Propofol-medetomidine-ketamine total intravenous anaesthesia in thiafentanil-medetomidine immobilised impala (*Aepyceros melampus*) of 120 minute duration

By

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

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Declaration of originality

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List of abbreviations

%	percentage
>	greater than
<	less than
=	equal to
^{o}C	degrees Celsius
µg	microgram(s)
boma	high-walled outdoor enclosure for housing wild ungulates
cAMP	cyclic adenosine monophosphate
ECG	electrocardiogram
EDTA	ethylenediaminotetreaacetic acid
$EtCO_2$	end-tidal carbon dioxide tension
ET-tube	endotracheal tube
F_EO_2	fractional expired oxygen concentration
FiO_2	fractional inspired oxygen concentration
GABA	gamma-aminobutyric acid
HCO_3^-	bicarbonate ion
IM	intramuscular
IQR	interquartile range
IV	intravenous
kg	kilogram(s)
kPa	kilo Pascals
L	litre(s)
mg	milligram(s)
min	minute(s)
mL	millilitre(s)
mmHg	millimetres Mercury
mmol	millimole(s)
n	number of animals
NMDA	N-methyl-D-aspartate
$PaCO_2$	arterial carbon dioxide tension
pH	negative log of hydrogen ion concentration
PaO_2	arterial oxygen tension
SC	subcutaneous
SpO_2	peripheral oxygen haemoglobin saturation
Vd	respiratory dead space volume (mL)
Vt	tidal volume (mL)

Summary

Propofol-medetomidine-ketamine total intravenous anaesthesia in thiafentanil-medetomidine immobilised impala (*Aepyceros melampus*) of 120 minute duration

By

ROXANNE KATE BUCK

Promoter: Dr Gareth Zeiler

Co-promoter: Dr Leith Meyer

Department: Companion Animal Clinical Studies

Degree: Master of Science (Veterinary Science)

Objective To characterise a propofol-medetomidine-ketamine total intravenous anaesthetic protocol in impala (*Aepyceros melampus*).

Study design Prospective clinical study.

Animals Ten adult female impala, weighing 39 (±4) kg.

Materials and methods Impala were immobilised with 2 mg thiafentanil and 2.2 mg medetomidine via projectile darts. Propofol was given to effect (0.5 mg kg^{-1} boluses) to allow

endotracheal intubation, following which oxygen was supplemented at 2 L min^{-1}. Anaesthesia was maintained with a constant rate infusion of medetomidine and ketamine at 5 µg kg^{-1} h^{-1} and 1 mg kg^{-1} h^{-1}, respectively, and propofol to effect (initially 0.2 mg kg^{-1} min^{-1}) for a period of 120 minutes. The propofol infusion was titrated according to reaction to nociceptive stimuli every 15 minutes. Cardiopulmonary parameters were monitored continuously and arterial blood gas samples analysed intermittently. At 120 minutes maintenance the thiafentanil and medetomidine were antagonised using naltrexone (10:1 thiafentanil) and atipamezole (5:1 medetomidine), respectively, and recoveries scored.

Results All impala were successfully immobilised, with a median (IQR) time to recumbency of 9.6 (7.2-14.4) minutes. The median (IQR) dose of propofol required for intubation was 2.7 (1.9-3.3) mg kg^{-1}. The propofol-medetomidine-ketamine combination ensured recumbency for the 120 minute period. Propofol titration showed an erratic downward trend; a minimum infusion rate was not determined. Heart rate, respiratory rate and arterial blood pressure were well maintained. Arterial blood gas analysis indicated marked hypoxaemia, hypercapnia and acidosis. All impala regurgitated frequently during the maintenance period. Recovery was calm and rapid in all animals. Median (IQR) time to standing from antagonist administration was 9.4 (8.2-10.6) minutes.

Conclusions and clinical relevance A propofol-medetomidine-ketamine combination can provide adequate anaesthesia for invasive procedures in impala for up to 120 minute duration. The propofol infusion should begin at 0.2 mg kg^{-1} min^{-1} and be titrated to clinical effect. Oxygen supplementation and airway protection with a cuffed endotracheal tube are essential.

Keywords *Aepyceros melampus*, impala, ketamine, medetomidine, propofol, TIVA

Literature review

Current practises in anaesthesia of wild ungulates

Many sedative and anaesthetic protocols have been used in wild ungulates. Traditionally, injectable anaesthetic agents have been used for immobilisation and short surgical procedures, with inhalation anaesthesia being used for maintenance during more invasive procedures (Auer et al. 2010).

