapnia and therefore it may be that it had little influence on blood pH in the impala at Time 1 (Haw et al. 2016b).

Irrespective of the cause of the respiratory acidosis, metabolic compensation, indicated by the significant rise in the bicarbonate ion concentration, occurred within a 120 minutes. This indicator of compensation is according to the traditional Henderson-Hasselbalch approach used to evaluate blood pH, whereby the body attempts to correct the increased hydrogen ion concentration by elevating the bicarbonate ion concentration to normalise the bicarbonate ion to carbonic acid ratio ($[HCO_3^-]$:$[H_2CO_3]$ ratio) back to 20:1 (Clarke et al. 2014). The $PaCO_2$ corresponds to H_2CO_3 and is merely substituted to simplify the calculation of the compensatory response (Constable 1999; Bush et al. 2004; Gonzalez et al. 2012). Therefore, any rise in $PaCO_2$ should be met by a rise in the HCO_3^- ions in uncomplicated respiratory acidosis, as demonstrated in these impala.

A simple change in the HCO_3^- does not completely explain the acid-base compensation that occurred in the impala. The apparent strong ion difference (SIDa) was higher and the total weak acids (Atot) were lower than that of published ranges for healthy control goats and calves. Both changes indicate an additional non-respiratory alkalinising effect (Kellum & Elbers 2009; Hickish & Farmery 2012; Constable 2014; Muir 2015). The measured electrolytes (sodium, potassium, calcium, chloride and phosphorus) were within accepted published ranges for impala (Karesh et al. 1997). Furthermore, all but the potassium and chloride concentration did not significantly change over time. Yet the decrease in the potassium level was not large enough to solely explain the increased apparent strong ion difference (SIDa) value. Therefore, the decrease in lactate ion concentration that occurred most likely contributed the most to the increased apparent strong ion difference (SIDa) at the 120 minute measurement. The drop in the chloride concentration could have been due to the increase in plasma bicarbonate, whereby the plasma attempts to maintain electrical neutrality by excreting chloride (Constable 1999; Corey 2003; Kellum & Elbers 2009; Hickish & Farmery 2012; Muir 2015). At the rate of administration used it is unlikely that the infused physiological saline increased sodium or chloride concentrations in the plasma of the impala, as reported in healthy dogs (West et al. 2013). The decreased total weak acids (Atot) over time was attributed to the decrease in albumin and globulin concentrations. A decrease in plasma protein levels has been described in animals undergoing general anaesthesia (Boscan et al. 2005), especially when alpha$_2$-adrenoceptor agonists such as medetomidine are administered (Kästner 2006).

The total weak acids (Atot) levels reported in this study were also lower compared to goats and calves. This difference could be attributed to different measurements of phosphorus, or different calculations used to determine the total weak acids (Atot). Furthermore, the rising bicarbonate ion concentration could cause the negatively charged proteins to move out of the plasma in order to maintain electrical neutrality, a plausible theory requiring further confirmation.

The decrease in the anion gap (AG) over time was attributed to the pronounced increase in the HCO_3^- concentration. Overall the anion gap (AG) was substantially lower than those reported for goats and calves (Stevens et al. 2009; Gonzalez et al. 2012; Muller et al. 2012; Tharwat & Al-Sobavil 2014; Trefz et al. 2015). However, the HCO_3^- concentrations and BE at the end of the anaesthesia were higher than a generally accepted upper limit of 30 mmol L^{-1} and 6.0 to 8.0 mmol L^{-1}, respectively for herbivores (Dugdale 2010; Clarke et al. 2014; Muir 2015) and the published ranges for healthy goats (Stevens et al. 2009; Gonzalez et al. 2012; Tharwat & Al-Sobavil 2014) and calves (Muller et al. 2012; Trefz et al. 2015). Both of these variables demonstrate that a metabolic compensatory response was initiated to correct the respiratory acidosis in the impala.

The worsening acidaemia, and precipitous drop in serum protein concentrations, especially albumin, may alter ionisation and protein binding of drugs, which could have profound effects on drug pharmacokinetics and dynamics (Ascenzi et al. 2014). These possible alterations warrant further investigation to gain better clarity of their clinical implications. In other words, did the impala in this study experience more pronounced respiratory depression due to alterations in the pharmacokinetics and dynamics of the drugs used to maintain general anaesthesia by causing a relative overdose?

Furthermore, the clinical implications of acidosis, regardless of cause, are serious and warrant careful consideration. The effects of acidosis on the cardiovascular system include negative inotropy, tachycardia and vasodilation which translates into a decreased blood pressure due to the reduction in cardiac output (decreased stroke volume) and systemic vascular resistance (Mitchell et al. 1972; Crimi et al. 2012). Oxygen binding to haemoglobin is altered, causing a right shift in the oxygen-haemoglobin dissociation curve (Bohr Effect; acidaemic plasma pH, usually brought about by increasing $PaCO_2$ levels at metabolically active tissue decrease haemoglobins affinity for oxygen, therefore increase its offloading to the tissue) (Clarke et al. 2014; Muir 2015). The right shift translates into a decrease affinity for haemoglobin to bind to oxygen, therefore less is transported to the tissue potentially resulting in

tissue hypoxia. Among other causes, the decrease in pH increases the stimulation to breathe which results in increased workload of the respiratory system and therefore global oxygen demand. Furthermore, the decrease in cardiovascular performance decreases oxygen delivery (Mitchell et al. 1972). Animals that cannot initiate compensatory responses to acidosis due to illness or the effects of anaesthetic drugs or both may suffer further physiological derangements which could lead to increased morbidity or mortality.

The shift in blood pH was evident in healthy impala undergoing immobilisation and general anaesthesia using two different drug protocols. The profound increase in $PaCO_2$, despite a seemingly normal respiratory rate for a medium sized ruminant suggests that monitoring for a respiratory acidosis is not reliable when using just the respiratory rate as an indicator of respiratory suppression. The gases found within the alveoli while breathing room air include nitrogen, oxygen and carbon dioxide (originating from the metabolically active tissue and transported to alveoli via the blood). If the amount of carbon dioxide increases to levels above 50 mmHg, as noted in these impala, then there is an increased competition for gases within the alveoli (Clarke et al. 2014; Muir 2015). This competition decreases the amount of oxygen that is available for absorption and can lead to hypoxaemia (Clarke et al. 2014; Muir 2015). Therefore, if these protocols are to be used in the field, oxygen supplementation should be considered mandatory (Buck et al. 2017; Zeiler et al. 2015). Despite the substantial increase in $PaCO_2$ over time the acidaemic shift in the blood pH was negligible due to the profound compensatory metabolic responses that we detected. These important responses require normal health and physiological function and therefore caution should be taken, and acid-base status carefully assessed, when immobilising ill or anorexic wild ungulates.

4.6 Conclusions

The profound increase in the partial pressure of carbon dioxide that worsened during the total intravenous anaesthesia in both protocols initiated a substantial metabolic compensatory response to prevent severe acidaemia. This compensation resulted in a clinically acceptable mild acidaemic state, which worsened over time but not between the protocols, in healthy impala. However, these important compensatory mechanisms require normal physiological function and therefore when immobilising ill or anorexic wild ungulates their acid-base status should be carefully assessed. In addition, whenever impala are immobilised with thiafentanil or etorphine based drug combinations respiration should be

closely monitored and butorphanol and oxygen supplementation should be considered when apnoea and hypoxia occurs.

CHAPTER 5

Captive management of wild impala (*Aepyceros melampus*) during intensive immobilisation and general anaesthesia study trials

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5.1 Abstract

Immobilisation and anaesthesia of impala (*Aepyceros melampus*) has become a popular research theme. This demand is brought about by the increased need to immobilise and anesthetise impala and other medium sized wild ungulates due to their increase value in game ranching and zoological collections. In order to improve our understanding of immobilisation and general anaesthesia in these species, it is paramount to be able to study them in a practical, safe research environment that does not cause harm or unnecessary stress to the animals. This study aimed to scrutinise our management and welfare practices and scientific methods of 15 wild-caught impala placed in captive confinement during a 16-week intensive research project. The scientific methods of the project were scrutinised to identify procedures that attributed to morbidity and mortality. Indicators of impala welfare during captivity were monitored by documenting serial physical (body weight, coat condition), physiological (biochemistry and haematology) and psychological (behavioural) aspects. Two impala suffered irreparable femoral fractures due to darting and were humanly euthanised. One impala suffered cardiovascular collapse during immobilisation and could not be resuscitated. The procedure of chemical capture had a morbidity and mortality rate of 14.8% and 4.4%, respectively. The impala maintained acceptable physical and physiological parameters including: stable body weights; well-groomed coats; values for total serum protein, serum creatinine and haematological parameters that were within reference ranges for the species. There were improvements in the impalas' psychological parameters which included a decrease in the number of aggressive interactions (head butting and ramming) and an increase in the number of reciprocal allogrooming interactions. The monitored welfare indicators suggest adaptation to captivity. The study showed that impala could be successfully managed in captivity for sixteen weeks. However, scientific methods (namely darting) increased the risk of injury and caused fatalities.

Keywords: *Aepyceros melampus*, impala, refinement, scientific methods, welfare

5.2 Introduction

Research in wild ungulates, like impala (*Aepyceros melampus*), has become increasingly important over the last few years (Meyer et al. 2008a, b; Meyer et al. 2015; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2017; Gerlach et al. 2017). This surge in invasive veterinary interventions in wild ungulates stems mainly from zoological collections and game farms. Furthermore, the author's experience proposes that extrapolation of data from studies in domesticated ungulates, such as goats, do not always reliably reflect physiological responses to field based immobilisation and anaesthetic procedures (Heard et al. 1990; Buss & Meltzer 2001; Meyer et al. 2015). Wild ungulates' anxious disposition and questionable ability to adapt to novel environments are leading differences compared to the more stoic and adaptive domesticated ungulates (Fraser et al. 2013). Therefore, in some instances, using domesticated animals as replacement models for wild ungulates can be fraught with difficulty when interpreting and extrapolating findings.

Impala have been used in numerous capture (physical and chemical immobilization) studies over the last six decades (Ables & Ables 1969; Janssen et al. 1993; Bush et al. 2004; Meyer et al. 2008b; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2017). They are medium in size, relatively easy to physically restrain, affordable to feed and herds can occupy a minimal space in outdoor enclosures (bomas). Experimental experience suggests that impala maintain their nervous and flighty disposition despite perceived adaptation to boma confinement, but they can suffer from mal-adaption, anorexia, injury sustained during frantic attempts to escape physical capture or confinement, nocturnal predation or capture myopathy (Murray et al. 1981; Knox et al. 1991; Knox et al. 1992; Meyer et al. 2008b). These points highlight the reasons why impala remain a popular research model for capture and anaesthesia studies but the risk of morbidity and mortality poses obvious welfare and ethical questions (Meyer et al. 2008b). Therefore, scrutinising scientific methods and welfare standards are needed to identify risks and to provide recommendations to refine future management and husbandry practices that will improve the welfare and ethical use of wild animals for research purposes.

Scrutinising and refining scientific methods includes improving statistics, improving experimental procedures and the study design (Koknaroglu & Akunal 2013). Animal welfare in research models are evaluated by monitoring various indicators specific for the species, such as: 1) the physical state (body weight, body condition scoring, coat condition, posture and lameness, evidence of diarrhoea); 2) the

physiological state (biochemistry and haematology); and 3) the psychological state (changes in behaviour) of the animal (Hawkins et al. 2011).

This study aimed to scrutinise our scientific methods and welfare of 15 wild-caught impala placed in captive confinement during a 16-week intensive research project investigating four drug combination trials and provide recommendation to refine the ethical use of wild antelope as a research model.

5.3 Materials and Methods

The study was approved by the University of Pretoria's research and animal ethics committees (V012-16 and V099-13) prior to commencement of the study. Fifteen adult female impala, which were in a good physical condition and from a single large wild herd, were acquired from an extensively managed game farm. The impala were chemically captured using thiafentanil (Thianil 1%; Wildlife Pharmaceuticals; Karino, Mpumalanga, 1240, South Africa; 0.025-0.050 mg kg^{-1}; intramuscularly) via darting, once recumbent they were placed in a wildlife transport trailer. Once in the trailer, the impala were treated with doramectin (Dectomax 1%; Zoetis; Sandton, Gauteng, 1246, South Africa; 0.2 mg kg^{-1}; sc) and ear tagged for identification. Haloperidol (Haloperidol 1%; Kyron Laboratories; Johannesburg, Gauteng, 2194, South Africa; 0.25 mg kg^{-1}; iv) and zuclopenthixol (Clupixol-Acuphase 5%; Lundbeck South Africa; North Riding, Gauteng, 2162, South Africa; 1.25 mg kg^{-1}; im) tranquilisers were administered. Once processed, the thiafentanil was antagonised using naltrexone (Trexonil 5%; Wildlife Pharmaceuticals; Karino, Mpumalanga, 1240, South Africa; 0.25-0.50 mg kg^{-1}; iv). A maximum of eight impala were loaded at one time. Each group was then transported to the study boma. All impala were captured and placed in the study boma within 36 hours, without injury or mortality.

A purpose built 2.7 meter high walled boma (450 m^2) was constructed for the study (**Figure 5.1**). The boma was subdivided by an internal wall, with swing-gates at each end, into a feeding area (150 m^2) and home area (300 m^2). Both the feeding and home area could be accessed by gates. The wall was built from diamond mesh (100 mm square) fencing spanned on 2.7 meter high metal fence posts along the perimeter of the boma boundary, internal wall and gates. Shade cloth (80%) was secured to the entire diamond mesh fencing from the ground to the top, to give the appearance of a solid walled structure. The boma floor was natural occurring turf which was water permeable. A permanent automated water trough provided water *ad libitum* (point e: **Figure 5.1**). The boma was scheduled to be cleaned when the build-up of uneaten food and faecal matter was subjectively deemed excessive.

The boma was positioned within a quiet area of the faculty campus, between sheep and equine paddocks.

Experimental procedures

The study series was made up of different drug protocol trials investigated by two research teams in an international collaborative effort. The overall study objectives were to study various physiological effects of randomly allocated immobilisation and general anaesthesia maintenance protocols, published elsewhere (Zeiler et al. 2015; Buck et al. 2017; Gerlach et al. 2017). The data reported in the drug protocol trials are independent and focused on describing the pharmacodynamic and practicality of each drug protocol. Data reported in this study focuses on the monitoring of animal welfare and overall project scientific method during the 16-week period, from May to August. Each research team was randomly allocated a total of ten impala, which were enrolled into the two cross-over designed drug protocol trials.

The study was divided into a six-week pre-trial period and an eight-week trial period, when investigation of the drug protocols took place.

Pre-trial period

Week one: The first week of the pre-trial period involved habituating animals to being moved within the boma and establishing a routine daily feeding schedule. Twice a day,

Figure 5.1 The housing structure used to confine impala (*Aepyceros melampus*) was a purpose built 2.7 meter high walled outdoor boma (450 m^2).
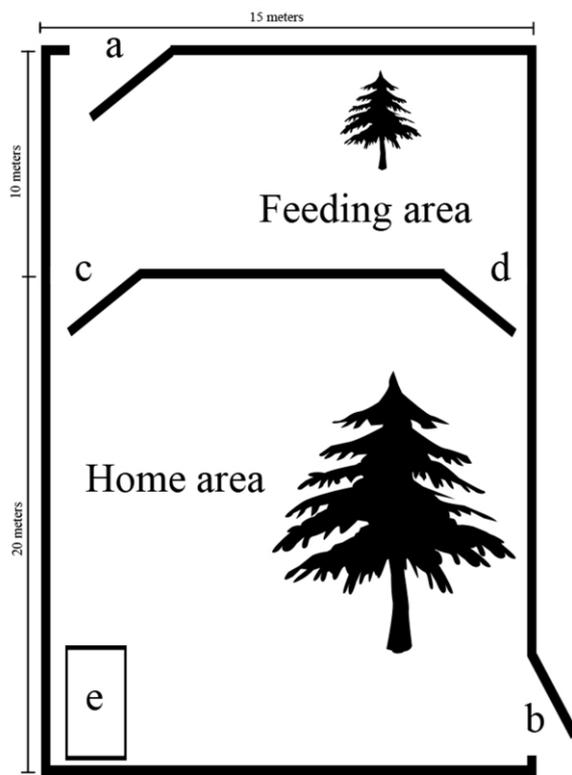
Footnote: The boma was subdivided by an internal wall with swing-gates (c and d) at either end; into a small feeding area (150 m^2) and a larger home area (300 m^2). Both the feeding and home area could be accessed by gates (a and b). There was an automated water trough (e) installed that allowed water *ad libitum*.

investigators would herd the impala into the home area of the boma and close off the feeding area by shutting the inter-leading swing-gates (gate c & d: **Figure 5.1**) between the two partitions. Then grass hay (*Eragrostis tef*) and lucerne hay (*Medicargo sativa*) were placed in piles scattered randomly around the feeding area. Antelope pellets (Alzu antelope pellets; Alzu; Johannesburg, Gauteng, 2194 South Africa; approximately 100g animal^{-1} day^{-1}) were added to the feeding routine in the afternoon. Once the food had been placed, the inter-leading swing-gates were opened and the investigators observed the impala. If the impala did not enter the feeding area within thirty minutes, they were slowly herded in by walking around the outside of the boma causing the impala to move from the home area to the feeding area. During feeding, the automated water trough was inspected from the outside of the boma. If the water trough required cleaning, only then would one of the investigators enter the home area via an access gate (gate b: **Figure 5.1**) leading directly into the home area.