A number of drug combinations have been used for immobilisation and occasional maintenance in wild ungulates. Potent opioids have been widely used to immobilise wild ungulates because they result in quick immobilisation. However, their use is associated with poor respiration, muscle rigidity and an inadequate plane of anaesthesia making them unsuitable for prolonged and intensive surgical procedures (Meyer et al. 2008a). Alternatives, such as combinations of ketamine and medetomidine have been shown to provide good respiration and muscle relaxation but the level of anaesthesia may be insufficient for major surgical procedures (Bush et al. 2004). Thus, despite wide-spread use of injectable agents for immobilisation, there is no widely accepted injectable protocol for maintenance of anaesthesia.

Inhalation maintenance of anaesthesia in wild ungulates is also not without its disadvantages. Inhalation agents have been shown to cause dose-dependent respiratory and cardiovascular depression making their use in high quantities undesirable (Clarke et al. 2014, Dzikiti 2013). Further, inhalation agents require bulky, sophisticated and expensive equipment such as anaesthetic machines, which are often impractical in field and zoo settings (Dzikiti 2013). Inhalation anaesthesia is also associated with increased atmospheric pollution and unscavenged or incorrectly used machines represent significant occupation health hazards to medical personnel (Dzikiti 2013).

Total intravenous anaesthesia

Total intravenous anaesthesia (TIVA) uses only intravenous anaesthetic agents to maintain an adequate depth of anaesthesia (Dzikiti 2013). TIVA requires less equipment than inhalation maintenance, making it a feasible option for field use. In its simplest form, TIVA requires only an intravenous catheter and drugs, although endotracheal intubation and oxygen supplementation are recommended during anaesthesia (Larenza et al. 2005) and infusion pumps allow increased precision in drug delivery (Dzikiti 2013). TIVA also lends itself to balanced anaesthesia protocols, whereby several drugs are combined at lower than normal doses to decrease the adverse side effects of each drug while still achieving the desired level of effect (Dzikiti 2013). TIVA protocols are also often associated with a smoother recovery (Dzikiti 2013). Drugs used to maintain TIVA should be short acting, non-accumulative, easy to titrate, quickly redistributed from the central compartment or metabolised and have a high therapeutic index (Clarke et al. 2014). Currently, there is scant information in the scientific literature detailing TIVA protocols in wild ungulates (Auer et al. 2010).

Drugs of interest

A novel protocol for maintenance of anaesthesia in wild ungulates is a propofol-medetomidine-ketamine total intravenous infusion.

Propofol (2,6-di-isopropylphenol) is an ultra-short acting injectable anaesthetic agent that induces central nervous system depression by enhancing the effects of the inhibitory neurotransmitter γ-aminobutyric-acid (GABA) through binding to $GABA_A$ receptors (Clarke et al. 2014). $GABA_A$ receptors are pentameric membrane proteins found throughout the central nervous system that function as GABA-gated chloride channels, which when activated cause a

hyperpolarisation of the neuron with decreased action potential transmission (Rudolph et al. 2001).

Following intravenous injection of propofol, the onset of action is rapid as a result of quick uptake into the central nervous system. Initial recovery is a result of tissue redistribution. Propofol is rapidly metabolised by the liver's cytochrome P450 system to inactive glucuronide conjugates that are excreted in the urine (Clarke et al. 2014). Extra-hepatic metabolism, possibly in well perfused tissues such as the lungs and kidneys, is thought to contribute to the swift metabolism. Clearance from plasma is rapid, such that residual sedation is hardly seen (Clarke et al. 2014) and rapid, smooth recovery characteristics are maintained in most species even following prolonged infusions (Grimm & Lamont 2007).

Major side effects of propofol are dose-dependent cardiovascular and respiratory depression. Propofol causes decreased arterial blood pressure, through arterial and venous vasodilation, as well as decreased myocardial contractility and dose-dependent respiratory depression which may result in hypoxia (Clarke et al. 2014).

Medetomidine (4-[1-(2,3-dimethylphenyl) ethyl]-1H-imidazole monohydrochloride) is an α_2-adrenoreceptor agonist that produces dose-dependent sedation, analgesia and muscle relaxation (Lemke 2007). Further, these effects can be reliably reversed by the α_2-adrenoreceptor antagonist atipamezole (Clarke et al. 2014). The effects of the drug are terminated by its removal from target tissues, which is paralleled by biotransformation in the liver (Lemke 2007). Inactive metabolites are excreted in the urine.

The sedative effects of medetomidine are the result of activation of α_2-adrenoreceptors in the brain while the analgesic effects are spinally mediated (Lemke 2007). The α_2-adrenoreceptors are transmembrane G-protein coupled receptors whose activation results in a conformational change causing a series of cellular responses, notably including decreased adenylyl cyclase activity and reduced intracellular cAMP concentration (Bylund 1992). Importantly, medetomidine markedly decreases the required dose of parenteral anaesthetics to maintain general anaesthesia (Jalanka & Roeken 1990).

The most important side effects of medetomidine administration are on cardiopulmonary function. Administration is usually accompanied by an initial vasoconstriction, hypertension and baro-reflex vagal nerve (parasympathetic) mediated bradycardia, followed by a central phase of decreased sympathetic tone, bradycardia and possible hypotension (Lemke 2007). The central phase is caused by a decrease in sympathetic tone resulting from activation of presynaptic α_2-adrenoreceptors decreasing noradrenaline release (Lemke 2007). Cardiac output can decrease dramatically as a result of the bradycardia and increased systemic vascular resistance (Clarke et al. 2014). In ruminants, tachypnoea has been observed in conjunction with increased respiratory effort and decreased arterial oxygen tension (PaO_2) which results in haemoglobin desaturation (Clarke et al. 2014). These hypoxic effects are thought to be brought about by pulmonary changes, including shunting, ventilation-perfusion mismatching and pulmonary oedema (Kästner 2006).

Ketamine hydrochloride is a cyclohexylamine derivative that produces a dissociative anaesthesia. It is thought to disrupt the transmission between parts of the brain with selective cortical and thalamic depression and simultaneous stimulation of the limbic system, thus producing cataleptic, analgesic and anaesthetic effects without any hypnosis (Clarke et al. 2014).

Antagonism of N-methyl-D-aspartate (NMDA) receptors is thought to be the most likely cause for most of the anaesthetic, amnesic, psychomimetic and neuroprotective effects of ketamine (Kästner 2007). NMDA receptors are ion-channel coupled receptors and ketamine acts as a non-competitive antagonist at the Ca^{2+} channel pore to inhibit excitatory potential (Hirota & Lambert 1996).

Recovery from a single injection of ketamine is by redistribution from the central compartment to peripheral compartments such as muscle and lipid tissue; hepatic metabolism becomes more important following repeated injection or continuous infusion (Grimm & Lamont 2007). Ketamine is metabolised primarily to norketamine and dihydronorketamine. The former is active, with one fifth to one third the activity of ketamine, and may contribute to prolonged anaesthetic effects associated with ketamine (Grimm & Lamont 2007).

Ketamine has a direct negative inotropic effect on the myocardium through disrupting the normal Ca^{2+} ion cycling during muscle contraction (Kongsayreepong et al. 1993). However, this is countered by a centrally-mediated sympathomimetic effect which results in an increased heart rate and blood pressure mainly thought to be due to the release of noradrenaline and release of catecholamines from the adrenal gland (Kästner 2007). As such, circulation is well maintained in animals with a functioning sympathetic nervous system. At normal clinical doses, respiration is well maintained (Clarke et al. 2014) as is the normal ventilatory response to hypoxia. However, in goats, higher arterial partial pressures of CO_2 have been documented with ketamine than propofol or thiopentone (Prassinos et al. 2005) and overdoses of ketamine or combinations with α_2-adrenoreceptor agonists have been known to result in respiratory depression (Clarke et al. 2014). Ketamine has potent somatic analgesic effects, even at subanaesthetic doses (Kästner 2007).

Important side effects of ketamine include hypersialosis, increased respiratory tract secretions and abnormal behaviour at induction and recovery (Kästner 2007). This abnormal behaviour may include ataxia, increased motor activity, hyperreflexia, sensitivity to touch and sometimes violent recovery; although premedication or co-administration of sedatives and tranquilisers may decrease the incidence and severity of these side effects (Kästner 2007).

Medetomidine-ketamine combinations have been used for a number of decades in wild ungulates (Jalanka & Roeken 1990). Although predominantly used for immobilisation, the combination has even been used for painful procedures, such as a femur fracture repair in an alpine ibex (Jalanka & Roeken 1990). Importantly, ketamine appears to counteract the bradycardia induced by the medetomidine, although decreased peripheral oxygen-haemoglobin saturation (SPO_2) and PaO_2 were noted when this combination was used in impala (Bush et al. 2004).

Propofol infusions are a novel idea in wild ungulates, although propofol has been used to maintain anaesthesia in a number of domestic species including goats (Dzikiti et al. 2009; Dzikiti et al. 2010; Larenza et al. 2005; Prassinos et al. 2005), which are small ruminants, like impala. Goats maintained under propofol continuous infusions showed a high clearance rate of propofol and recovery was rapid after termination of anaesthesia (Prassinos et al. 2005). Goats maintained with a combination of propofol and ketamine also showed good or excellent recovery (Larenza et al. 2005). Rapid, calm recovery after anaesthesia is particularly important in ruminants, because of their predisposition to ruminal tympany and regurgitation of ruminal contents. The rapid recovery characteristic is also vital in wild ungulates to ensure reintegration into the herd and resumption of normal behaviour, particularly if released back into the wild.