Week two: The daily feeding routine continued as in week one, with the exception that the inter-leading swing-gates were closed once the impala had moved into the feeding area. The impala were confined in the feeding area for at least 30 minutes, yet not exceeding 90 minutes, twice daily.

Week three: Daily activity continued as per week two. All impala, except one (the smallest impala excluded from study series enrolment), were chemically captured once for blood sampling and weighing (control values). To confirm drug dose and combinations for the investigational studies various combinations of the following drugs were used for immobilisation: etorphine (Captivon 0.98%; Wildlife Pharmaceuticals; Karino, Mpumalanga, 1240, South Africa; 0.05 mg kg^{-1}; im), thiafentanil, butorphanol (Butorphanol 1% compounded; V-Tech Pharmacy; Centurion, Gauteng, 0157, South Africa; 0.15 mg kg^{-1}; im), ketamine (Ketamine Fresenius 10%; Intramed; Port Elizabeth; 6000; RSA; 4 mg kg^{-1}; im) and medetomidine (Medetomidine 1% compounded; Kyron Laboratories; Johannesburg, Gauteng, 2194, South Africa; 0.055 mg kg^{-1}; im) (first capture of study: **Table 5.1**).

Table 5.1 Drug protocols used to chemically capture impala (*Aepyceros melampus*) during six different captures. The drug protocols from the second to the fifth capture were randomly allocated to impala by two different research teams investigating different immobilisation and total intravenous anesthesia protocols.

Number of impala	Immobilisation protocol (im)		Maintenance protocol (iv)		Antagonists		References
	Drug	Dose	Drug	Dose	Drug	Dose	
			Week 3: First capture of study				
4	Thiafentanil	0.05	None		Naltrexone (iv)	0.5	
	Medetomidine	0.055			Atipamezole (im)	0.28	
2	Medetomidine	0.2	None		Atipamezole (im)	1.0	
	Ketamine	4.0					
4	Medetomidine	0.2	None		Atipamezole (im)	1.0	
	Ketamine	4.0			Naltrexone (iv)	0.5	
	Butorphanol	0.15					
4	Medetomidine	0.030	None		Atipamezole (im)	0.15	
	Ketamine	2.0			Naltrexone (iv)	0.5	
	Etorphine	0.025					
			Week 7: Second capture of study*				
11	Medetomidine	0.2	Medetomidine	0.0012	Atipamezole (im)	1.2	Gerlach et al. 2017
	Ketamine	4.0	Ketamine	2.4	Naltrexone (iv)	0.3	
	Butorphanol	0.15	Midazolam	0.036			
			Week 9: Third capture of study**				
10	Thiafentanil	0.05	Propofol	12.0	Naltrexone (iv)	0.5	Buck et al. 2016
	Medetomidine	0.055	Ketamine	1.5	Atipamezole (im)	0.28	
			Medetomidine	0.005			
			Week 11: Fourth capture of study*				
11	Etorphine	0.038	Medetomidine	0.0012	Atipamezole (im)	1.2	Gerlach et al. 2017
	Medetomidine	0.2	Ketamine	2.4	Naltrexone (iv)	0.75	
	Midazolam	0.2	Midazolam	0.036			
			Week 13: Fifth capture of study**				
10	Etorphine	0.05	Etorphine	0.04	Naltrexone (iv)	1.0	Zeiler et al.2015
	Medetomidine	0.055	Ketamine	1.5	Atipamezole (im)	0.28	
			Medetomidine	0.005			
			Week 16: Sixth capture of study				
12	Thiafentanil	0.038	None		Naltrexone (iv)	0.38	

All doses reported as mg kg^{-1}; All maintenance dose rates reported as mg kg^{-1} hour^{-1}; *Week 7 and 11 protocols were randomly assigned to impala during these weeks by research team one; **Week 9 and 13 protocols were randomly assigned to impala during these weeks by research team two.

Week four to six: Daily activity continued as in week two, with the exception that the inter-leading swing-gates were not closed while the food was being placed. Once the food was placed, then the impala were led into the feeding area and confined, as described during week two. During this time, to further habituate the impala to the darting procedure, the carbon dioxide powered rifle (Model JM; Dan-inject International SA; Kruger National Park, Skukuza, Mpumalanga, 1350, South Africa) was dry fired (no dart in the chamber) at the 5 bar pressure setting once to five times outside the boma per day.

Trial period

Week seven to fourteen: The two research teams captured data on alternating weeks. Data collection was done within one week followed by a one-week rest period. During the data collection week, the enrolled impala within that trial underwent an immobilisation followed by a 120 minute total intravenous general anaesthesia (second to fifth capture of study: **Table 5.1**). Prior to darting, all impala were confined in the feeding area. Once the selected impala was immobilised, the rest of the herd was herded into the home area. The immobilisation drug combination was delivered via a 3 ml dart (S300 Syringe Dart; Dan-inject International SA; Kruger National Park, Skukuza, Mpumalanga, 1350, South Africa) projected into the muscles of the pelvic limb using a carbon dioxide powered rifle. The impala tended to congregate at the area between the tree and boundary wall by gate d during darting. The person that darted the impala stood on a ladder outside the boma wall at the level of gate c and darted at an elevated height of 2.7 meters over a distance ranging from 10 to 15 meters, therefore the rifle pressure was set to 5 bars. If the impala did not become recumbent within 15 minutes after darting, a second dart (half-strength or full strength depending on the degree of ataxia shown) was administered. Once immobilised, blood was collected (after immobilisation) and the impala was weighed and then enrolled into the total intravenous anaesthesia study. Another blood sample was collected just prior to discontinuing the total intravenous infusion (after anaesthesia). Once the 120 minute anaesthesia was completed, the impala was returned to the feeding area for recovery. Gates c and d were opened prior to administering antagonists during recovery (**Table 5.1**). The recovery period was observed until one hour after the studied impala returned to the herd (waiting in home area) on their own (not herded). During the one week rest period, husbandry continued as described in week four to six of the habituation period.

Weeks fourteen to sixteen: Two weeks after the last drug trial (a rest period) all impala were chemically captured (sixth capture of study: **Table 5.1**) and transported back to the game farm from which they came and were released back into the wild. One year later, the owner was contacted to obtain information about the studied impala, such as if they were still alive, integrated back into the herd and breeding.

Data collection

Data collected to scrutinise scientific method included evaluating the boma construct, the daily husbandry routine and the number of: immobilisations, darts used, adverse events and mortalities. Adverse events were defined as darting injuries (intra-osseous, intra-peritionial and skin tagging), cardiopulmonary depression or collapse and bone fracture.

Morbidity and mortality rates of immobilisation were calculated as follows:

- Morbidity rate = total number of adverse events/total number of immobilisations

- Mortality rate = total number of deaths/total number of immobilisations

Data collected to scrutinise animal welfare included variables monitoring the physical state, the physiological state and the psychological state of the impala. The physical state of the impala were monitored by collecting: 1) serial body weight (at each capture), 2) body condition score (at each capture: scored from 1 [emaciated] to 5 [obese] where 3 is ideal body condition), 3) subjective coat condition scoring (at each capture: groomed and clean, not groomed and dirty, generalised or focal alopecia, macroscopic evidence of ectoparasites), 4) noting posture and lameness (daily: normal stance, hunched back, non-weight or weight bearing lameness) and 5) noting evidence of diarrhea (daily: fecal pellet inspection, soiled perineal area).

The physiological state was monitored by measuring biochemical and haematological parameters at each capture. Blood samples were collected into a serum tube (Serum tube BD Vacutainer 5.8 mL; BD; Woodmead, Gauteng, 2191, South Africa) and ethylenediaminotetreaacetic acid tube (EDTA tube BD Vacutainer 5.8 mL; BD; Woodmead, Gauteng, 2191, South Africa). After immobilisation blood samples for biochemistry (serum proteins, creatinine) and haematology (erythrogram and leukogram) were analysed using daily calibrated bench top analysers (Cobas, Integra 400 Plus; Roche Products (Pty)

Ltd.; Sandton, Gauteng, 1246, South Africa; and ADVIA 2120 Haematology System; Siemens; Isando, Gauteng, 1609, South Africa; respectively). Total cortisol concentrations (control samples taken soon after immobilisation during week 3; trial samples taken soon after immobilisation and at the end of anaesthesia weeks 7, 9, 11 and 13) were analysed (Immulite 1000; Siemens; Isando, Gauteng, 1609, South Africa) using a chemiluminescent enzyme immunoassay.

The psychological state of the impala were subjectively monitored by noting their change in behaviour of 1) feeding (daily: herd pellet consumption), 2) antagonistic interactions (daily: presence or absence of head butting and ramming), 3) grooming interactions (daily: present or absence of individual and reciprocal allogrooming) and 4) anxiety (daily: presence or absence of anorexia, withdrawing from herd, escape attempts through jumping; during data capture: response to darting). For subjective comparison a weekly summative report of the overall herd psychological state was compiled from data that was recorded during all days.

<u>Statistical analysis</u>

Data was assessed for normality by plotting histograms and the Anderson-Darling test for normality. Herd data over time was derived from impala (n = 7) that were enrolled in all capture weeks to analyse the physical (body weight, body condition score) and physiological data (serum proteins, creatinine, cortisol and haematology). Parametric data was described using mean (minimum: maximum). Variables (body weight, body condition score, total serum proteins, albumin, creatinine, cortisol and haematology) were analysed over time (over weeks 3, 7, 9, 11, 13) using an unpaired one-way analysis of variance (ANOVA). Week 3 data (first capture) was used as a control and post hoc multiple comparisons were compared using Dunnett's method for significant findings. The immobilisation (number of immobilisations, darts used, adverse events and mortalities), weekly synopses of psychological state variables (feed intake, presence of antagonistic, grooming and anxiety behaviour), coat condition, posture and lameness and diarrhoea events were presented descriptively. Data were analysed using commercially available software (MiniTab 17.1.0; MiniTab Incorporated; State College, Pennsylvania, 16801, USA) and results were interpreted at the 5% level of significance.

5.4 Results

The boma division allowed successful management of the impala by providing a dedicated feeding area and confinement during darting. The weekly husbandry routine of progressively introducing different stimulations and activities over time successfully kept the herd unified and no deaths resulted from direct husbandry related interventions. Sixty eight immobilisations occurred in the herd during the entire study period when they were in the boma (**Table 5.2**). Darting of the impala was carried out by one of the investigators throughout the study period.

Table 5.2 Immobilisation outcome data of impala (*Aepyceros melampus*) enrolled into an intensive research study.

Parameter	Week 3	Week 7	Week 9	Week 11	Week 13	Week 16	Total
Immobilisations	14	10 (+ 1†)	10	10 (+ 1‡)	10	12	68
Darts used	22	15	11	14	13	15	90
Adverse events							
Darting injuries							
Intra-osseous							4
Scapula	1						
Ilium	1						
Femur		1			1		
Peritoneal			1		1		2
Skin Tagging	1			1		1	3
Cardiovascular derangements				1*			1
Aetiology of mortalities							
Euthanised		1			1		2
Cardiac arrest				1			1

(+ 1†): one impala died and was replaced by another impala not enrolled into any trial; (+ 1‡): an incorrectly identified impala was accidently darted but not enrolled in any trial and subsequently died from cardiac arrest; *indicates mortality.

A total of 90 darting events were required to immobilise the impala (sometimes animals had to be darted more than once) during the study. Three impala died during the study, one animal suffered cardiovascular collapse during the immobilisation; and two suffered inoperable comminuted fractures to their femur bone due to darting injuries and were humanly euthanised using sodium pentobarbitone (Euthapent 20%; Kyron Laboratories; Johannesburg, Gauteng, 2194, South Africa; 200 mg kg^{-1}; iv). A total of 10 adverse events were recorded, including darting injuries and cardiovascular derangements. The morbidity and mortality rate based on immobilisation were 14.8% and 4.4%, respectively.

Monitoring the physical state of the herd demonstrated a stable herd weight (p = 0.803) increasing from 36.1 kg to 39.1 kg and an overall body condition score (p = 0.327) of 3 throughout the study (physical state, **Table 5.3**). The condition of the impalas' coats were groomed and cleaned. However, in week twelve, one impala demonstrated non-pruritic generalised alopecia over the dorso-lateral thoracic and lumbar regions. She was treated with doramectin (Dectomax 1%; Zoetis; Sandton, Gauteng, 1246, South Africa; 0.2 mg kg^{-1}; sc) while immobilised, and was recovering well two weeks later. No signs of lameness were detected during the study. Defecation was normal in all impala during the study.

Some of the monitored physiological parameters (albumin, creatinine, haematocrit and erythrocyte count) did demonstrate statistically significant increases over time, yet all were within clinically acceptable reference ranges for impala, as shown in **Table 5.3**. The total serum protein and differentiated white cell count remained within normal reference ranges and did not significantly change over time (**Table 5.3**). The cortisol concentrations after immobilisation were no different to the control samples (p = 0.517) and did not differ over the weeks (7-13) when the animals were immobilised for the trials (one-way ANOVA; p = 0.462). The cortisol concentrations at the end of anaesthesia (second sample) were significantly decreased from control concentrations (p < 0.001), but did not differ over the weeks (7-13) when the animals were immobilised for the trials (one-way ANOVA; p = 0.218).

The psychological state of the herd, based on the weekly synopses, highlighted that all daily pellet rations were consumed and no sign of anorexia were noted in any of the impala (**Table 5.3**). The antagonistic interactions were initially noted as random bullying (ramming and head butting of random impala by random impala) in all the impala when moving the impala into the feeding area (during confinement habitation and darting period). The bullying of the darted impala by the remaining herd escalated once the drug effect (onset of ataxia and wondering) became evident. Over time, from week 9 onwards, bullying only occurred towards the darted impala, once drug effect was evident. Individual grooming was noted throughout the study and reciprocal allogrooming was noted from week 9 onwards. Herd reactivity to the investigators presence outside the boma and during darting changed from continuous trotting in a hastily manner with escape attempts through jumping into the walls (especially the corners of the boma) to a calm herd, hardly making any reaction by week 9 onwards.

The food (hay and lucerne) that was not consumed by the impala formed a layer of bedding which the animals utilised. The boma required only two cleaning sessions to rake out faecal pellets (faecal load

mainly in one designated place (latrine) in the home area, away from food and water source) and uneaten food.

Communication with the owner of the game farm one year after returning the remaining impala confirmed that all released animals were still alive, had reintegrated well within the original herd and most had fawns at foot.

Table 5.3 Serial parameters monitored in impala (*Aepyceros melampus*) to help indicate welfare aspects in animals undergoing a 16-week long study investigating the effects of immobilisation and anaesthesia drug combinations.

Parameter	Unit	Week 3		Week 7		Week 9		Week 11		Week 13		p value	Ref Range		Ref
		Mean	Min Max	Mean	Min Max	Mean	Min Max	Mean	Min Max	Mean	Min Max		Mean	±SD	
Physical state															
Weight	kg	36.1	29.1 40.5	37.1	29.3 44.3	37.4	30.4 41.5	37.9	31.8 42.3	39.1	31.4 43.3	p = 0.803			
Body condition score	none	3	3 4	3	2 3	3	3 4	3	3 3	3	3 3	p = 0.327			
Physiological state															
Biochemistry variables															
Total serum protein	g L⁻¹	58.1	49.8 62.3	57.7	51.3 64.0	62.6	58.7 66.9	59.8	55.2 64.4	59.0	51.7 63.1	p = 0.289	63 62	±9.2 ±7.7	Hattingh et al. 1983 Sleenman 1993
Albumin	g L⁻¹	37.4	33.1 40.5	40.5*	37.5 42.4	44.5*	41.7 46.5	42.5*	40.9 43.6	42.3*	39.0 44.3	p < 0.001	56.6	±6.3	Hattingh et al. 1983
Globulin	g L⁻¹	20.8	16.7 24.1	17.2	13.4 23.2	18.1	14.7 21.3	17.3	14.3 21.1	16.8	12.7 20.2	0.161	-	-	-
Creatinine	µmol L⁻¹	101.0	84.0 133.0	124.8	91.0 141.0	133.5*	102.0 159.0	137.8*	123.0 154.0	136.8*	109.0 155.0	p = 0.006	175.0	±35.4	Janssen et al. 1993
Cortisol after immobilisation	nmol L⁻¹	84.4	21.5 188.0	59.7	25.3 165.0	84.5	35.6 173.0	63.5	14.1 136.0	80.0	21.8 136.0	p = 0.517	114 66	±22 ±47	Knox et al. 1993# Meyer et al. 2008b**
Cortisol after Anaesthesia	nmol L⁻¹	84.4	21.5 188.0	8.4*	3.1 18.0	7.6*	2.8 14.3	7.6*	4.7 13.4	11.4*	5.3 21.7	p < 0.001	11.0 19.4	±3.0 -	Hattingh et al. 1990 Hattingh 1988

Table 5.3 continued on next page

Table 5.3 (continued) Serial parameters monitored in impala (*Aepyceros melampus*) to help indicate welfare aspects in animals undergoing a 16-week long study investigating the effects of immobilisation and anaesthesia drug combinations.