Further, although propofol can cause hypotension as a result of decreased vascular resistance, combinations of ketamine and propofol in goats have shown mean arterial blood pressures that were significantly higher than those of goats anaesthetised with sevoflurane (Larenza et al. 2005). It was proposed that the sympathetic action of the ketamine countered the vasodilator activity brought about by the propofol.

Outcome of literature review

To be effective, a TIVA protocol for wild ungulates must allow for flexible control of anaesthetic agent to achieve desired level of anaesthesia and extension of anaesthetic duration with a calm, complete and quick reversal that allows for immediate release. There is currently very little literature available on the use of TIVA in wild ungulates and none on the use of propofol continuous infusions. Thus the proposed drug combination of propofol-medetomidine-ketamine for maintenance in immobilised impala warrants scientific investigation to characterise clinical effects and make recommendation on its use in the field.

Introduction

Historically, management of wild ungulates has been most focussed on capture and translocation. As a result, the major focal point of anaesthetic research and practice in these species has been on immobilisation, with little emphasis on maintenance of anaesthesia for longer periods. However, with their increased numbers and value in zoo and game ranching collections (Bezuidenhout 2013), the ability to safely anaesthetise wild ungulates for more invasive procedures such as surgical implantation of transmitters, orthopaedic surgery or caesarean section is rapidly becoming more important (Meyer et al. 2008a).

Impala (*Aepyceros melampus*) are sentinels for many diseases in southern Africa (particularly Foot and Mouth Disease) and are a commonly encountered species in game ranching (Bush et al. 2004). Impala are a flighty species, well known to be highly excitable and reactive to capture (Meyer et al. 2008b). This feature makes them highly susceptible to stress-related complications (Bush et al. 2004, Meyer et al. 2008b). The difficulties associated with their immobilisation and anaesthesia make impala a good model for wild ungulate anaesthesia.

In this study, a propofol-medetomidine-ketamine TIVA was investigated for its use as a field or hospital anaesthetic maintenance protocol. Propofol was incorporated into the combination to provide hypnosis. Its inclusion allowed for the dose of ketamine to be decreased from an anaesthetic to an analgesic dose, with the aim of minimising the amount of circulating active metabolites that may form during prolonged anaesthesia. Ketamine is a potent somatic analgesic, even at subanaesthetic doses. Medetomidine was included for its analgesic, muscle relaxant and anaesthetic sparing effects.

To mimic filed conditions, the impala were first immobilised before being maintained under general anaesthesia. A commonly used potent opioid and α_2-adrenoreceptor agonist combination of thiafentanil and medetomidine was used to achieve rapid immobilisation (Meyer et al. 2008a).

Thiafentanil oxalate is a potent pure μ-agonist opioid that is associated with fast induction when administered intramuscularly to wild ruminants. Thiafentanil can be associated with respiratory depression, hypotension or hypertension, poikilothermia and either tachycardia or bradycardia (Lance & Kenny 2012). Thiafentanil has a shorter duration of action than carfentanil and etorphine, and as such renarcotisation has never been documented as a problem with this agent (Grimm & Lamont 2007).

The μ-antagonist naltrexone effectively reverses the actions of thiafentanil in two to ten minutes (Lance & Kenny 2012). In addition butorphanol, a κ-agonist μ-antagonist has been shown to reverse excessive sedation associated with pure μ-agonists (Grimm & Lamont 2007) and has been used successfully in other species to partially reverse the respiratory effects of the potent opioids (Lance & Kenny 2012).

Aims and objectives

The primary aim of this study was to evaluate the efficacy of a novel combination of propofol-medetomidine-ketamine for intravenous maintenance of anaesthesia in immobilised impala for use in the field or hospital setting.

The goals and objectives of the anaesthesia included:

1. Maintenance of adequate depth of anaesthesia and analgesia for invasive or painful procedures, as assessed by palpebral reflexes and response to deep pain (claw clamping).
2. Maintenance of stable and optimal cardiopulmonary function during surgical anaesthesia, as indicated by arterial blood pressure and arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) tensions, respectively.
3. Recovery that is calm, rapid, and allows immediate release and reintegration into the herd following termination of anaesthesia.

Hypotheses

H_0: A propofol-medetomidine-ketamine total intravenous infusion will maintain adequate general anaesthesia and stable cardiopulmonary function in anaesthetised impala.

H_1: A propofol-medetomidine-ketamine total intravenous infusion will not maintain adequate general anaesthesia nor stable cardiopulmonary function in anaesthetised impala.

Benefits arising from the study

The principle benefit from this study was to expand our knowledge and understanding of anaesthetic and cardiopulmonary clinical properties of injectable anaesthetic combinations in impala and to develop a TIVA protocol for use in the field. A reliable TIVA protocol could improve the administration and safety of field anaesthesia for impala and other wild ungulates by providing the veterinarian with an effective method of maintaining surgical anaesthesia for prolonged periods without the bulky, expensive equipment needed for inhalation anaesthesia.

Materials and methods

Experimental design

A prospective, descriptive clinical trial was performed.

Animals

Healthy adult female impala weighing a mean (±SD) of 39.0 (±4.27) kg were used. The animals were wild, but habituated to boma housing and management procedures. The impala were externally sourced, from a nearby game ranch, at the start of the trial and sold again at the end of the trial. Fifteen impala were purchased; however, ten impala were selected for use in the trial after a six week boma habituation period. The reason for selecting a subset of animals was to ensure similar sizes of animals used. The other animals remained as part of the herd, and were also sold at the end of the study.

After the habituation period, all impala were immobilised for physical examination. Each impala was weighed and colour-coded ear tags were placed to facilitate easy identification of each animal. Age was estimated by dental and physical examination. In addition, blood samples were drawn in sodium ethylenediaminotetraacetic (EDTA) acid and serum tubes for complete blood count and serum biochemistry, respectively, to determine general health status of the animals. The impala were also treated for ecto- and endoparasites with a long acting macrocyclic lactone (0.02 ml kg^{-1} subcutaneously; Dectomax 1%; Pfizer Laboratories; South Africa).

Housing

For the duration of the trial, the impala were housed on the Onderstepoort Veterinary campus in northern Pretoria, South Africa, in a specially constructed housing pen (boma). The walls of the boma were 2.7 meters in height to prevent escape through jumping. The shade-cloth walls also gave the impression of being solid, which served to both discourage breakout and provide

protection from the elements. There were also numerous trees in the boma, which provided further shade and shelter. To facilitate darting and immobilisation, the boma was subdivided into two sections, one larger area (20 m x 30 m) and one smaller "capture" area (10 m x 30 m) with two interconnecting gates between them.

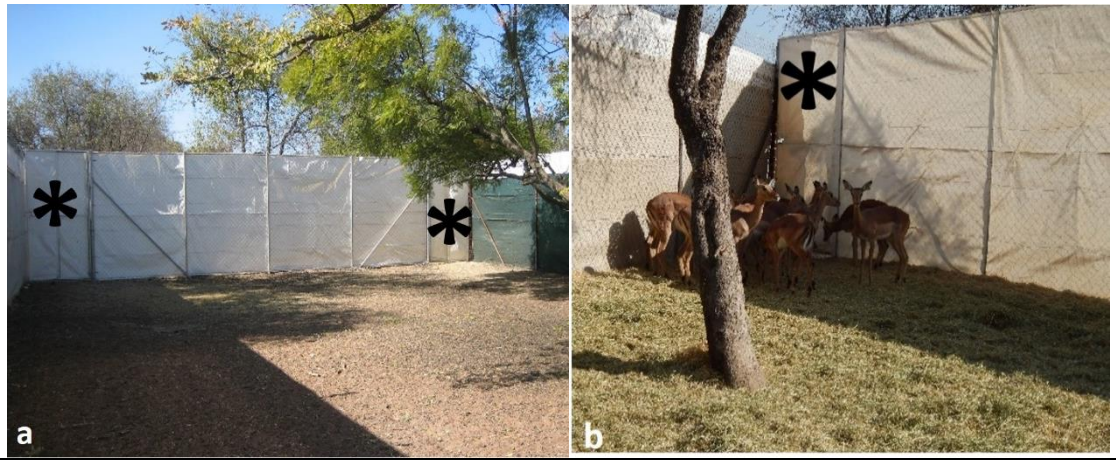


Photo 1 The high-walled outdoor enclosure (boma) the impala were housed in for the duration of the trial showing **(a)** home area and **(b)** capture area. ***** *Indicates interconnecting gates*

Throughout the duration of their stay, the impala had free access to fresh water, hay and lucerne *ad libitum.* In addition, they were supplemented with commercially available antelope pellets as needed to maintain body condition.

To reduce stress associated with changes in environment, the impala were allowed an adaptation period in the boma of six weeks before the trial commenced. During this time, the interconnecting gates were left open, to allow the impala access to the entire area and thus ensure familiarisation with all parts of the boma.