Parameter	Unit	Week 3 Mean	Min Max	Week 7 Mean	Min Max	Week 9 Mean	Min Max	Week 11 Mean	Min Max	Week 13 Mean	Min Max	p value	Ref Range Mean	±SD	Ref
						Haematology variables									
Haematocrit	L L^{-1}	0.34	0.27 0.42	0.37	0.30 0.44	0.44*	0.42 0.46	0.39	0.34 0.48	0.44	0.40* 0.48	0.004	0.44 0.43	±0.06 ±0.07	Sleenman 1993 Gamble et al. 1994
Erythrocyte count	x10^{12} L^{-1}	19.5	16.2 24.3	20.7	17.5 23.8	24.2*	22.9 24.9	21.8	18.0 25.6	23.9*	22.8 25.0	0.002	23.3 24.0	±5.3 ±3.9	Sleenman 1993 Gamble et al. 1994
Leukocyte count	x10^{9} L^{-1}	5.2	3.6 6.8	10.8	2.4 43.8	6.4	5.5 7.2	4.6	2.9 5.3	6.4	5.5 7.6	0.616	3.4 9.1	±1.5 ±4.8	Sleenman 1993 Gamble et al. 1994
Neutrophil count (Segmented)	x10^{9} L^{-1}	1.3	0.7 2.1	2.9	0.4 12.3	1.2	0.9 1.6	1.0	0.4 1.9	1.4	0.6 2.0	0.547	2.2†	±1.3	Gamble et al. 1994
Neutrophil count (Band)	x10^{9} L^{-1}	0.0	0.0 0.0	0.2	0.0 0.9	0.0	0.0 0.0	0.1	0.0 0.1	0.0	0.0 0.0	0.139			
Lymphocyte count	x10^{9} L^{-1}	3.4	2.7 5.2	7.5	1.8 29.8	5.0	4.3 5.5	3.5	2.1 4.3	4.9	3.8 5.7	0.622	6.7	±4.3	Gamble et al. 1994
Monocyte count	x10^{9} L^{-1}	0.1	0.0 0.2	0.2	0.1 0.9	0.1	0.0 0.2	0.1	0.0 0.2	0.2	0.1 0.5	0.447	0.06	±0.11	Gamble et al. 1994
Eosinophil count	x10^{9} L^{-1}	0.1	0.0 0.3	0.0	0.0 0.2	0.0	0.0 0.1	0.0	0.0 0.0	0.0	0.0 0.0	0.603	0.16	±0.25	Gamble et al. 1994

Table 5.3 continued on next page

Table 5.3 (continued) Serial parameters monitored in impala (*Aepyceros melampus*) to help indicate welfare aspects in animals undergoing a 16-week long study investigating the effects of immobilisation and anaesthesia drug combinations.

Parameter	Unit	Week 3		Week 7		Week 9		Week 11		Week 13		p value	Ref Range	Ref
		Mean	Min Max	Mean	Min Max	Mean	Min Max	Mean	Min Max	Mean	Min Max		Mean ±SD	
						Psychological state								
Weekly synopsis	Feeding; Antagonistic interactions; Grooming; Anxiety.	All daily pellet rations were consumed; Ramming and head butting of impala when moving into feeding area for darting, interaction increased towards darted impala during immobilisation and reintegration to herd after recovery; Individual grooming behaviour of impalas noted; Herd reactive (continuous trotting hastily around the boma) to activity of investigators outside the boma, escape attempts through jumping into the wall at the corners of the boma, especially during darting.		All daily pellet rations were consumed; Ramming and head butting of impala when moving into feeding area for darting, interaction increased towards darted impala during immobilisation and reintegration to herd after recovery; Individual grooming behaviour of impalas noted; Herd less reactive (short trotting episodes hastily around the boma) to activity of investigators outside the boma, no escape attempts through jumping into the wall noted		All daily pellet rations were consumed; Ramming and head butting of darted impala during immobilisation and reintegration to herd after recovery; Individual an reciprocal allogrooming behaviour of impalas noted; Herd calm and hardly reactive to activity of investigators outside the boma, no escape attempts through jumping into the wall noted		All daily pellet rations were consumed; Ramming and head butting of darted impala during immobilisation; Individual an reciprocal allogrooming behaviour of impalas noted; Herd calm and hardly reactive to activity of investigators outside the boma, no escape attempts through jumping into the wall noted		All daily pellet rations were consumed; Sporadic ramming and head butting of darted impala during immobilisation; Individual an reciprocal allogrooming behaviour of impalas noted; Herd calm and hardly reactive to activity of investigators outside the boma, no escape attempts through jumping into the wall noted				

Ref Range: reference range of various haematology and biochemistry values in impala; Ref: published reference; Week 3: control sample; p value: significance of unpaired one-way analysis of variance with post hoc Dunnett's method where * indicates significant difference from control sample values; Min: minimum; Max: maximum; SD: standard deviation; kg: kilogram; mg L^{-1}: milligram per litre; umol L^{-1}: micromole per litre; nmol L^{-1}: nanomole per litre; #: after net capture; **: after chemical capture; †: segmented and band neutrophils.

5.5 Discussion

The impala herd was successfully managed during the 16-week boma occupancy. The boma construct and positioning, daily husbandry routine, progressive introduction of various stimuli and overall management were successful and support an effective application of good scientific methods. However, the procedure of darting and chemical capture caused injury and death. Monitoring of the welfare (physical, physiological and psychological states) of the impala suggested adequate boma adaptation and herd unification. The study demonstrated two paramount clinically relevant outcomes. The first is that impala, if managed correctly, can be successfully kept in a boma for sixteen weeks for studies that require regular testing of immobilisation and anaesthetic combinations. The second is that current methods used to capture impala in bomas (namely darting) remain high-risk interventions which require improvement.

There is a paucity of published criteria used to monitor refinement of procedures in wild ungulates, such as impala, that are temporarily confined in bomas for research purposes. Refinement is an all-encompassing term that describes both improvement of scientific method (statistics, procedural interventions, and experimental design) and welfare (monitoring physical, physiological and psychological states) aspects that are required to ensure ethical outcomes with valid and meaningful results (Smaje et al. 1998; Lloyd et al. 2008; Hawkins et al. 2011). The scientific methods used and welfare of the impala of the present study were scrutinised to identify aspects that require refinement to improve the ethical use of wild ungulates enrolled into future studies.

A boma is a standard type of outdoor housing to keep wild ungulates temporarily captive (Bothma & van Rooyen 2005). The boma size, an important welfare aspect to consider, was compared to 1) boma sizes used in other research studies in impala, 2) natural wild conditions, 3) zoological paddock enclosure recommendations and 4) current "Norms and Standards" recommended for captive wild herbivores in southern Africa (Bothma & van Rooyen 2005). Boma sizes reported in impala studies range from 285 m^2 to 800 m^2 surface area (Gandini et al. 1989; Heard et al. 1990; Knox et al. 1991; Knox et al. 1992; Bush et al. 2004). The surface area per impala ranged between 8.6 and 25 m^2/impala, in these studies. In natural wild populations there are 33 to 109 impala/km^2, which translates into 9174 to 30303 m^2/impala, respectively (Rduch 2016). Zoological paddock recommendations for permanent housing of impala in enclosures are 500 m^2 for up to 10 impala, with an additional 40 m^2 for each additional impala over 10 (Zoolex 2017). This translates into a 700 m^2 boma for 15 impala or 47

m^2/impala. The southern Africa "Norms and Standards" recommend the size of a boma is 2 m^2/50kg live weight for temporary housing of antelope (Bothma & van Rooyen 2005), which translates into 24 m^2 for 15 impala weighing 40 kg each, or 0.63 m^2/impala. Therefore, the boma that we used in our study (30 m^2/impala) was greater in surface area per animal than the southern Africa "Norms and Standards" criteria and those used in other reported studies, but was smaller than zoological paddock recommendations. Furthermore, within the home area, the impala created a latrine area away from the food and water points thereby reducing the requirements for frequent disturbances when cleaning, which may have been less likely if the boma was much smaller in size. Other characteristics of a good research boma for impala are: 1) shelter from the elements (ours had two large broad-branched trees and semi-solid shade cloth boma walls that provided shade); 2) two partitions separated by a high wall to allow the impala a sense of escape when humans entered the boma; 3) inter-leading swing-gates that allow confining impala to one of the partitions during study and husbandry tasks; 4) high walls to prevent escape and injury through jumping (these occur more often if animals perceive a chance of escape); 5) ample gates to allow easy access from outside to both partitions of the boma; 6) and allowance of enough natural ventilation (boma size and shade cloth walls). Furthermore, the pliable shade cloth-diamond mesh walling of the boma had an advantage compared to solid-rigid walled bomas, by: allowing the impala to partially see movement outside the boma which minimised startling and allowed the animals to be herded from the outside of the boma; and limited catastrophic injuries (neck and limb fractures) from occurring during attempts to escape through jumping.

Darting is a necessary procedure used in wildlife management practice to administer immobilisation combinations or other medications remotely. We used a 5 bar setting to project a 3 ml dart a distance of 10 to 15 m that was determined by ranging before the trial and which was in accordance with manufacturer recommendations. A lower pressure, at this distance, would have reduced accuracy and would have resulted in a slower dart and arched trajectory to which impala have an uncanny ability to easily dodge. Darting the impala from a closer point 5 to 7 m away (along the 15 m wall of the feeding area, behind the tree, for example) could have allowed a lower rifle pressure to be set. However, the tree that could have concealed the darter, would have hindered dart projection. Also, a smaller dart could not be used (to decrease the kinetic energy) due to the constraints of the studied immobilisation combinations requiring 3 ml. Despite adhering to all good darting practices by experienced veterinarians, accidents occur and catastrophic injuries are experienced (Karlsson & Stahling 2000;

Kreeger 2002). Refinement of darting procedures warrants further scientific investigation to decrease these rates to levels less than 2%, which are stated to be acceptable (Arnemo et al. 2006).

The monitored variables to determine the overall welfare of the impala suggested that they progressively adapted to the boma. The physical and physiological parameters were within normal anticipated limits throughout the study, and similar to those expected in wild impala living in optimal conditions (Hattingh et al. 1983; Sleenman 1993; Gamble et al. 1994; Karesh et al. 1997; Barnett 2007; Kock & Burroughs 2012; Fraser et al. 2013; Koknaroglu & Akunal 2013). The etiology of the non-puritic alopecia experienced by the one impala remains unknown. Parasites and fungal causes were tested for, but ruled out. Other possible causes included: drug reactions, hormonal changes and season changes (shedding of winter coat). The cortisol levels measured soon after immobilisation were similar to impala undergoing net and chemical capture which suggests that the impala did not become accustomed to the darting procedure (Knox & Zeller 1993; Meyer et al. 2008b). Basal cortisol concentrations were not measured in this study. However, values obtained from head shot impala and impala sampled rapidly after hand capture were similar to the cortisol concentration obtained at the end of anaesthesia (Hattingh 1988; Hattingh et al. 1990). Interpretation of serum cortisol concentrations as a definitive indicator of chronic stress has short-comings (Hart 2012). Therefore, these values should be interpreted with caution (Mormede et al. 2007; Hart 2012). Pooled faecal glucocorticoid metabolite measurements may have been a more appropriate method of determining stress responses over time for the herd, but a valid method for measuring these metabolites is yet to be established for impala. The psychological state determined by noting the incident of aggressive behaviour that changed from random (any impala) to focused (towards immobilised impala) ramming and head butting events suggests a herd social hierarchy was established and that they became acclimatised to the daily routine (Dunbar 1981). Furthermore, the observed reciprocal allogrooming that occurred also suggests behavioural acclimation (Hart & Hart 1992; Hart et al. 1992). Despite detecting a change in behaviour over time, our observations were limited in that we only assessed the impala during a short time period each day and therefore we cannot comment on the behaviour in between these monitored times. The author's (Meyer) previous experience with impala suggests that impala genders should not be mixed during a protracted study in a confined space, especially during the rutting season. The herd aggression, particularly among rams tends to increase dramatically during rutting, thus making mixed gender herds difficult to house for research purposes. Also, the use of long acting tranquilisers is

recommended at first introduction to a boma as this facilitates acceptance to a confined environment (Knox et al. 1990; Kock & Burroughs 2012).

We believe the management and welfare aspects require minimal refinement and were satisfactory in this study. Although, the scientific methods caused the most harm to the impala and do require further refinement through thorough investigation.

After the study, the reintegrated impala appeared to have returned to a normal social and physical state. An encouraging outcome was that many of the impala had fawn at foot. The return to a normal social behaviour and appearance of being fit and healthy suggests that there were no long-term ill effects to the intensive study.

5.6 Conclusions

By employing methods that appropriately habituate animals to confinement and management procedures ensured that wild impala can be housed within a boma, at relatively high stocking densities for a 16-week period, for capture-related research, without compromising animal welfare. However, the scientific methods employed in this study did result in injury and deaths. We found that the procedure of darting impala had an unacceptably high mortality rate of 4.4%. Further refinement of drug administration, particularly darting, in impala and possibly other medium sized wild ungulates, should be an area of focus.

CHAPTER 6

Chemical capture of impala (*Aepyceros melampus*): a review of factors contributing to morbidity and mortality

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6.1 Abstract

The objective was to review the factors that contribute to morbidity and mortality of impala (*Aepyceros melampus*) undergoing chemical capture, and discuss how they are potentially mitigated. The following databases were searched: Pubmed, Science Direct, Google Scholar and Onderstepoort Veterinary Academic Hospital records. The keywords (found on the bottom of the page) were used alone and in various phrases to query each database. The narrative review concluded that impala are an important species of antelope in Africa and are often captured during management procedures, veterinary interventions and research projects. Chemical capture is a preferred technique over physical capture and restraint for veterinary interventions as it allows for easier handling and better clinical assessment and treatment. However, this capture technique results in high mortality (4%) and morbidity rates (23%) which translates into animal welfare and economic concerns. Investigation of environmental, drug and drug delivery, and animal factors to elucidate the origin of these high rates was reviewed. The greatest risks emanate from the drug and drug delivery factors where potent opioids (etorphine and thiafentanil) cause profound respiratory compromise, that, if left, untreated often translates into fatalities. Furthermore, the procedure of darting, an essential tool in game capture, can cause irreparable fractures and other fatal injuries mainly through accidental misplacement of the dart into a long bone, thoracic or peritoneal cavity. Impala are anxious and flighty and this demeanour (animal related factor) can contribute towards mortality and morbidity rates. Impala that mount an inappropriate stress response to capture tend to die, therefore procedures that induce an intense stress response (awake clinical examinations) should be avoided. Sequela of a heightened stress response include capture-induced hyperthermia, myopathies, fractures, maladaptation to confinement or new environments and death. Impala serve as a useful model for improving immobilising and anaesthetic drug protocols, darting techniques or new methods of remote injection in wildlife. However, the risks associated with chemical capture in this species should be understood and all efforts to mitigate these should be employed.

Keywords: *Aepyceros melampus*, capture, immobilisation, morbidity, mortality

6.2 Introduction

Impala (*Aepyceros melampus*) are an African species of antelope that play a significant role in extensive ecosystems and contribute to local and international economies through zoological collections and game farming (Zeiler et al. 2015). Impala are regularly captured for disease monitoring, clinical and reproductive examination and treatment and translocations during routine management procedures.

Chemical capture remains the preferred capture technique for impala undergoing veterinary intervention as this allows for easier handling and better clinical assessment and treatment, and is believed to cause less stress when compared to physical capture and restraint techniques. However, in some ungulate species physical capture techniques have been shown to be safer compared to chemical capture (Kock et al. 1987; Jacques et al. 2009). Conversely, impala are known to die more readily during physical capture and restraint; Murray et al. (1981) dubbed the cause of these deaths as "fatal stress", where they recorded up to 30% deaths in physically captured and restrained wild impala.

Chemical capture and general anaesthesia are of the most challenging procedures when routine veterinary interventions are required in wild antelope (Ball & Hofmeyr 2014), and many factors need to be considered to ensure success. Factors include environmental conditions, drug and drug delivery systems and animal characteristics (Arnemo et al. 2006).

Factors associated with the rates of chemical capture-induced morbidity and mortality in impala will be reviewed. The aim of this review is to provide information that can help reduce the risk associated with impala capture thereby improving animal welfare and the success of capture and veterinary interventions.

6.3 Morbidity and Mortality Rates

Published data on rates and causes of capture-related morbidity and mortality in impala are sporadic (**Table 6.1**). Knottenbelt (1990) highlighted that veterinary interventions (capture and transport) were the leading cause of death, mostly due to stress, physical injury and maladaptation to novel environments. In impala, physical capture, with or without tranquilisation, results in a higher mortality rate compared to chemical capture techniques. The current published chemical capture mortality rates (average of 4%; **Table 6.1**) are in agreement with those reported anecdotally by experienced wildlife veterinarians of around 3.0% (0.5 to 7.0%) (unpublished data). These figures are related deaths

occurring within the immediate peri-capture period and do not take medium to long-term survival rates (>14 days post-capture) into consideration.

6.4 Factors Contributing to Morbidity and Mortality

Table 6.1 Morbidity and mortality rates of impala (*Aepyceros melampus*) undergoing either physical (alone or with administration of a tranquiliser) or chemical capture.