Procedures

The study was approved by the University's Animal Ethics and Research Committees (Protocol number: V042-14). The impala were housed for a total of three months. The impala underwent

four clinical trials encompassing four different immobilisation and three maintenance protocols with a 14 day washout period between trials. The current study details one of the trials. In this trial each of the ten selected impala was anaesthetised once with the protocol. The trial took place at 1252 meters above sea level; the barometric pressure ranged from 665.4 to 670.8 mmHg (88.7-89.4 kPa). The order of impala to be anaesthetised was randomly selected by computer generated randomisation (Excel Microsoft Office 2010; Microsoft Corporation; WA, USA).

Data collection for the study was divided into three phases, namely immobilisation, maintenance and recovery. There was an onsite data collection sheet for each individual animal (see Addendum). Data recorded included date of the trial, animal identification, sex and body mass. All clinical variables were recorded on this sheet for the duration of anaesthesia. In addition, doses of all drugs and infusion rates were recorded, as well as times of darting, recumbency, start of infusion, end of anaesthetic, reversal, extubation, sternal and standing following antagonism of the immobilising agents. Any necessary deviations from the protocol or rescue interventions required were also recorded.

Immobilisation

To reduce the incidence of bloat and regurgitation, concentrate food was withheld for at least 12 hours before all anaesthesias. Thirty minutes prior to each immobilisation, the impala herd was moved to the smaller “capture” area of the boma to facilitate easier darting.

The impala were immobilised via remote injection (darting). A carbon dioxide powered projector (Dan-Inject 0037; Mod JM; Denmark) was used to deliver a 3 mL air-pressurised dart (Dan-Inject)

into the muscles of the hind quarters. The preselected animal for study was darted by an experienced veterinarian.

The immobilisation agents used were a standard combination of thiafentanil (2 mg; Thianil 10 mg mL^{-1}; Wildlife Pharmaceuticals; South Africa) and medetomidine (2.2 mg; Medetomidine 10 mg mL^{-1}; Kyron Prescriptions; South Africa) in the same dart. The volume was diluted with sterile water for injection (Kyron water for injection; Kyron Laboratories; South Africa) to a total volume of 3 mL per dart. The quality of immobilisation was scored (Table 1). Any animal that did not go down within 15 minutes of the initial dart was darted again with either a full or a half-dose dart depending on the level of ataxia and sedation. Any animal that did not go down after a second dart was darted with reversal agents and excluded from the trial.

Table 1 Scoring system used to describe quality of capture. To allow better description, separate scores were assigned for immobilisation quality and ability to intubate.

Immobilisation quality		**Ability to intubate**	
Score	Description	Score	Description
1	Calm transition, no excitement*, remains "down" once recumbent	1	Orotracheal intubation easy at first attempt
2	Mild excitement before becoming recumbent; maintains recumbency	2	Orotracheal intubation achieved after 2-3 attempts
3	Pronounced excitement; attempts to stand after becoming recumbent	3	Additional propofol required before orotracheal intubation successful
4	Profound excitement; does not become recumbent	4	Orotracheal intubation unsuccessful

*Excitement included paddling or fighting

Once the impala was recumbent, the rest of the herd was released into the home area of the boma. The impala was then approached and blind-folded and ear plugs (wads of cotton swabs) were placed to reduce any effects of external stimuli. A cursory clinical examination of temperature, pulse, respiration and SpO_2 was performed. An indwelling cannula (20 Gauge) was aseptically placed percutaneously into a cephalic vein. The impala was then transported from the boma to the procedure room, a distance of approximately 650 m (by pickup truck).

Instrumentation

Once in the procedure room, the impala was weighed and then placed on the working table in right lateral recumbency. An indwelling cannula (22 Gauge) was placed aseptically into an auricular artery. Thereafter, the impala was intubated with a size 8, cuffed polyvinyl chloride endotracheal (ET) tube (Teleflex Medical; Germany) using a visual guided technique (Miller size 4 blade illuminated laryngoscope and wire stylet inside the ET tube). If depth of anaesthesia was insufficient to allow intubation, propofol (Fresenius Propoven 1%; Fresenius Kabi; South Africa) was administered intravenously; a dosage of 4 mg kg^{-1} was calculated for each impala and administered in 0.5 mg kg^{-1} boluses every 10 seconds until anaesthetic depth was sufficient to allow orotracheal intubation. Where propofol was needed, the amount given was recorded. If any swallowing, chewing or spontaneous movement occurred, immediately after intubation, additional propofol was administered (0.5 mg kg^{-1} boluses until an adequate plane of anaesthesia was reached). This amount was recorded as part of the induction dose. Ability to intubate was scored (Table 1). The impala remained intubated for the duration of the anaesthesia to ensure the airway remained patent, protect against regurgitation, facilitate delivery of oxygen and enable measurement of end-tidal carbon dioxide and oxygen tension.