Study number	n =	Physical capture alone	Physical capture + tranq	Chemical capture	Overall	References
			Mortality rates (death due to capture)			
1	47	-	-	4%	4%	Pienaar et al. 1966
2	14	-	-	14%	14%	Ables & Ables 1969
3	522	30%	22%	-	10%	Murray et al. 1981
4	36	-	-	0%	0%	Cheney & Hattingh 1987
5	15	30%*	50%*	-	40%*	Knox et al. 1990
6	25	33%**	31%**	-	32%**	Knox et al. 1991
7	44	-	-	0%	0%	Janssen et al. 1993
8	35	-	-	0%	0%	Bush et al. 2004
9	26	-	-	11%	11%	Meyer et al. 2008a
10	15	-	-	0%	0%	Meyer et al. 2008b
11	19	-	-	0%	0%	Meyer et al. 2010
12	15	-	-	4%†	20%	Zeiler & Meyer 2017c
Ave		**31%**	**34%**	**4%**	**11%**	
			Morbidity rates (injury or physiological derangements due to capture)			
4	36	-	-	66%	66%	Cheney & Hattingh 1987
8	35	-	-	0%	0%	Bush et al. 2004
13	10			10%	10%	Perrin et al. 2010
12	15	-	-	14%	14%	Zeiler & Meyer 2017c
Ave		**-**	**-**	**23%**	**23%**	

n =: number of impala enrolled into study; tranq: tranquilisation; Ave: average; *Four drugs tested in four trails; results presented in this table is an average of all four trials; **a non-tranquiliser drug was studied to determine if habituation to confined housing could be improved; † Study calculated the overall mortality rate by dividing the number of deaths by the total number of immobilisations, however, overall three out of fifteen impala died.

Footnote: The rates referenced in this table were either directly quoted from the article, or calculated due to enough information being provided. Morbidity rate (%) = number of injured/number of impala captured x 100; mortality rate (%) = number of impala dead/number of impala captured x 100.

Mortality rates associated with capture ideally should be less than 2% (Spraker 1993). Ethical consideration mandates systematic exploration aimed at environmental, drug and drug delivery and animal factors that could be mitigated to decrease these rates, a sentiment already highlighted a decade ago (Arnemo et al. 2006).

6.4.1 Environmental Factors

Impala, in the wild, and in extensive game farming enclosures, prefer the ecotone between grassland and woodland (Kock & Burroughs 2012), favouring areas where there is abundant cover from the elements.Impala, in the wild, and in extensive game farming enclosures, prefer the ecotone between grassland and woodland (Kock & Burroughs 2012), favouring areas where there is abundant cover from the elements. Alternatively, in intensive game farming, research facilities and zoological collections impala are often housed in confined high-walled enclosures such as a boma, or display enclosures. Impala may injure themselves during chemical capture in many different ways in these vastly different environments. Distal long bone fractures (**Figure 6.1a**) and joint dislocations are frequently reported by field practitioners, thought to be due to uneven footing during escape attempts (flight response to darting) or getting their limbs trapped in fences. Cervical fractures (**Figure 6.2**) and dislocations, through jumping into walls or fences during frantic escape attempts, are also commonly reported. In our experience, recovery after successful surgical repair of fractures and dislocations is rare and animals are often euthanised. Less catastrophic injuries such as non-fatal horning injuries, skin laceration and abrasions are also reported and contribute to capture morbidity. Erroneously, in the field, these risk factors are often considered a normal outcome of capture, and their mitigation is often not explored or instituted.

Impala herds tend to remain together during the chase prior to darting, but darted animals tend to leave the herd once the drug(s) begin to take effect. Thus, there is an increased risk of losing an animal in extensive environments, which could place an immobilised animal at risk of morbidity and mortality. Tracking a darted impala is particularly important if there are predators in the vicinity of capture (Cronje et al. 2002). Furthermore, there are moral and legal motives to ensure that darted animals are found to prevent unwanted scavenging or even human consumption of drugged animals (Ables & Ables 1969).

Environmental temperature is considered a major contributing factor to capture-induced hyperthermia (defined as an acute precipitous rise in body temperature; rectal temperature: mild: 39.5 to 40.0°C; moderate 40.1 to 41.0°C; severe >41.1°C; Kock & Burroughs 2012). Recommendations to capture during the cooler parts of the day, such as at sunrise and sunset (Murray et al. 1981) or at ambient air temperatures below 25°C (Kock & Burroughs 2012) have been proposed. Furthermore, impala are thought to be a heat-sensitive antelope and recommendations such as not to capture during hot ambient

Figure 6.1 Common long bone fracture type presentations during chemical capture of impala (*Aepyceros melampus*) in extensive (**a:** distal limb fractures by tripping during escape attempts or horning injury) and intensive (**b:** misplaced dart into the proximal limb bones) housing environments.

Figure 6.2 Cervical fracture sustained in an impala (*Aepyceros melampus*) during chemical capture in an intensive housing environment where impala attempted to escape by jumping against solid walls of the enclosure.

temperatures (> 37°C) exist (Kock & Burroughs 2012). However, there is no scientific evidence to confirm that high environmental temperatures result in capture-induced hyperthermia. Rather, high environmental temperatures cause an additional increase in body temperature only once animals are captured (Cheney & Hattingh 1987). Not only environmental (ambient) temperature, but also environmental humidity (outdoors and inside transport vehicles or trailers) and radiant, convective and conductive heat loads must be considered, especially in the period directly after the initial capture, as these may either hinder cooling or increase an animal's body temperature further. Furthermore, an animal's body temperature just prior to capture is rarely considered (Meyer et al. 2008a). Impala, like other antelope, have a 24 hour temperature rhythm and are at their hottest core body temperature (approximately 38.5°C) around sunset (Meyer et al. 2008a; Shrestha et al. 2012), at a time when environmental temperatures are often low. More importantly, there is robust evidence that capture-induced hyperthermia develops during heightened stress responses during capture and temporary confinement (Meyer et al. 2008a), discussed further in the "*Animal factors*" section.

Hypothermia is another complication of immobilisation especially in cold environmental conditions, even more so during precipitation. Furthermore, some immobilising drug combinations

could accelerate the shift of core temperature to the peripheral body and accelerate heat loss (Grimm 2015). Hypothermia may result in altered pharmacokinetic (prolonged duration of action of drugs causing delayed recovery or an unpredictable response to pharmacological antagonists) and physiological (arrhythmias, acidosis, hypoventilation, hypotension) effects that, if untreated, could result in death (Caulkett & Arnemo 2015). It is challenging to remedy hypothermia under field conditions due to limitations in carrying appropriate equipment into the field (Caulkett & Arnemo 2015). Blankets (to dry and keep dry) and cover (tarpaulin, gazebo etc.) should be available when capturing, especially in rainy seasons, to minimise this morbidity.

Capture in extensive environments can be unpredictable. Therefore, gaining a working knowledge of the terrain (rivers, valleys, cliffs and ravines), predator load and other dangerous animal (hippopotamus, rhinoceros and elephant etc.) whereabouts at the proposed capture site will help in decreasing risks during chemical capture (Kock & Burroughs 2012).

6.4.2 Drug and Drug Delivery Factors

Countless drug combinations have been used and proposed as preferred combinations to chemically capture impala over the last seven decades. A general consensus is to induce recumbency (immobilise) in the shortest time possible to limit injury and prevent the loss of an animal and the unwanted side effects associated with prolonged induction. However, the ideal immobilisation agent has not yet been discovered (**Table 6.2**). Published drug and drug combinations currently used to chemically capture impala, and their pharmacodynamics effects that alter cardiovascular and respiratory physiology, are highlighted in **Table 6.3**.

Table 6.2 Characteristics of an ideal theoretical immobilisation drug and how frequently used drugs, or drug classes, to immobilise impala (*Aepyceros melampus*) compare to this ideal drug.

Characteristic	Potent opioids	Ketamine**	Midazolam*	Alpha$_2$-adrenoceptor agonists*	Azaperone*
Drug formulation concentration	9.8-10 mg ml^{-1}	<250 mg ml^{-1}	20, 40, 50 mg ml^{-1}	10, 20, 40, 50 mg ml^{-1}	40, 100 mg ml^{-1}
Wide safety margin	+++	+++	+++	+	++
Suitable for administration in most species	++	+++	++	++	++
Able to be absorbed from most injection sites (intramuscular, subcutaneous)	+++	++	++	++	++
Safe if administered intravenously	+	++	+++	++	++
Allow maintenance of righting reflex	++	+	+	+	++
Retain essential body function	++	+++	+++	+	++
Stable high concentration to allow low volume administration	+++	+	+++	+++	++
Stable at a range of environmental temperatures and pressures	+++	+	+	++	++
Non irritant	+++	+	++	+++	++
Soluble in water	+++	+++	++	+++	+++
Miscible with other drugs	+++	+++	++	+++	+++
Easily reversible or have antagonists with minimum untoward effects	+++	-	++	+++	-
Provide muscle relaxation	-	-	+++	+++	+
Provide analgesia	++	+++	-	+	-
Safe for handler of drug	-	++	++	+	++

*Drug can be compounded at high drug formulation concentrations for injection; **Powder formulation in bottle which can be mixed with water for injection to desired concentration; +++: strongly agree; ++: agree; +: somewhat agree; -: disagree. Ideal immobilisation drug (Harthoorn 1968; Haigh 1990)

Opioid drugs used for immobilisation

Potent opioids, etorphine and thiafentanil, are considered the drugs of choice to induce immobilisation in impala. When these drugs are used alone, they result in muscle rigidity (a mu-opioid receptor effect), but can fail to fully immobilise impala, especially when conservative doses are used (Woolf et al. 1973). The degree of muscle rigidity ranges from occasional myoclonic contractions, most noticeable in the distal limbs, to generalised full-body muscle catatonia and catalepsy (Harthoorn 1967). Furthermore, the appearance of struggling against the immobilising drug effect is pronounced. Typical potent opioid

induction progresses from mild ataxia, noticed within minutes of administration, to a star-gazed high-stepping Hackney-like gait prior to moving into sternal or lateral recumbency, often due to tripping over obstacles (Harthoorn 1967; Janssen et al. 1993). Impala that fail to be induced by potent opioids, regardless of dose (Harthoorn 1967), may trot around in a Hackney-like gait for a prolonged period of time. These impala are at risk of suffering from physical injury, capture-induced hyperthermia and myopathy during this state (Haigh 1990). Rescue intervention during this state is done by administering additional drugs (etorphine, thiafentanil, ketamine, midazolam or medetomidine) via darting or via intramuscular or intravenous injection, if animals can be captured and restrained by hand (Haigh 1990). Because of the unpredictable effects when potent opioids are used on their own, they are normally combined with synergistic drugs (sedatives or tranquilisers) to improve the quality of induction into immobilisation (**Table 6.3**).

Cardiovascular effects of etorphine and thiafentanil, when used alone, are similar in impala (Cheney & Hattingh 1987; Janssen et al. 1993). Arterial blood pressure is well maintained (mean arterial blood pressure approximately 120 mmHg; Janssen et al. 1993), often tending towards systemic hypertension (Brown et al. 2007). Heart rates are approximately 100 beats min^{-1} for both potent opioids, a rate often above pre-drug control values of 75 beats min^{-1} (Cheney & Hattingh 1987; Janssen et al. 1993).

Respiratory depressant effects are pronounced and are thought to be the leading cause for potent opioid related deaths in impala (Pienaar et al. 1966). Early in the immobilisation phase, bouts of apnoea (no visible breath attempt for 60 seconds), Biot's (cyclical deep gasps followed by apnoea) and apneustic (slow breathing with prolonged end-inspiratory pauses) breathing patterns may be observed. The potent opioids are thought to disrupt normal respiratory rhythm generation by interfering with the pre-Bötzinger complex (slow inspiratory effort or struggling to initiate an inspiratory effort) and Kölliker-Fuse nucleus (transition from inspiration to expiration) through activation of mu-receptors (Pattinson 2008). Furthermore, the opioids also decrease the central and peripheral chemoreceptor response to arterial carbon dioxide and oxygen gas tensions (Pattinson 2008). However, within 10 to 15 minutes after the start of etorphine immobilisation, minute volume (10.9 L min^{-1}; mean body weight 36 kg; Meyer et al. 2010) is often comparable to acceptable limits calculated for small stock ruminants (6.3 to 10.4 L min^{-1}: Hales & Webster 1967; Bakima et al. 1988) and respiratory rates are between 12 to 20 breaths min^{-1}. The return of an apparent normal respiratory rate and rhythm is likely due to the waning plasma and biophase concentration of the potent opioid, or acute desensitisation of the mu-opioid receptor

effect (Virk & Williams 2008). Despite seemingly adequate measured minute ventilation, respiratory compromise is indicated by pronounced hypoxemia (mean ±SD; PaO_2 = 40 ±3 mmHg), poor oxygen haemoglobin saturation (SpO_2 = 76 ±3%) and hypercapnia ($PaCO_2$ = 51 ±2 mmHg) that do not easily correct in potent opioid immobilised impala (Meyer et al. 2010). Goats immobilised with etorphine demonstrate similar cardiopulmonary responses. More complete investigation of the respiratory effect of etorphine found pulmonary hypertension (mean pulmonary artery pressure 23 ±6 mmHg) a plausible cause of right-to-left intrapulmonary shunting and inadequate gas diffusion which manifests as abnormal gas tensions and gradients (Vodoz et al. 2009; Meyer et al. 2015). Despite the known cardiopulmonary effects of potent opioids, they remain the preferred capture drug in impala, especially during capture in extensive environments. Lastly, etorphine and thiafentanil immobilisation can be successfully antagonised with either naltrexone (mu-, kappa-, and delta-opioid receptor competitive antagonist) or diprenorphine (mixed mu-antagonist, kappa-agonist), the preferred reversal drugs.

Table 6.3 Drug combinations used to chemically immobilise impala (*Aepyceros melampus*) during chemical capture.

Resource Number	Combination	Mean Weight (kg)	Dose (mg)	Source (research study, textbook)	Antagonists	Dose (mg)	Time to recumbency (minutes)	Comments	References
				Opioid based combination					
1	Etorphine Midazolam	40.0	2.0 15.0	Textbook	Diprenorphine (iv)	6.0	-	Apnoea	Kock & Burroughs 2012
2	Thiafentanil Midazolam	40.0	2.0 15.0	Textbook	Naltrexone (iv)	20.0	-	Apnoea	Kock & Burroughs 2012
3	Etorphine Azaperone	38.0	1.5 40.0	Study	Diprenorphine (iv)	3.0	7.9 ±3.2	Hypoxaemia and apnoea common; stress increases body temperature	Meyer et al. 2008b
4	Etorphine Acepromazine	31.0	0.47 4.7	Study	Naltrexone (im)	19.0	7.1 ±2.4	Good muscle relaxation, predicatble and calm induction and recovery. Hypoxaemia a concern and oxygen supplementation recommended.	Perrin et al. 2015
5	Etorphine Medetomidine	38.0	1.5 2.0	Study	Diprenorphine (iv) Atipamezole (im)	3.0 10.0	9.2 ±3.8	Hypoxaemia and apnoea common; stress increases body temperature	Meyer et al. 2008b
	Etorphine Medetomidine	40.0	2.0 2.2	Study	Naltrexone (iv) Atipamezole (im)	42.0 13.0	13.9 ±2.7	Hypoxaemia and apnoea common; Butorphanol (iv) 1:1 etorphine dose resolved apnoea without arousal	Zeiler et al. 2015
6	Etorphine Medetomidine Midazolam	40.0	1.5 8.0 8.0	Study	Naltrexone (iv) Atipamezole (im)	30.0 40.0	9.5 ±2.9	Hypoxaemia and apnoea common; Butorphanol (iv) 1:1 etorphine dose resolved apnoea without arousal	Gerlach et al. 2017
7	Thiafentanil Azaperone	38.0	1.2 40.0	Study	Naltrexone (iv)	12.0	3.9 ±1.1	Hypoxaemia and apnoea common; stress increases body temperature	Meyer et al. 2008b
8	Thiafentanil Medetomidine	38.0	1.2 2.0	Study	Naltrexone (iv) Atipamezole (im)	12.0 10.0	4.4 ±3.1	Hypoxaemia and apnoea common; stress increases body temperature	Meyer et al. 2008b
	Thiafentanil Medetomidine	40.0	2.0 2.2	Study	Naltrexone (iv) Atipamezole (im)	20.0 11.0	9.6 ±3.2	Hypoxaemia and apnoea common; Butorphanol (iv) 1:1 thiafentanil dose resolved apnoea without arousal	Buck et al. 2016
9	Fentanyl Azaperone	40.0	10.0 30.0	Textbook	Diprenorphine (iv)	6.0	-	Apnoea thought to be less of a concern compared to etorphine and thiafentanil. Yet apnoea remains a concern	Kock & Burroughs 2012

Table 6.3 continued on next page

Table 6.3 (continued) Drug combinations used to chemically immobilise impala (*Aepyceros melampus*) during chemical capture.

Resource Number	Combination	Mean Weight (kg)	Dose (mg)	Source (research study, textbook)	Antagonists	Dose (mg)	Time to recumbency (minutes)	Comments	References
				Non-opioid based combinations					
10	Ketamine Medetomidine	33.0	185.0* 9.0*	Study	Atipamezole (im)	18.0*	4.5 ±1.5	Re-sedation noticed hours after antagonist administration at 2:1 ratio; recommend a 5:1 ratio instead	Bush et al. 2004
4	Ketamine Medetomidine	31.0	136.0* 3.4	Study	Atipamezole (im)	12.0	17.4 ±5.3	Unpredictable and highly variable induction and recovery quality. Caution advised when using this protocol. Oxygen supplementation is advised.	Perrin et al. 2015
6	Ketamine Medetomidine Butorphanol	40.0	160.0 8.0 6.0	Study	Atipamezole (im) Naltrexone (iv)	40.0 12.0	11.0 ±6.4	Mild hypoxemia and bradycardia common; antagonism was complete with no re-sedation reported	Gerlach et al. 2017

*article gives dose per kilogram only (retrospective calculations) therefore recalculated to total dose administered based on mean weight and dosages rates published.

Non-opioid drugs used for immobilisation

Cyclohexylamine based drug protocols for impala were historically described with phencyclidine, and now with ketamine. Because of ketamine's convulsive and generalised hypertonic effects (Kock & Burroughs 2012) it is normally administered in combination with drugs known to have muscle relaxant properties (benzodiazepine agonists and alpha$_2$-adrenoceptor agonists). That said, Bush et al. (2004) reported that intramuscular ketamine (8 mg kg^{-1}) alone, in impala, resulted in ataxia lasting up to 10 minutes, with no impala achieving recumbency and none demonstrating overt hypertonia or convulsions. Williams and Riedesel (1987) also noted the apparent inability of ketamine (6 mg kg^{-1}) to induce a state of immobilisation in a 2-month-old white-tailed deer. To the authors' knowledge there is no report on physiological data in impala that only received ketamine. Renewed interest in ketamine based combinations has arisen due to the difficulties in obtaining etorphine or thiafentanil in many countries (Perrin et al. 2015; Gerlach et al. 2017). Recently, ketamine-medetomidine and ketamine-medetomidine-butorphanol drug combinations have been evaluated (Perrin et al. 2015; Gerlach et al. 2017). Both combinations demonstrated prolonged induction times compared to the potent opioid combinations. Both report an elevated mean arterial blood pressure (140 ±15 mmHg: Gerlach et al. 2017; 115 ±11 mmHg: Perrin et al. 2015) and decreased heart rate (41 ±5 beats min^{-1}: Gerlach et al. 2017; 44 ±15 beats min^{-1}: Perrin et al. 2015). Despite hypoxaemia (SpO$_2$ = 93 ±7%; PaO$_2$ = 42.9 ±7.2 mmHg: Gerlach et al. 2017; SpO$_2$ = 93 ±7: Perrin et al. 2015) hypoventilation was not a characteristic finding (minute volume = 10.3 ±2.1 L min^{-1}: Gerlach et al. 2017). Considering the variability in recumbency times (Bush et al. 2004; Perrin et al. 2015; Gerlach et al. 2017) and apparent resistance to clinical effects of ketamine and medetomidine when administered alone (Bush et al. 2004) these protocols require further development before they can be recommended for chemical capture in extensive environments, but would be useful in intensive environments. To the authors' knowledge, there have been no mortalities documented using these and other ketamine combinations. Furthermore, medetomidine (when combined with opioid or non-opioid combinations) can be successfully antagonised with intramuscular atipamezole (five times the medetomidine dose) without apparent untoward effect in impala (Bush et al. 2004; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2016; Gerlach et al. 2017).

<u>Drugs used to counter respiratory compromise</u>

Opioid-induced respiratory compromise is common and often life threatening in impala. Immediate intervention is required in impala suffering apnoea, especially if associated with marked muscle rigidity (abdominal muscle rigidity is thought to splint the diaphragm, causing a decrease in functional residual capacity, thus decreasing alveolar oxygen reserves; Sirian & Wills 2009). In the field where emergency equipment is limited, a single intravenous bolus of butorphanol (0.5 to 1:1 etorphine or thiafentanil dose) is enough to relax the opioid immobilised impala and improve the breathing pattern without causing arousal. This dose can be repeated at 2 minute intervals until the impala is relaxed and in a stable regular breathing pattern (Zeiler et al. 2015; Buck et al. 2016). Butorphanol, at the same dose, displayed similar effects in etorphine-immobilised goats (Haw et al. 2016b). Butorphanol, a mixed mu-antagonist (or partial mu-agonist) and kappa-agonist, is thought to competitively antagonise adverse mu-opioid receptor effects of the potent opioids in a dose dependent manner (Haw et al. 2016b). From the authors' clinical experience higher butorphanol doses (>1:1) result in arousal. In controlled clinical settings, prompt tracheal intubation and intermittent positive pressure ventilation is effective and could be complemented by a single butorphanol bolus and, or, oxygen supplementation. However, these interventions are not always feasible during field capture operations. The use of doxapram, an analeptic, has been advocated for years as a successful treatment for apnoea (believed to be a respiratory "kick start") in chemically caught wildlife (Swan 1993). Doxapram (1 mg kg^{-1}) raised the oxygen without changing carbon dioxide arterial tensions (Meyer et al. 2010), but the rise was transient (lasting approximately 3 minutes) and increased by only 8 ±7 mmHg in hypoxaemic (PaO$_2$ = 40 ±3 mmHg) impala. Also, despite a species variation to doxapram, this drug has untoward effects that include hypertension, muscle spasticity and stimulates catecholamine release that increases metabolism which can negate the perceived benefit of the increased arterial oxygen tension (Yost 2006). Serotonergic receptor agonists metoclopramide (5-HT$_4$), buspirone (5-HT$_{1A}$) and pimozide (5-HT$_7$) have also been evaluated as interventions to counter opioid-induced respiratory depression in impala (Meyer et al. 2010). Metoclopromide and buspirone, but not pimozide, transiently and partly attenuated etorphine-induced hypoxaemia through improving alveolar gas diffusion, but not minute ventilation (Meyer et al. 2010). Similar effects, with greater attenuation, occurred in goats receiving the potent serotonergic agonists zacopride (5-HT$_4$) and 8-OH-DPAT (5-HT$_{1A}$ and 5-HT$_7$) (Meyer et al. 2005). Another novel agent worth investigating in impala is the ampakine CX1942, a novel positive allosteric modulator of the

AMPA receptors important in respiratory rhythmogenisis and inspiratory drive (Pace et al. 2007). In goats, it attenuated etorphine-induced respiratory depression and hypoxaemia without stimulating arousal (Haw et al. 2016a). These potent serotonergic and ampakine agonists show promise in treating opioid-induced respiratory compromise, but may also be used in combination with the potent opioids to prevent its occurrence, however, further research is needed.

Considerations and drugs used for general anaesthesia

The environment in which animal(s) recover is an important consideration before administering general anaesthesia to an impala. Recovery in a confined housing structure, free from predators, decreases the risk of morbidity and mortality associated with recovery. Prolonged drug effect (with or without active metabolites) is less of a recovery concern in controlled environments. However, during recoveries in extensive environments, especially where predators are present, all but the shortest acting drugs should be considered and, preferably, drugs that can be fully antagonised, should be used (Caulkett & Arnemo 2015). Inhalation anaesthesia (isoflurane or sevoflurane in oxygen) is optimal in controlled circumstances. Using inhalation anaesthesia allows for rapid and complete recovery from drug effect and also allows for oxygen supplementation. However, administering inhalation anaesthesia is not always practical in the field and therefore total intravenous techniques have become attractive options. Constant rate infusions of mixtures of etorphine-ketamine-medetomidine (Zeiler et al. 2015), propofol-ketamine-medetomidine (Buck et al. 2016) and ketamine-midazolam-medetomidine (Gerlach et al. 2017) have successfully kept impala under general anaesthesia for up to 120 minutes, with calm and rapid recoveries reported. However, securing the airway with a cuffed endotracheal tube and providing oxygen support is strongly advised for all these combinations. Hand boluses of ketamine and, or potent opioids is another common technique that can be used for general anaesthesia or prolonging immobilisation, but ideally should be reserved for cases that require less than 60 minutes of general anaesthesia. Administering intravenous boluses of ketamine (even as low as 0.5 mg kg^{-1}) in an immobilised impala often results in apnoea (Personal observation). Also, that intravenous boluses of potent opioids can cause systemic hypertension, pulmonary hypertension and apnoea. These effects usually worsens existing hypoxaemia, therefore, boluses should be used with caution and at the lowest dose possible to avoid these physiological "seesaw" effects.

<u>Post-capture tranquilisation and sedation</u>

Tranquilisers and sedatives play an integral role in managing impala post-capture (**Table 6.4**) as they reduce stress-related complications and enhance adaption into captivity. Since the inception of their use in routine practice, mortality rates have decreased during translocation and confined housing (Gandini et al. 1989; Knox et al. 1990). Benzodiazepine agonists (diazepam and midazolam), butyrophenones (azaperone and haloperidol), short-acting (acetylpromzine), medium-acting (zuclopenthixol acetate) and long-acting (perphenazine enanthate) phenothiazine derivatives are the most commonly used sedatives and tranquilisers in wildlife (Kock & Burroughs 2012), yet there is scant evidence documenting their pharmacodynamic effects in impala. However, anecdotal evidence suggests that these drugs have limited cardiovascular, respiratory and thermoregulatory effects in impala, when administered at the current published doses. Empirical evidence of self-limiting extrapyramidal signs (allotriophagia, shivering, tremors, ground pawing and star gazing) in impala have been observed. However, these events are often of limited clinical significance and resolve within a few hours. More importantly, self-limiting anorexia (lasting up to 24 hours) is a common finding in impala receiving the medium and long-acting phenothiazine derivatives (Knox et al. 1990). Despite these side effects, which are normally short-lived, the use of these tranquilisers, at the correct dose, provide important benefits when they are used in healthy impala. However, post-veterinary interventions, when injured or ill impala are convalescing in confined housing, they often do not respond well to prolonged tranquilisation. Clinical observation suggests that the incident of rumen stasis, ileus and eventual death is higher in tranquilised impala. In these cases, low doses of benzodiazepine agonists should be considered, as and when required, as the sedative of choice. Because pain contributes to anorexia, and thus rumen stasis, analgesics should be administered in all cases where the perception of pain is likely, regardless of animal behaviour.

Table 6.4 Tranquilisers and sedatives often used in impala (*Aepyceros melampus*), especially during transport and relocation to novel areas or short and long-term confinement.

Drug	Dose (mg)			Duration of action	Comment
	Adult male	Adult Female	Sub-adult (Juvenile)		
Benzodiazepine agonists					
Diazepam	14 (iv)	12 (iv)	6 (iv)	1-4 hours	Intravenous route recommended. Preferred sedative for injured or ill impala.
Midazolam	15(iv, im)	15(iv, im)	6 (iv, im)	1-4 hours	Same clinical effect as diazepam, but can also be administered intramuscularly. Preferred for injured or ill impala.
Butyrophenones					
Azaperone	20-40 (iv, im)	15-30 (iv, im)	-	2-4 hours	If used, it is often administered in the dart.
Haloperidol	15 (iv, im)	10 (iv, im)	5 (iv, im)	8-12 hours	Short acting tranquiliser administered intravenously to captured (physical or chemical) impala. Often administered in combination with a longer acting tranquiliser. Popular choice for transportation.
Phenothiazine derivatives					
Zuclopenthixol acetate	50 (im)	40 (im)	-	2-3 days	Self-limiting anorexia (<24 hours) in healthy impala. Often used in combination with a short acting tranquiliser or sedative such as haloperidol or diazepam. Caution advised in injured and ill impala.
Perphenazine enanthate	50 (im)	40 (im)	-	7-10 days	Anorexia. Not often used in impala.

References for the tranquilisers and sedatives (Gandini et al. 1989; Knox et al. 1990; Meyer et al. 2008a, b; Kock & Burroughs 2012).

<u>Analgesia</u>

There is little scientific evidence of the efficacy of analgesic drugs in wild antelope. Analgesic drugs have been used empirically in impala that are injured or undergoing surgery. Injectable non-steroidal-anti-inflammatory-drugs (NSAIDs), meloxicam (0.5 mg kg^{-1}, subcutaneously; Zeiler et al. 2015; Buck et al. 2016; Gerlach et al. 2017) and carporfen (2.0 mg kg^{-1}; subcutaneously) have been administered without apparent ill effect. The authors have routinely used local anaesthetics (lidocaine at 2-4 mg kg^{-1} or ropivacaine at 2 mg kg^{-1}) for regional and infiltrative block techniques, yet not for axial anaesthesia as impala show distress if they cannot stand post-surgery. The use of opioids in impala is not routinely practiced, mostly because long acting antagonists (naltrexone or diprenorphine) are used to reverse immobilisation, which then limits subsequent opioid use (morphine, butorphanol, buprenorphine) within the first 24 hours post antagonist use. Theoretically, diprenorphine, which appears to have mixed receptor (opioid receptor mu-antagonist and kappa-agonist) binding properties in some species, may be the antagonist of choice in painful impala, but its usefulness needs to be confirmed. Further research on other drugs (like tramadol) or oral administration of NSAIDs needs to be assessed for managing pain, post-operatively. Oral administration of some NSAIDs in sheep (Stock et al. 2013) and calves (Coetzee et al. 2009; Stock et al. 2014) have excellent bioavailability and efficacy and therefore further research to determine their hypoalgesic and antihyperalgesic effects in wild antelope is warranted.

<u>Drug delivery</u>

Drug delivery devices used for chemical capture include hand injection (impala in transport crates or net captured), pole mounted syringes (impala in transport trailers) and projectile darts (gas or powder charged dart guns). Hand injection and pole syringe delivery techniques usually do not cause clinically significant trauma, other than soft tissue bruising. However, important to consider, is the stress and anxiety created by approaching impala within their flight zone (Gandini et al. 1989). The stress response can be profound and has resulted in an unacceptably high mortality rate (Murray et al. 1981; Knox et al. 1990). The dart is a common and often preferred technique of administering drug combinations to impala. The act of darting impala has the greatest risk of causing injury, sometimes with fatal outcomes, during chemical capture (Zeiler & Meyer 2017c). Long bone fractures due to a misplaced dart is a common finding (Figure 1b). The mass of the dart and the trajectory velocity are thought to be the most important considerations when deciding on which darting system to use (Kock & Burroughs 2012).

Trauma is directly proportional to the dispersion of the kinetic energy (Ek) of the dart into the surrounding tissues at the moment of impact. The kinetic energy of the dart can be represented by the equation used in dart ballistics (Kock & Burroughs 2012):

$$Ek \text{ (joules)} = \tfrac{1}{2} \, m \times V^2$$

where m = mass (kg) and V = velocity (m s^{-1} in the direction of travel).

Impala are masters of avoiding darts and thus often require a fast dart projection, where impact velocities of 40-50 m s^{-1} should suffice for good needle penetration and dart discharge without causing excessive trauma in most cases (Cattet et al. 2006). Therefore, to attain this safe velocity range, the animals should not be darted from too far away (the further away, the greater the projection and impact velocity) and the lightest dart (normally the smallest volume dart, such as a 0.5 or 1.0 ml dart for impala) should be chosen to minimise the kinetic energy. Clinical experience in darting impala suggests that their bones are rigid and brittle and prone to shattering when subjected to a misplaced dart impacting a long bone. The ash content analysis of impala femur bone tissue confirms the suspicion that the bones are rigid and brittle (Brits et al. 2014). Relatively short needles (~ 20 mm long needles) should be used to minimise the risk of hitting bone.

6.4.3 Animal Factors

Impala are an inherently "highly-strung and anxious" antelope that often react fractiously to capture and confinement (Meyer et al. 2008b). This behavioural trait makes the treatment and management of systemically ill or injured impala complicated and risky. The highest risk for mortality is darting a distressed anxious impala whereby the undesirable drug effects (hypertension, tachycardia and hypoxemia) combined with profound sympathoadrenal activation (increased circulating catecholamines and glucocorticoids increase metabolism and oxygen demand) can culminate in acute (within five minutes of darting) heart failure and death (Personal observation). Additionally, stress has been shown to be linked to capture myopathy, capture-induced hyperthermia, maladaptation and ill thrift, all potentially culminating in death (Kock & Burroughs 2012; Paterson 2014). Furthermore, male impala, particularly during the rutting season (mating season), are at risk of these complications as they are aggressive (cause horning injuries in competing males), anxious and are often physiologically

compromised as they often lose body condition and total body water volume due to time spent guarding territory and mating (Jarman & Jarman 1973).

Capture myopathy (exertional rhabdomyolysis) is a serious and ultimately life-threatening syndrome experienced in captured wildlife. Impala are prime candidates to suffer from capture myopathy due to their pronounced stress response and overall fear of capture and confinement. Once their flight zone has been breached, they will invariably attempt frantic escape attempts. These attempts result in overexertion and a profound stress response (sympathoadrenal system activation). Both are key risk factors in the development of capture myopathy (Blumstein et al. 2015). Clinically the syndrome has four recognised presentations: 1) *capture shock syndrome*; 2) *ataxic myoglobinuric syndrome*; 3) *ruptured muscle syndrome*; 4) *delayed-peracute syndrome* (Spraker 1993). This syndrome is normally diagnosed on post-mortem investigation where characteristic dull white striations in cardiac and major skeletal muscle groups are seen (Beringer et al. 1996; Paterson 2014). However, in early phases diffuse haemorrhage, congestion and oedema may be mainly present.