Following ET-tube placement, oxygen was supplemented at 2 L min^{-1} via a feeding tube (10 French gauge) inserted through a pitot pneumotachograph attached to the end of the ET-tube (Photo 2).

Photo 2 Setup of spirometer and side stream gas sampler connected to the end of the ET-tube. Oxygen supply (via feeding tube inserted through ET-tube to depth of thoracic inlet) indicated by arrow.

Additional indwelling cannulas (20 Gauge) were aseptically placed percutaneously into the remaining cephalic vein and the lateral saphenous vein of the non-dependent pelvic limb. The cephalic veins were used for delivery of propofol (alone) and medetomidine-ketamine infusions. Balanced isotonic crystalloid fluids (Sodium Chloride Fresenius 0.9%, Fresenius Kabi; South Africa) were administered via the lateral saphenous vein for the duration of anaesthetic maintenance at 5 mL kg^{-1} h^{-1}. Separate infusion pumps (Infusomat Space; B Braun; Germany) were used for delivery of each fluid.

Cardiopulmonary parameters were monitored continuously throughout anaesthetic maintenance using a multiparameter monitor (Cardiocap 5; Datex-Ohmeda; Finland). Heart rate was measured from an electrocardiogram with the ECG electrodes placed in a base-apex configuration.

In addition to the multiparameter monitor, pulse rate and peripheral oxygen haemoglobin saturation were also measured with an infrared pulse oximeter transmission probe (NONIN

PureSat 2500 A; NONIN Medical INC; MN, USA) placed on the vulva. Measured pulse rate was correlated with palpated pulse rate and with heart rate from the ECG.

End-tidal carbon dioxide and fractional expired oxygen, and expiratory tidal volume were evaluated using a side-stream gas sampler (200 mL minute^{-1}) and a pitot pneumotachograph, respectively, attached to the end of the ET-tube (Photo 2). Respiratory rate was taken from the capnograph and correlated with visual assessment.

A calibrated strain gauge transducer (DTX Plus disposable transducer; BD Medical; South Africa), zeroed to the level of the sternum, was coupled to the multiparameter monitor to measure direct arterial blood pressure from the auricular artery.

A rectal thermometer probe (Physitemp Model BAT-12; Physitemp Instruments; NJ, USA) was used to monitor rectal temperature to indicate body temperature. In an attempt to maintain body temperature between 37.5 °C and 39.5 °C throughout the anaesthetic period, a circulating warm water blanket (Micro-Temp LT; Cincinnati Sub-Zero; OH, USA) and a forced warmed air blanket (Bair Hugger Model 505; Arizant Healthcare; MN, USA) were used as needed.

Maintenance

General anaesthesia was maintained for 120 minutes (time started from the initiation of the maintenance infusion which begun post endotracheal intubation) using an intravenous infusion. The impala remained in right lateral recumbency, onto a padded surface, with the dependent thoracic limb pulled forward, for the duration of anaesthesia. Oxygen supplementation was maintained at 2 L min^{-1} for the duration of anaesthesia.

To prepare the Medetomidine-Ketamine (MK) solution, 2.5 mg medetomidine (Domitor 1 mg mL^{-1}, Pfizer Laboratories (PTY) Ltd; South Africa) and 750 mg ketamine (Ketamine Fresenius 100 mg mL^{-1}, Bodene trading as Intramed; South Africa) were mixed in one 1 L isotonic saline bag (Sodium Chloride Fresenius 0.9%), to give a concentration of 2.5 µg mL^{-1} medetomidine and 0.75 mg mL^{-1} ketamine. An equivalent volume of saline was removed from the bag, prior to injecting the medetomidine and ketamine. After the drugs were added, the infusion bag was weighed on a precision scale to ensure a final total fluid volume of 1000 mL (where 1 mL = 1 g) prior to initiation of the infusion. The ketamine was administered at a fixed infusion rate of 1 mg kg^{-1} h^{-1} and the medetomidine at 5 µg kg^{-1} h^{-1}; which equated to 2 mL kg^{-1} h^{-1} of the MK solution. The delivery rate of the MK solution remained constant throughout the 120 minute general anaesthesia. A 1 mg kg^{-1} ketamine bolus was administered IV prior to the start of the infusion.