Capture-induced hyperthermia is a common finding in captured impala. Many environmental, drug and animal factors have been suspected to cause capture-induced hyperthermia, as discussed in the "*Environmental factors*" section. However, evidence rather suggests that animals with a greater stress response (elevated catecholamines and/or cortisol) are more prone to develop rapid-onset capture hyperthermia, irrespective of whether they are chased, chemically captured or not, and regardless of the drug(s) used during chemical capture (Meyer et al. 2008a, b). As discussed previously, environmental conditions, and the effects of drugs on thermoregulation, most likely have a greater effect post-capture by influencing an animal's ability to thermoregulate. Regardless of the cause, rectal temperatures should always be regularly or continuously monitored in captured impala. Impala having rectal temperatures exceeding 40.5°C (Normal expected mean ±SD is 38.9 ±0.25°C; Meyer et al. 2008b) require cooling interventions (Kock & Burroughs 20120. Treating animals with capture-induced hyperthermia in the field is most effectively achieved by dousing an animal with water and rubbing the hair coat to allow the water to make contact with the skin (Sawicka et al. 2015). Cooling can also be enhanced by 1) placing the impala in a shaded area, 2) providing rapid air movement after water dousing (using a fan, leaf blower or exposing an animal to ventilation during transport, etc.) to increase evaporative cooling, 3) using drugs that cause vasodilation and 4) to consider antagonising the immobilising drugs to provide an early recovery (Kock & Burroughs 2012).

The darting order, especially during simultaneous capture of a few animals, is important to consider. Dominant rams and females should be darted first and only once drug effects are noticed, then the remaining herd should be darted. This approach tends to decrease the incident of horning and head-butting injuries and general herd distress (Murray et al. 1981).

Immobilised impala are prone to rumen bloating and regurgitation, which could result in serious complications that have led to mortalities (collapsed cardiovascular system and or aspiration pneumonia). Two interventions may minimise this risk, namely withholding food and water (applicable to confined animals) prior to immobilisation and guarding the airways. The time when last food and water was consumed are unknown in wild impala that need to be captured or require emergency veterinary care and therefore careful attention to guarding the airway becomes a priority. Guarding of the airway by either early tracheal intubation with a cuffed endotracheal tube (best) or placing the animal in sternal recumbency and holding the head above shoulder height (decreases the risk of passive regurgitation and allows eructation), provided the nose points downwards and is lower than the angle of the jaw to promote flow of saliva (and regurgitate) out of the oropharynx (Kock & Burroughs 2012).

6.5 Conclusions

Environmental, drug and drug delivery, and animal factors were reviewed to highlight potential concerns contributing to the high mortality (4%) and morbidity rates (23%) of impala during chemical capture and immediate confinement. All factors considered, the drug and drug delivery and animal factors contribute most to the mortality and morbidity rates. Chemical capture through darting increases the chance of the impala sustaining a fatal injury by frantic escape attempts or accidental misplacement of a dart. All currently recommended drug combinations for impala chemical capture cause apnoea, hypoxaemia and hypertension. Immobilised impala must be continuously monitored for apnoea, hypoxia and hyperthermia, common complications of immobilisation that can rapidly progress with fatal outcomes. The innate nervous disposition of the impala cannot be altered and could be classed as a known risk factor that could compound the pathophysiological effects of the drugs used and increased morbidity and mortality risks. Individual inappropriate stress responses to chemical capture and confinement accelerates the 1) development of cardiorespiratory failure and capture-induced hyperthermia, 2) injuries sustained through escape attempts, and 3) increases the chance of anorexia and maladaptation. Pain perception in impala contributes to poor adaption to confinement and promotes anorexia, rumen stasis and death. Therefore, judicious prolific use of analgesics such as non-steroidal-

anti-inflammatory drugs, local anaesthetics or opioids should be practiced. Much has already been learned about wild antelope capture and confinement from impala. These lessons should be applied by those working with impala as this specie is known to have a greater risk of morbidity and mortality compared to other wild antelope. By doing so veterinary management of these animals will be improved and the success of further research into wild antelope capture, confinement, welfare and veterinary management will be enhanced.

CHAPTER 7

Discussion and general conclusions

The focus of the investigations were to 1) determining the physiological effects of the drugs used for immobilisation and general anaesthesia and 2) the welfare and ethics of using impala as a research model, to understand the risks we subject impala to during intensive study conditions. The overall objective was to determine if impala are an appropriate species of animal to be used as a live animal research model, especially when researching various drug combinations for chemical immobilisation and general anaesthesia. Each of the five reported studies contributed to answer the complex question: are impala a practical choice of specie to be used as a live animal research model?

The thesis also highlights the practicality of using impala as a research model. Through the experience gained from this study series, a number of practical recommendations are proposed to mitigate unnecessary suffering and improve welfare standards to ensure successful and ethical use of impala in future studies.

7.1 Drug effects of chemical immobilisation and general anaesthesia

The investigations into the cardiopulmonary effects of the immobilisation and general anaesthesia drug combinations yielded interesting, yet predictable results where impala suffered from hypoxaemia, hypercapnia and acidaemia (Meyer et al. 2008b; Perrin et al. 2015; Zeiler et al. 2015; Buck et al. 2017; Gerlach et al. 2017). Some of the findings when using thiafentanil-medetomidine and etorphine-medetomidine were unexpected, such as: the prolonged time to recumbency, and the apparently normal minute volume despite hypercapnia being present (Zeiler & Meyer 2017a). Also, this was the first report of a total intravenous anaesthesia protocol for use in impala, and the acid-base status of impala undergoing immobilisation and general anaesthesia (Zeiler et al. 2015; Zeiler & Meyer 2017b). The outcomes of the first three investigations provide a better understanding of the effects the drugs have on impala and allow clinically relevant recommendations to be proposed, which could improve the safety and welfare of impala immobilisation and general anaesthesia.

The hypoxaemia, hypercapnia and acidaemia were not unexpected and these derangements are common findings in drug combinations that include a potent opioid (Meyer et al. 2008a; Kock &

Burroughs 2012; West et al. 2014). However, various oxygenation and ventilation indices were calculated in an attempt to better understand the causal mechanisms of the hypoxaemia and hypercapnia (Armstrong et al. 2007). Also, two clinically relevant approaches were used to analyse the acidaemia (Henderson 1908; Hasselbalch 1916; Stewart 1978). The traditional aetiology of these gas derangements (hypoxaemia and hypercapnia) is opioid-induced respiratory depression, as noted in primates when they receive clinical doses of non-potent opioids (morphine, fentanyl, butorphanol etc.) for analgesia or anaesthesia induction (Ko et al. 2003). The effects of this opioid-induced respiratory depression is characterised in primates by a shift to the right in the line that describes the ventilatory (minute volume) response to increasing arterial carbon dioxide ($PaCO_2$), indicating that the threshold for carbon dioxide to stimulate a breath (or maintain minute volume) must be higher than the normal 35-45 mmHg range (Ko et al. 2003; Clarke et al. 2014; Grimm et al. 2015). Some opioids also blunt the ventilatory response, whereby the gradient of the slope is flatter than the normal ventilatory response (Grimm et al. 2015). In general, primates (human and non-human) are likley more sensitive to the respiratory depressant effects of opioids than are commonly domesticated species (equine, canine, feline, bovine and caprine) (Lin & Walz 2014; Grimm et al. 2015). Hence, extrapolating information from primates to other species may not be appropriate when trying to identify the aetiology of blood gas derangements. Furthermore, when primates receive the more potent opioids (fentanyl, for example), then the aetiology of the gas derangements becomes more complex. Thus, calling the response opioid-induced respiratory depression or hypoventilation (defined as a decrease in minute volume) is inadequate and the term opioid-induced respiratory impairment is more accurate (Ko et al. 2003). The term opioid-induced respiratory impairment allows for other aetiologies to be considered as the mechanism behind the gas derangements, these mechanisms probably operate alongside opioid-induced hypoventilation. The term hypoventilation has been defined as inadequate alveolar minute ventilation compared to the production of carbon dioxide from cellular metabolism thereby hypercapnia develops (Grimm et al. 2015). However, hypercapnia can result from aetiologies other than hypoventilation, such as hyper-metabolism or hyperthermia (Grimm et al. 2015). Unfortunately we did not measure metabolism and did not detect hyperthermia that could explain the hypercapnia. The oxygenation indices (described in chapters 2 and 3) indicated that the alveoli-capillary interface did not allow sufficient oxygen to enter the capillary blood, thereby contributing to the hypoxaemia we measured (Armstrong et al. 2007). Also, when oxygen was supplemented, the response was poor, suggesting that

hypoventilation was not a major contributor to the oxygenation derangements (Tang et al. 2005; Armstrong et al. 2007; Grimm et al. 2015). Oxygen supplementation during simple hypoventilation usually increases PaO_2 towards the expected levels and may be accompanied by a low $P(A-a)O_2$ gradient (Dugdale 2010; Grimm et al. 2015). In our impala, we did not notice this typical response and the $P(A-a)O_2$ gradient was wider than expected (Zeiler et al. 2015). The $PaCO_2$ progressively increased over time and was well above 45 mmHg, which, on cursory examination, suggests simple hypoventilation (the most common cause of hypercapnia in patients under general anaesthesia) (Grimm et al. 2015). Carbon dioxide is very soluble and can easily cross barriers between tissues, such as at the alveolar-capillary interface. Therefore, in patients suffering simple hypoventilation, normally the $P(a-et)CO_2$ gradient remains within normal limits (Armstrong et al. 2007; Grimm et al. 2015). However, in our impala, the $P(a-et)CO_2$ gradient was wider than expected (Zeiler et al. 2015). Considering these oxygenation and ventilation indices, it is clear that simple hypoventilation was not the only aetiology causing the observed gas derangements. Furthermore, we measured tidal volumes and calculated minute volumes and they were within measured impala and small stock ranges (awake), which again indicates that simple hypoventilation was not a major contributor to the observed gas derangements (Hales & Webster 1967). However, we did not measure alveolar tidal and minute volumes, only total minute volumes, but dead space to tidal volume ratios were approximately 30% calculated using a modified Bohr-Enghoff equation; this suggests adequate alveolar tidal ventilation (Enghoff 1983). We applied the single-breath test to determine the end-tidal carbon dioxide concentration using the capnograph (Aitken & Clark-Kennedy 1928). The single-breath test to obtain a single end-tidal carbon dioxide concentration, instead of a mixed expired carbon dioxide concentration, has been successfully described in mechanically ventilated dogs (Mosing et al. 2010). The major limitation of our dead space to tidal volume ratio measurements is that our impala were spontaneously ventilating and we used the single-breath end-tidal concentration values. Therefore, the calculated ratio can only suggest that the ratios were within normal limits for domesticated small stock ruminants (Grimm et al. 2015).

The major clinical relevance of these findings is that an impala that appears to be breathing at a normal respiratory rate may in fact still suffer from profound hypoxaemia and hypercapnia. The oxygenation and ventilation indices I calculated suggest that other aetiologies must be considered as the cause of these gas derangements, but they do not allow for an exact mechanism to be proposed (Armstrong et al. 2007). The three most likely causes include pulmonary hypertension (drug enduced effect by the

potent opioids or medetomidine causing a severe ventilation to perfusion mismatch), increase in the alveoli-capillary diffusion barrier (due to pulmonary hypertension or leukocyte degranulation) and severe right-to-left intrapulmonary shunting (atelectasis due to lateral recumbency position during the general anaesthesia or pulmonary hypertension) (Duggan et al. 2005; Kathirgamanathan et al. 2009; Grimm et al. 2015).

Pulmonary hypertension has been described in goats receiving etorphine alone (Meyer et al. 2015), and the reported gas derangements were similar to my finings reported in this thesis. Pulmonary hypertension has been shown to cause clinically relevant hypoxaemia, although there are proposed mechanisms to explain how pulmonary hypertension could cause these derangements such as 1) an increase in right-to-left intrapulmonary shunting (Vodoz 2009), 2) increase interstitial fluid movement that widens the alveoli-capillary diffusion barrier (Meyer et al. 2015), and 3) a decreased time for oxygenation to take place (Kästner 2006). Therefore, pulmonary hypertension might be the initiating cause of the gas derangements and therefore research should be focused on preventing the potent-opioid induced pulmonary hypertension and not only focused on hypoventilation. Meyer et al. (2015) did describe that pulmonary hypertension in etorphine treated goats results in the hypoxaemia. They also noted that there was a decrease in the measured minute volume from 13.7 ±3.3 L min^{-1} to 7.6 ±2.7 L min^{-1} and termed this decrease as hypoventilation. Yet, the measured minute volumes where well within expected ranges for concisous domesticated small stock ruminants, which range from 6.3 to 10.4 L min^{-1} (Bakima et al. 1988; Hales & Webster 1967). The initial reading was higher than expected, being 13.7 L min^{-1}, which suggests that the goats were in fact hyperventilating (increase in minute volume). The explanation for the hyperventilation is perhaps due to the instrumentation of the goats being performed while they were awake, and that the placement of the arterial and pulmonary artery catheter caused some stress. Furthermore, the stress could have continued when the tight fitting face mask was placed to measure the minute volume during the baseline readings. Therefore, once the etorphine was administered, the goat's conscious perception became obtunded and the minute ventilation decreased, yet was well within the expected minute ventilation for small stock. Again, Meyer et al. (2015) proved that pulmonary hypertension plays a significant role in causing the gas derangements. However, that said, the reader must be aware that the potent opioids can induce profound alterations in the respiratory pattern that are evident within the first 10 minutes of immobilisation (Zeiler & Meyer 2017a). These alterations include apnoea, apneustic, Biot's and Cheney-Stokes breathing patterns (Grimm et al.

2015). These altered breathing patterns within the early phase of immobilised impala, using potent-opioid drugs, are dangerous and must be corrected immediately. Butorphanol, when administered 1:1 by weight to the potent-opioid (etorphine and thiafentanil) resolves apnoea and improves the breathing pattern without causing arousal (Zeiler & Meyer 2017a). Furthermore, thiafentanil-based combinations may require more than one butorphanol administration to correct the breathing pattern (Zeiler & Meyer 2017a). Once the breathing pattern is resolved, the minute volume stabilises to a normal range, but the gas derangements do not correct with the improved minute ventilation.

Investigation into the increase in the alveoli-capillary diffusion barrier and severe right-to-left intrapulmonary shunting is warranted in impala immobilised with potent-opioids alone or in various combinations. Although, both aetiologies could emanate from the pulmonary hypertension, technologies exist that can aid in the diagnosis of the gas derangements, such as electrical impedance tomography and volumetric capnography (Mosing et al. 2017). These technologies could be included in impala that are instrumented with a pulmonary artery catheter, arterial catheter and spirometer to improve our overall detection of the aetiology of the gas derangements (Grimm et al. 2015). The microanatomy of the alveolar-capillary interface and overall macroanatomy of the cardiovascular and pulmonary systems in antelope are worthy of consideration. A better understanding of the anatomy and physiology will allow for targeted research aimed to correct these derangements. Research teams are already attempting to resolve the apnoea and altered respiratory patterns noticed during the early phase of immobilisation (Meyer et al. 2010; Haw et al. 2016a, b). These interventions include administering serotonin agonists, ampikines, opioid mixed agonist-antagonists and analeptics. These interventions have shown variable results, but the serotonergic agonists and ampikines seem promising for resolving the overall gas derangements during the early phase of immobilisation (Meyer et al. 2010; Haw et al. 2016a). Some serotonergic agonists transiently improve the $P(A-a)O_2$ gradient and oxygenation without changing the minute ventilation. However, some serotonergic agonists cause profound pulmonary hypertension (Brunton et al. 2005), therefore the the mechanism behind the improvement in oxygenation is complex and warrants further investigation. Another, yet unmeasured or investigated plausible aetiology for the gas derangements according to the Author, is the effect of local system pressure gradients and its ability to drive gas across a diffusion barrier. As described above, oxygen and carbon dioxide diffuse between the alveolar gas and the pulmonary capillary blood down partial pressure gradients. However, the system pressures, i.e. the actual pressure in the alveoli (which

normally approximates atmospheric air pressure) and in the pulmonary capillary (which is intermediate between pulmonary arterial and venous pressures, and which fluctuates above atmospheric pressure), might play a role in how easily each gas can diffuse along its own partial pressure gradient, through a tissue barrier from one state (gas) to another state (liquid). In pulmonary hypertension, the system pressure within the capillary is greater than normal, and therefore the partial pressure gradient of oxygen might need to be larger to encourage the same amount of oxygen to diffuse from the alveoli (normal system pressure) into the capillary blood (higher system pressure).

The reported acid-base status of immobilised and anaesthetised impala where the resultant pH was interpreted by using the Henderson-Hasselbalch and the Stewart approaches (Zeiler & Meyer 2017b). The acidaemia was not unexpected and it was broadly classified as originating from hypercapnia, which traditionally indicates respiratory depression. However, as noted above, we did not detect respiratory depression by the classic definition of hypoventilation (inadequate alveolar ventilation compared to carbon dioxide production). Also, carbon dioxide readily crosses a wider alveoli-capillary diffusion barrier due to is high solubility, but we did detect a wide $P(a-et)CO_2$ gradient, which therefore suggests that carbon dioxide was prevented from being exhaled. A wide $P(a-et)CO_2$ gradient and wide $P(A-a)O_2$ gradient can be explained by a large right-to-left intrapulmonary shunt fraction of greater than 20% (Theodore et al. 2013; Tang et al. 2015). Furthermore, maximum carbon dioxide diffusion deficits occur at shunt fractions greater than 50% (Tang et al. 2005). Also, $PaCO_2$ rises and $P(a-et)CO_2$ gradient widens when patients are supplemented with oxygen (Yamauchi et al. 2011). Carbon dioxide is a by-product of normal cellular metabolism and therefore the increase in $PaCO_2$ could be related to an increase in cellular metabolism (Grimm et al. 2015). The potent-opioids can increase sympathetic tone, which in turn could increase cellular metabolism (Buss et al. in press). Although the body temperature of the impala was within the normal range for awake impala (Meyer et al. 2008a; Shrestha et al. 2012), profound muscle rigidity was present which could have resulted in an increase in muscle tissue metabolism causing an increase in $PaCO_2$ production. Regardless, the increase in $PaCO_2$ was the most likely contributor to the acidaemia, as predicted by the Henderson-Hasselbalch approach (Henderson 1908; Hasselbalch 1916). The respiratory acidosis was partialy opposed by an increase in bicarbonate ions suggesting a partial compensatory metabolic response (Henderson-Hasselbalch approach) and increased SIDa and decreased Atot and AG (Stewart approach) (Stewart 1978). The acidaemia has clinical implications whereby it can cause a decrease in cardiovascular performance (decreased

inotropy and vasodilation resulting in hypotension and decrease in cardiac output [Mitchell et al. 1972; Crimi et al. 2012]) and altered oxygen binding to haemoglobin (when $PaCO_2$ is high, haemoglobin acts as a buffer and this reduces its ability to transport oxygen due to the Bohr Effect [Clarke et al. 2014; Muir 2015]). Both result in a decrease in oxygen delivery to the tissues. Furthermore, albumin decreased over time, which could have important intravascular oncotic (Muir 2015) and drug transporting effects (Ascenzi et al. 2014) that could alter the pharmacokinetics of the immobilising drugs. Much about antelope acid-base balance remains unknown and there is a need to determine the effects of acid-base status on cardiovascular and respiratory stability in immobilised and anaesthetised impala, as well as antelope. The preliminary findings of our acid-base status study suggested that acid-base status could play a significant role in how healthy impala respond to the drugs used to achieve chemical immobilisation and general anaesthesia, and as yet the clinical significance of this is unknown. However, the compensatory mechanisms mentioned require normal physiological function and, therefore, when immobilising ill or anorexic wild impala, or other antelope, their acid-base status should be carefully assessed (Zeiler & Meyer 2017b).

The times to recumbency were unexpectedly longed, compared to reports where the potent opioids were used alone (Janssen et al. 1993; Meyer et al. 2010), or in combination with medetomidine (Meyer et al. 2008b), at similar doses as used in this study. Furthermore, the thiafentanil-medetomidine was not quicker at inducing recumbency in the impala compared to etorphine-medetomidine, which contradicts other data (Meyer et al. 2008b) and recommendations that thiafentanil alone or in combination with sedatives results in a faster time to recumbency (Burroughs et al. 2012). Plausible causes for these unexpected times to recumbency were:

> 1) The female impala herd appeared to be calm and did not seem to mount a profound flight response (Haigh 1990); or

> 2) the impala were anxious but did not exhibit flight response behaviours, similar to findings reported by Ables & Ables (1969) where female impala appeared more "resistant" to drug effects as they mounted a profound stress response to darting; or

> 3) misplaced darts or failure of dart discharge were possible, but ruled out by visual inspection of the dart, but we used plain needles and therefore the needle could have moved out of the muscle during the injection phase; or

4) physicochemical drug interactions within the dart which made the combination less effective (Yaksh & Wallace 2011), however, these drug combinations have been mixed in a dart, at higher concentrations, without apparent interaction (e.g., crystallisation) or having reduced efficacy (Meyer et al. 2008b); or, most likely,

5) diluting the drugs (7x dilution) with water to ensure the 3 ml dart was full (a study limitation whereby we standardised the dart size across all trials in the global study project) could have prolonged absorption, despite the total dose being adequate to achieve rapid immobilisation (Meyer et al. 2008b).

The rate of absorption from an intramuscular site of injection, among other factors, is proportional to the drug concentration gradient across the barrier of absorption (muscle cell walls and endothelium) and the surface area, as described in Fick's Law of passive diffusion (Brenner & Stevens 2013). The finding that time to recumbency was long for both drug combinations highlights the importance using care when extrapolating from other species or from other studies within the same species. The variations in study design and population and the methodology and equipment all contribute to an outcome that is unique for that study. Therefore, outcomes can differ greatly between two studies investigating a similar research question, and this phenomenon is particularly evident in wildlife orientated studies. My unexpected results highlight the nature of this phenomenon. Regardless of this phenomenon, the information being published invariably has value and contributes to improving our understanding of immobilisation and general anaesthesia of antelope.

This thesis highlights a first report on a total intravenous anaesthesia protocol that maintained general anaesthesia in impala for 120 minutes (Zeiler et al. 2015). Subsequently, I have used this constant rate infusion to maintain general anaesthesia in free-ranging impala, wildebeest and sable undergoing various soft tissue surgeries with success. Therefore, this field ready protocol can be recommended, but here are cautions that must be heeded. The first major caution is to ensure that the airway is guarded with a cuffed endotracheal tube. Passive regurgitation is common, especially if the animal is placed into lateral or dorsal recumbency during the surgery. The second caution is to have provision for mechanically ventilating the patient for the first 10-15 minutes after immobilisation. If mechanical ventilation is not possible, then butorphanol administered at a 1:1 dose ratio with the potent opioid can be used to help stabilise the respiratory rate and breathing pattern (Zeiler et al. 2015; Haw et al. 2016b;

Buck et al. 2017). Wildlife practitioners can make use of this infusion protocol to prolong immobilisation, or even administer the infusion at dose rates that can induce general anaesthesia for field surgery. This decreases the need to transport impala, or other ungulates, to a veterinary practice for soft tissue surgery. Transporting flighty species, like impala, poses risk for increased morbidity and mortality. Also, there are logistical and financial implications that might deter potential clients from consenting to surgery at a veterinary practice. Therefore, there is an advantage in being able to offer a predictable field-ready drug combination for permitting soft tissue surgery in the field.

7.2 Welfare and ethics of using impala as a research model

This is the first report describing the captive management of impala during an intensive series of studies investigating various immobilisation and general anaesthesia protocols (Zeiler & Meyer 2017c). The free-ranging impala adapted to captive confinement, at relatively high stocking densities for a relatively long period (sixteen weeks), for capture-related research. The methods used to capture the free-ranging impala stems from experience gained in previous studies (Meyer et al. 2008a, b) and thus allowed us to habituate the impala and allow them to maintain optimal health throughout this study series.

Adaption was facilitated by implementing methods that appropriately habituated the impala to confinement and boma management procedures before beginning the intensive series of studies. The process of establishing a daily routine helped in calming the naïve herd to the boma. We began by establishing a fixed daily feeding and watering routine, whereby the impala appeared to adapt to the human-impala interaction. Then, we began to slowly introduce different management procedures each week, such as confining the impala herd to various sections of the boma and firing an unloaded dart projector to minimise startling once the drug trials began. The success of this methodology ensured that we kept the impala under standardised conditions that did not compromise their welfare. We determined this by monitoring various indicators of animal welfare such as: 1) the physical state (body weight, body condition scoring, coat condition, posture and lameness, evidence of diarrhoea); 2) the physiological state (biochemistry and haematology); and 3) the psychological state (changes in behaviour) of the animal (Hawkins et al. 2011). We found all of the monitored indicators to be useful and that we could detect that the impala adapted to captivity progressively over time.

The success of the adaption could also be related to the fact that we purposely acquired an all female herd. Female impala do not demonstrate strong territorial behaviour and there were no male impala to

encourage reproductive behaviours (Skinner 1971; Jarman & Jarman 1973). Therefore, the level of fighting between the impala was minimal. However, they appeared to establish a sort of hierarchal dominance system, because when the impala were placed under stressful conditions (darting or confinement to sections of the boma which increased human-impala interaction) in the first few weeks there seemed to be an increase in head-butting and ramming. Female impala lack horns, and therefore the injuries sustained from this antagonistic behaviour were limited to tissue bruising. No evidence of limping or alterations in gait were evident, again suggesting that the seriousness of the suspected injuries were minimal. We cannot recommend the use of breeding adult males as a research model in captivity, especially if they are part of a mixed herd (Jarman 1970; Murray et al. 1981). Despite food and water not being a limiting resource in captivity, the ability to mate with females is, and therefore male impala will still demonstrate rutting during the breeding season. This behaviour will increase the stress levels within the herd and therefore make standardising the conditions of the study challenging. Other measurements of welfare could also be considered, for example monitoring the rumen environment and microflora could play a significant role in understanding impala adaptation to novel environments, especially if they are repeatedly immobilised. One of the leading causes of death in impala that have undergone a transport to and from a veterinary practice for surgery, is rumen stasis and acidosis. It is unknown what effects immobilisation and general anaesthesia have on the rumen environment in healthy impala, let alone those that are injured, sick and experiencing pain. The value of extrapolating information from domestic ungulates is questionable because the drugs that are used and their diets are very different compared to impala (Cersosimo et al. 2015). Also, we already know that administering anaesthetic drugs to domesticated ungulates alters rumen motility. Likewise, stress and pain are known causes for decreased rumen motility (Kock & Burroughs 2012; Lin & Walz 2014). Other behavioural traits, such as time dedicated to being vigilant, if monitored, could have aided us in detecting subtle herd anxiety. Therefore, we recommend incorporating these two additional indicators of welfare (rumen biology and vigilance) in future studies investigating adaption of wild ungulates to captivity. Overall the impala in our study demonstrated adaptation to captivity and showed that our routine husbandry care did not cause morbidity or mortality. However, our experimental procedures did cause the death of three impala and caused repeated drug-induced hypoxaemia, hypercapnia and acidaemia.

We also monitored our scientific methods and procedures and found that the procedure of darting impala had an unacceptably high mortality rate of 4.4%. Therefore, we confirm that impala are at increased risk of morbidity and mortality whenever human interventions related to capture are performed, as noted previously by Knottenbelt (1990). Darting and chemical capture caused death in our impala, therefore we found it necessary to systematically review chemical capture of impala to determine if there are other factors that can place impala at risk of morbidity and mortality. Environmental, animal, drug, and drug delivery factors were reviewed to highlight potential concerns contributing to the high mortality (4%) and morbidity rates (23%) of impala during chemical capture and immediate confinement. The drug and drug delivery, and animal factors contributed most to the mortality and morbidity rates. Chemical capture through darting increases the chance of the impala sustaining a fatal injury by frantic escape attempts or accidental misplacement of a dart (Zeiler & Meyer 2017c). All currently recommended drug combinations for the chemical capture of impala cause apnoea, hypoxaemia and hypertension (Perrin et al. 2015; Buck et al. 2017; Gerlach et al. 207). Immobilised impala must be continuously monitored for apnoea, hypoxia and hyperthermia, common complications of immobilisation that can rapidly progress with fatal outcomes (Meyer et al. 2008a). The innate nervous disposition of the impala cannot be altered and could be classed as a known risk factor that could compound the pathophysiological effects of the drugs used and increased morbidity and mortality risks. Individual inappropriate stress responses to chemical capture and confinement accelerates the 1) development of cardiorespiratory failure and capture-induced hyperthermia (Meyer et al. 2008a), 2) injuries sustained through escape attempts (Murray et al. 1981), and 3) increases the chance of anorexia and maladaptation. Pain perception in impala contributes to poor adaptation to confinement and promotes anorexia, rumen stasis and death (Lin & Walz 2014; West et al. 2014). Therefore, judicious prolific use of analgesics such as non-steroidal-anti-inflammatory drugs, local anaesthetics or opioids should be practiced.

Much has already been learned about wild antelope capture and confinement from impala. These lessons should be applied by those working with impala, as this species is known to have a greater risk of morbidity and mortality compared to other wild antelope (Murray et al. 1981). By doing so, veterinary management of these animals will be improved and the success of further research into wild antelope capture, confinement, welfare and veterinary management will be enhanced.

7.3 Final summary and recommendations

Firstly, we conclude that all investigated drug combinations used for immobilisation and general anaesthesia caused clinically relevant hypoxaemia, hypercapnia and acidosis. Advanced investigation of cardiopulmonary integration by calculating various oxygenation and ventilation indices suggest the aetiology of the hypoxaemia, hypercapnia and acidosis are due predominantly to either pulmonary hypertension or increased alveolar-capillary diffusion barrier or right-to-left intrapulmonary shunting, and also, only somewhat, to hypoventilation. Apnoea and pronounced muscle rigidity are more common during thiafentanil-medetomidine compared to etorphine-medetomidine drug combinations for immobilisation. Butorphanol (at 1:1 potent opioid dose ratio) is an effective rescue intervention to resolve apnoea and muscle rigidity. Oxygen supplementation is somewhat effective in treating the hypoxaemia. In all cases, tracheal intubation with a cuffed endotracheal tube should be considered essential to secure the airway. The acidaemia appears to be primarily due to an increasing $PaCO_2$ and thus can be classified as respiratory in origin. However, hypoventilation was not a key finding in the immobilised impala under general anaesthesia, indicating the seriousness of the right-to-left shunt possibly contributing to the hypercapnia, or increased cellular metabolism. Regardless of the aetiology of the hypercapnia, both Henderson-Hasselbalch (raise bicarbonate ion) and Stewart (raised strong ion difference, decreased total weak acids and anion gap) analysis of acid base status confirmed that metabolic compensation was present. The clinical significance of the acidaemia, although not severe in this series of studies, should be heeded as it could cause decreased cardiovascular performance and oxygen delivery to metabolising tissues. These findings indicate that we place the impala under physiological risks (hypoxaemia, hypercapnia, acidaemia) when chemically capturing and anaesthetising them using total intravenous anaesthesia techniques. In healthy impala, these physiological risks appear to be manageable.

Secondly, we conclude that free-ranging impala can be captured and placed in captive confinement for a sixteen week period. Abiding to a regular daily management routine (feeding, habituation of humans and various sounds like firing an unloaded dart gun) facilitated rapid adaption to confinement. The adaption was objectively and subjectively measured by monitoring various biomarkers of welfare such as 1) the physical state (body weight, body condition scoring, coat condition, posture and lameness, evidence of diarrhoea); 2) the physiological state (biochemistry and haematology); and 3) the psychological state (changes in behaviour) of the animal. Despite the impala adapting to captive

confinement, we report three deaths. These deaths were a direct result of our scientific methods, whereby we opted to administer the studied drug combinations through darting. The act of chemical capture by darting resulted in fatal injuries (inoperable long bone fractures) and cardiovascular collapse (unresolved apnoea causing myocardial hypoxia and cardiac arrest) that caused mortalities. Because the procedure of darting is essential to wildlife capture, research should be focused on improving the safety of darting and investigating drug combinations that do not cause hypoxaemia, hypercapnia and acidaemia.

Finally, are impala an appropriate choice of specie to be used as a live animal research model for capture, immobilisation and anaesthesia studies?

The unique and novel findings from this series of studies highlights that impala remain an important research model that can inform us of the effects and consequences of capture, immobilisation and anaesthesia in other medium sized antelope. They habituate readily to captive management without compromising their welfare. Therefore, with implementing a routine management programme, free-ranging impala are a practical choice for enrolment into investigations under captive conditions related to wild ungulates, especially medium sized antelope. However, impala are at risk of mortality if the research focus is related to immobilisation and general anaesthesia. Therefore, the information provided within this thesis will assist future researchers to refine their scientific methods and procedures to minimise the risk for morbidity and mortality associated with immobilisation and general anaesthesia. Heeding our observations and recommendations will enhance the success and ensure the ethical use of free-ranging impala as a research model.

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ADDENDUMS

Animal Ethics Certificates

A.1   Certificate V099/13

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	Determination of the efficacy of novel drug combinations for induction and long-term anaesthesia of impala (Aepyceros melampus) infield conditions
PROJECT NUMBER	V099-13
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. L Meyer

STUDENT NUMBER (where applicable)	——————————————————————————
DISSERTATION/THESIS SUBMITTED FOR	Academic

ANIMAL SPECIES	Impala (Aepyceros melampus)	
NUMBER OF ANIMALS	15	
Approval period to use animals for research/testing purposes		March 2014-August 2014
SUPERVISOR	Dr. L Meyer	

KINDLY NOTE:

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

APPROVED	Date	25 November 2013
CHAIRMAN: UP Animal Ethics Committee	Signature	

Animal Ethics Committee

PROJECT TITLE	Determination of the efficacy of novel drug combinations for induction and long-term anaesthesia for impala (*Aepyceros melampus*) in field conditions
PROJECT NUMBER	V099-13 (Amendment 1)
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. L Meyer

STUDENT NUMBER (where applicable)	--	
DISSERTATION/THESIS SUBMITTED FOR	Academic	

ANIMAL SPECIES	Impala (*Aepyceros melampus*)	
NUMBER OF ANIMALS	To be reported	
Approval period to use animals for research/testing purposes		April – December 2014
SUPERVISOR	Dr. L Meyer	

<u>KINDLY NOTE:</u>

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

APPROVED	Date	28 April 2014
CHAIRMAN: UP Animal Ethics Committee	Signature	

A.2 Certificate V012/16

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	Practicality of using impala *(Aepyceros melampus)* as an anaesthesia research model for other wild ungulates
PROJECT NUMBER	V012-16
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. G Zeiler

STUDENT NUMBER (where applicable)	UP_21039993
DISSERTATION/THESIS SUBMITTED FOR	PhD

ANIMAL SPECIES	n/a	
NUMBER OF ANIMALS	n/a	
Approval period to use animals for research/testing purposes		February 2016 – February 2017
SUPERVISOR	Dr L Meyer	

KINDLY NOTE:

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

APPROVED	Date	1 March 2016
CHAIRMAN: UP Animal Ethics Committee	Signature	

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Proof of published articles

A.3 Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (*Aepyceros melampus*)

Page 1 of 8   Original Research

Comparison of thiafentanil-medetomidine to etorphine-medetomidine immobilisation of impalas (*Aepyceros melampus*)

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Impalas (*Aepyceros melampus*) are increasingly valuable in the South African wildlife industry, and there is a greater need to chemically immobilise them, ideally with minimal risk. This study aimed to compare the times to recumbency and physiological effects of thiafentanil-medetomidine versus etorphine-medetomidine immobilisation. A combination of thiafentanil (2 mg) + medetomidine (2.2 mg) and etorphine (2 mg) + medetomidine (2.2 mg) was administered (to nine impalas; crossover design) via a dart. After darting, a stopwatch was started to record times to recumbency (time from darting until recumbent without attempts to stand). If apnoea was present, the impalas received one or more boluses of butorphanol (1:1 potent opioid dose). Data collection included arterial blood gas analysis and a number of butorphanol boluses. Two-sample *t*-tests were used to compare differences between combinations. The time to recumbency for thiafentanil-medetomidine was 12.2 (± 6.8) min and no different from 14.5 (± 5.2) min for etorphine-medetomidine ($p = 0.426$). The thiafentanil-medetomidine combination required more butorphanol boluses (median: 2; interquartile range: 2–3) compared to etorphine-medetomidine (median: 0; interquartile range: 0–1) ($p = 0.001$). Despite butorphanol treatment and resolution of apnoea, all impalas suffered hypoxaemia (PaO$_2$ ± 44.0 mmHg). Thiafentanil-medetomidine did not immobilise impalas more rapidly than etorphine-medetomidine, and resulted in more apnoea that required rescue butorphanol boluses. Marked hypoxaemia resulted from both combinations, mainly because of right-to-left intrapulmonary shunting and not because of hypoventilation. Butorphanol and oxygen supplementation should be considered as essential rescue interventions for all impalas immobilised with these potent opioid combinations.

Introduction

Impalas (*Aepyceros melampus*) have been commonly used as a research animal model for antelope immobilisation in recent years (Meyer et al. 2008; Zeiler et al. 2015). This increased interest is because of impalas and other antelopes being increasingly valuable in the South African wildlife industry; thus, they are commonly immobilised for translocation, and owners are more prepared to consent to invasive surgical interventions and other veterinary treatments in injured animals. To achieve these goals, antelopes need to be immobilised safely. Because of an inherent flight response and nervous disposition, impalas make an excellent animal model to study immobilisation protocols in antelopes (Knox, Hattingh & Raath 1990).

Thiafentanil and etorphine are potent opioids commonly used to immobilise antelope because of their rapid, predictable and reversible effects (Pienaar et al. 1966). Thiafentanil is a fentanyl analogue (of the 4-anilidopiperidine opioid class) and, like fentanyl, has an exclusive affinity for the mu-opioid receptor (Vardanyan & Hruby 2014). It is the next generation of opioid immobilisation drugs, which is claimed to induce more rapid immobilisation and shorter duration of action compared to etorphine (Lance & Kenny 2012). Also, claims that thiafentanil produces less respiratory and cardiac depression, when compared with other potent opioids such as fentanyl, carfentanil and etorphine, exist (Lance & Kenny 2012). Furthermore, thiafentanil is claimed to be the opioid drug of choice in impala immobilisation (Burroughs et al. 2012). Whereas etorphine is a semi-synthetic derivative of the opioid alkaloid thebaine (belonging to the phenanthrene chemical opioid class), which is a non-specific agonist at the mu-, kappa- and delta opioid receptors (Blane et al. 1967; Yaksh & Wallace 2011), medetomidine is a specific alpha$_2$-adrenoceptor agonist used as a synergist with potent opioids in immobilisation drug combinations. It is used for its reliable sedative, good muscle relaxation and analgesic properties (Kästner 2006).

A.4 Etorphine-ketamine-medetomidine total intravenous anaesthesia in wild impala (*Aepyceros melampus*) of 120-minute duration

ETORPHINE–KETAMINE–MEDETOMIDINE TOTAL INTRAVENOUS ANESTHESIA IN WILD IMPALA (*AEPYCEROS MELAMPUS*) OF 120-MINUTE DURATION

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Abstract: There is a growing necessity to perform long-term anesthesia in wildlife, especially antelope. The costs and logistics of transporting wildlife to veterinary practices make surgical intervention a high-stakes operation. Thus there is a need for a field-ready total intravenous anesthesia (TIVA) infusion to maintain anesthesia in antelope. This study explored the feasibility of an etorphine-ketamine-medetomidine TIVA for field anesthesia. Ten wild-caught, adult impala (*Aepyceros melampus*) were enrolled in the study. Impala were immobilized with a standardized combination of etorphine (2 mg) and medetomidine (2.2 mg), which equated to a median (interquartile range [IQR]) etorphine and medetomidine dose of 50.1 (46.2–50.3) and 55.1 (50.8–55.4) µg/kg, respectively. Recumbency was attained in a median (IQR) time of 13.9 (12.0–16.5) min. Respiratory gas tensions, spirometry, and arterial blood gas were analyzed over a 120-min infusion. Once instrumented, the TIVA was infused as follows: etorphine at a variable rate initiated at 40 µg/kg per hour (adjusted according to intermittent deep-pain testing); ketamine and medetomidine at a fixed rate of 1.5 mg/kg per hour and 5 µg/kg per hour, respectively. The etorphine had an erratic titration to clinical effect in four impala. Arterial blood pressure and respiratory and heart rates were all within normal physiological ranges. However, arterial blood gas analysis revealed severe hypoxemia, hypercapnia, and acidosis. Oxygenation and ventilation indices were calculated and highlighted possible co-etiologies to the suspected etorphine-induced respiratory depression as the cause of the blood gas derangements. Impala recovered in the boma post atipamezole (13 mg) and naltrexone (42 mg) antagonism of medetomidine and etorphine, respectively. The etorphine-ketamine-medetomidine TIVA protocol for impala may be sufficient for field procedures of up to 120-min duration. However, hypoxemia and hypercapnia are of paramount concern and thus oxygen supplementation should be considered mandatory. Other TIVA combinations may be superior and warrant further investigation.

Key words: *Aepyceros melampus*, etorphine, impala, ketamine, medetomidine, TIVA.

INTRODUCTION

Etorphine immobilization in impala (*Aepyceros melampus*) has been studied since the 1960s.[1,7] Despite decades of use in impala, etorphine immobilization has only been described and no reports are available mentioning its use for the long-term maintenance of surgical anesthesia. The lack of its incorporation into anesthetic maintenance protocols is perhaps because of pronounced

dose-related respiratory depression.[4,23,24] In order to decrease the dose of etorphine and thus its undesirable characteristics, it can be used in a combination with other drugs with known analgesic and anesthetic sparing effects, such as a low dose of ketamine (a cyclohexamine dissociative anesthetic) or medetomidine (an alpha₂-adrenoceptor agonist) to achieve surgical anesthesia.[16]

In South Africa, because of the importance of antelope for conservation and the value of these animals in zoo and private wildlife collections, veterinary intervention is often required.[5,20] These animals, in particular impala, are prone to injury and are often presented to veterinarians for surgical intervention. Long-duration transport to veterinary facilities, field equipment, manpower limitations, and high cost make surgery on wildlife a challenge. A reliable field anesthetic protocol that does not require the logistical challenges of inhalation agents may make surgical interventions more accessible to antelope situated in remote

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A.5 Blood acid-base status in impala (*Aepyceros melampus*) immobilised and maintained under total intravenous anaesthesia using two different drug protocols

RESEARCH ARTICLE **Open Access**

Blood acid-base status in impala (*Aepyceros melampus*) immobilised and maintained under total intravenous anaesthesia using two different drug protocols

Gareth E. Zeiler[*] and Leith C. R. Meyer

Abstract

Background: In mammals, homeostasis and survival are dependent on effective trans-membrane movement of ions and enzyme function, which are labile to extreme acid-base changes, but operate efficiently within a narrow regulated pH range. Research in patients demonstrating a pH shifts outside the narrow regulated range decreased the cardiac output and systemic vascular resistance and altered the oxygen binding to haemoglobin. These cardiopulmonary observations may be applicable to the risks associated with anaesthesia and performance of wildlife ungulates on game farms. The aim of this study was to compare blood pH changes over time in impala immobilised and anaesthetised with two different drug protocols (P-TMP – immobilisation: thiafentanil-medetomidine; maintenance: propofol-ketamine-medetomidine; P-EME – immobilisation: etorphine-medetomidine; maintenance: etorphine-ketamine-medetomidine). Additionally, we discuss the resultant blood pH using both the Henderson-Hasselbalch and the Stewart approaches. Two data collection time points were defined, Time1 before maintenance of general anaesthesia and Time 2 at end of maintenance of general anaesthesia. We hypothesise that blood pH would not be different between drug protocols and would not change over time.

Results: Significant differences were detected over time but not between the two drug protocols. Overall, the blood pH decreased over time from 7.37 ± 0.04 to 7.31 ± 0.05 ($p = 0.001$). Overall, over time arterial partial pressure of carbon dioxide changed from 51.3 ± 7.5 mmHg to 72.6 ± 12.4 mmHg ($p < 0.001$); strong ion difference from 44.6 ± 2.4 mEq/L to 46.9 ± 3.1 mEq/L ($p < 0.001$); anion gap from 15.0 ± 3.1 mEq/L to 10.9 ± 2.2 mEq/L ($p < 0.001$); and total weak acids from 16.1 ± 1.2 mmol/L to 14.0 ± 1.1 mmol/L ($p < 0.001$). The bicarbonate changed from 29.6 ± 2.7 mEq/L to 36.0 ± 4.1 mEq/L ($p < 0.001$); and lactate changed from 2.9 ± 1.5 mEq/L to 0.3 ± 0.03 mEq/L ($p < 0.001$) over time.

Conclusions: The profound increase in the partial pressure of carbon dioxide that worsened during the total intravenous anaesthesia in both protocols initiated a substantial metabolic compensatory response to prevent severe acidaemia. This compensation resulted in a clinically acceptable mild acidaemic state, which worsened over time but not between the protocols, in healthy impala. However, these important compensatory mechanisms require normal physiological function and therefore when immobilising ill or anorexic wild ungulates their acid-base status should be carefully assessed.

Keywords: Blood pH, Impala, *Aepyceros melampus*, Immobilisation, General anaesthesia, Henderson-Hasselbalch, Stewart approach

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A.6 Captive management of wild impala (*Aepyceros melampus*) during intensive immobilisation and general anaesthesia study trials

CAPTIVE MANAGEMENT OF WILD IMPALA (*AEPYCEROS MELAMPUS*) DURING INTENSIVE IMMOBILIZATION AND GENERAL ANESTHESIA STUDY TRIALS

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Abstract: Immobilization and anesthesia of impala (*Aepyceros melampus*) has become a popular research theme. This demand is brought about by the increased need to immobilize and anesthetize impala and other medium-sized wild ungulates because of their increased value in game ranching and zoological collections. To improve our understanding of immobilization and general anesthesia in these species, it is paramount to be able to study them in a practical, safe research environment that does not cause harm or unnecessary stress to the animals. This study aimed to scrutinize our management and welfare practices and scientific methods of 15 wild-caught impala placed in captive confinement during a 16-wk intensive research project. The scientific methods of the project were scrutinized to identify procedures that attributed to morbidity and mortality. Indicators of impala welfare during captivity were monitored by documenting serial physical (body weight, coat condition), physiological (biochemistry and hematology), and psychologic (behavioral) aspects. Two impala suffered irreparable femoral fractures during darting and were humanely euthanized. One impala suffered cardiovascular collapse during immobilization and could not be resuscitated. The procedure of chemical capture had a morbidity and mortality rate of 14.8% and 4.4%, respectively. The impala maintained acceptable physical and physiologic parameters, including stable body weights, well-groomed coats, and values for total serum protein, serum creatinine, and hematologic parameters that were within reference ranges for the species. There were improvements in the impala's psychological parameters, which included a decrease in the number of aggressive interactions (head butting and ramming) and an increase in the number of reciprocal allogrooming interactions. The monitored welfare indicators suggest adaptation to captivity. The study showed that impala could be successfully managed in captivity for 16 wk. However, scientific methods (namely darting) increased the risk of injury and caused fatalities.

Key words: *Aepyceros melampus*, impala, refinement, scientific methods, welfare.

INTRODUCTION

Research in wild ungulates, such as impala (*Aepyceros melampus*), has become increasingly important during the past few years.[5,12,13,14,17,43] This surge in invasive veterinary interventions in wild ungulates stems mainly from zoological collections and game farms. Furthermore, the authors' experience proposes that extrapolation of data from studies in domesticated ungulates, such as goats (*Capra aegagrus hircus*), do not always reliably reflect physiologic responses to field-based immobilization and anesthetic procedures.[3,40,42] Wild ungulates' anxious disposition and questionable ability to adapt to novel environments are leading differences compared with the more stoic and adaptive domesticated ungulates.[9]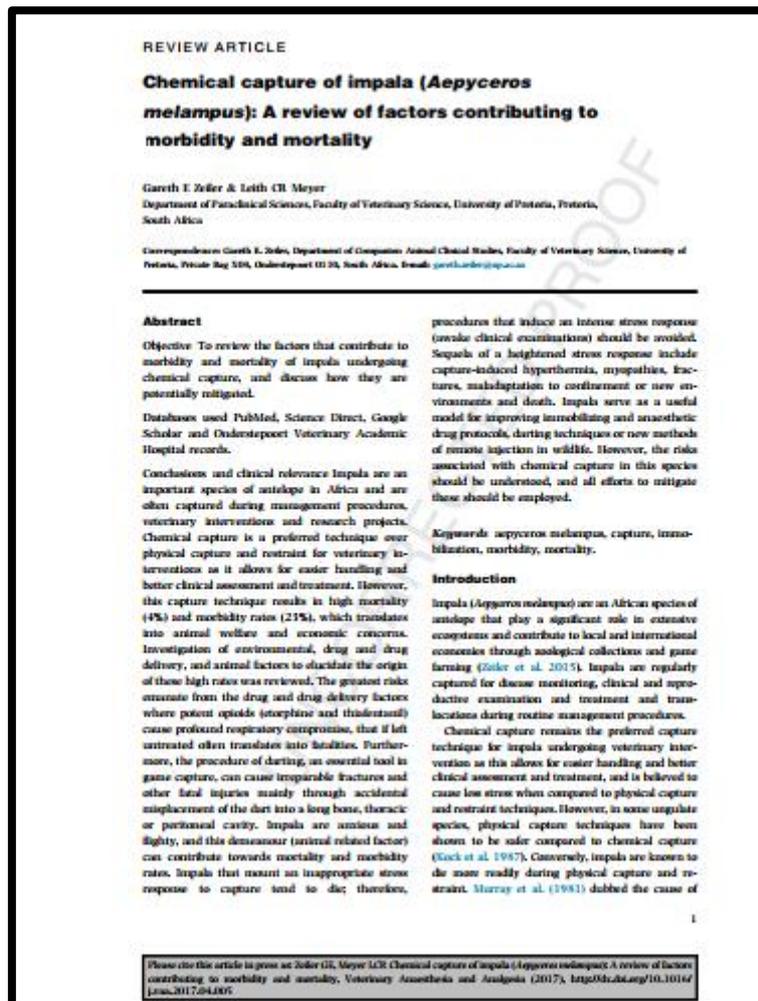 Therefore, in some instances, using domesticated animals as replacement models for wild ungulates can be fraught with difficulty when interpreting and extrapolating findings.

Impala have been used in numerous capture (physical and chemical immobilization) studies during the past six decades.[13,6,20,34,37,41,43] They are medium in size, relatively easy to physically restrain, affordable to feed, and herds can occupy a minimal space in outdoor enclosures (bomas). Experimental experience suggests that impala maintain their nervous and flighty disposition despite perceived adaptation to boma confinement, but they can suffer from maladaptation, anorexia, injury sustained during frantic attempts to escape physical capture or confinement, nocturnal predation, or capture myopathy.[16,20,34,38] These points highlight the reasons why impala remain a popular research model for capture and anesthesia studies, but the risk of morbidity and mortality poses obvious welfare and ethical questions.[34] Therefore, scrutinizing scientific methods and welfare standards are needed to identify risks and to provide recommendations to refine future management and husbandry practic-

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A.7 Chemical capture of impala (*Aepyceros melampus*): a review of factors contributing to morbidity and mortality