Propofol (Fresenius Propoven 1%) in 50 mL bottles was used. Propofol was initially delivered at 0.2 mg kg^{-1} min^{-1} (0.02 mL kg^{-1} min^{-1} or 1.2 mL kg^{-1} hr^{-1}). The propofol infusion rate was altered in response to changes in anaesthetic depth and response to noxious stimuli, to maintain a level of surgical anaesthesia. Every fifteen minutes during the anaesthetic maintenance period, deep pain was tested. To do this, a digit was clamped using vulsellum forceps (Photo 3) applied just below the coronary band (over phalanx 2). The forceps were closed tightly to the first ratchet for 60 seconds, or until purposeful movement was seen. Purposeful movement was defined as gross movement of the head or limbs. To ensure uniformity, the same person (primary investigator) applied the clamp each time. The four digits

Photo 3 Application of vulsellum forceps just below coronary band to test deep pain.

on the non-dependent thoracic and pelvic limbs were used consecutively; in the following order: axial claw of left thoracic limb, abaxial claw of left thoracic limb, abaxial claw of left pelvic limb, axial claw of left pelvic limb. This order was then repeated.

If movement occurred, the propofol infusion was increased by 20% and held constant for 15 minutes; where after deep pain was retested. If there was no response to the stimulus, the propofol infusion was decreased by 20% and the stimulus reapplied in 15 minutes. Changes in propofol delivery rates were recorded.

Both the MK solution and propofol infusions as well as the crystalloid fluids were stopped at 120 minutes. At this point, the total amounts of propofol, MK solution and saline that had been delivered were recorded.

A single dose of meloxicam (0.5 mg kg^{-1}; Metacam 20 mg ml^{-1}, Ingleheim Pharmaceuticals; South Africa) was administered subcutaneously at the end of anaesthesia.

Monitoring

Physiological parameters monitored continuously throughout the anaesthetic period included heart rate, peripheral oxygen haemoglobin saturation, respiratory rate, end-tidal carbon dioxide, fractional expired oxygen, direct arterial blood pressure and rectal temperature. Measurements were recorded every five minutes onto the individual data collection sheet for each animal.

Depth of anaesthesia was assessed based on medial palpebral reflex and response to painful stimuli. Response to deep pain was determined as described above and recorded every fifteen minutes. The palpebral reflex was evaluated every five minutes.

Samples were drawn for arterial blood gas determination every thirty minutes. Samples were collected anaerobically in pre-heparinised syringes (Heparin Sodium Fresenius 1000 IU ml^{-1}; Bodene, trading as Intramed; South Africa) and analysed immediately on-site with a portable blood gas analyser (EPOC Reader Blood Analysis; Epocal; Canada). The catheter in the auricular artery was used to obtain the samples. The initial (baseline) sample was taken before starting the maintenance infusion and oxygen supplementation. The EPOC BGEM blood gas smart cards (EPOC BGEM; Epocal; Canada) measured the arterial oxygen tension (PaO_2), arterial carbon dioxide tension ($PaCO_2$), pH and lactate and calculated bicarbonate (HCO_3^-)-, and base excess (blood and extracellular fluid). The arterial blood samples were not corrected to body temperature but reported at 37°C (alpha-stat).

The arterial oxygen tension to fractional inspired oxygen ratio (PaO_2:FiO_2) was calculated. End-tidal oxygen fractions were used to estimate FiO_2 as follows: $FiO_2 = F_EO_2 + 5\%$. A conservative correction of 5% was used based on an average uptake of oxygen in mammalian lungs (Jacobs et al. 1999). The dead space to tidal volume ratio (Vd/Vt) was calculated using the formula $Vd/Vt = (PaCO_2\text{-}EtCO_2)/PaCO_2$ (Cross & Plunkett 2008).

Any side effects observed or interventions needed throughout the time of anaesthesia were recorded.

Recovery

The MK solution and propofol infusions were stopped at 120 minutes. Thereafter, the impala was disconnected from the monitoring equipment and infusion pumps and transported back to the boma. Upon arrival, the impala were placed in the capture area in sternal recumbency supported against the boma fence and with the head supported on hay.

Photo 4 Positioning of an impala for recovery

The total dose of medetomidine (immobilisation plus infusion dose) was antagonised with atipamezole (5:1 mg medetomidine, IM; Atipamezole 50 mg ml^{-1}; V-Tech Pharmacy; South Africa) ten minutes following the end of the maintenance infusion. Naltrexone (10:1 mg thiafentanil, IV; Trexonil 50 mg ml^{-1}; Wildlife Pharmaceuticals; South Africa) was used to reverse the thiafentanil. This was administered five minutes after the atipamezole, following which the intravenous cannula was removed. The endotracheal tube was removed, with the cuff partially inflated, at the first sign of swallowing.

Recovery was observed and scored (Table 2). To facilitate scoring, recovery was divided into early recovery (the period from administration of antagonists to the first attempts to stand) and late recovery (from the first attempt to stand until reintegration into the herd). Once walking, the impala was allowed to return to the herd. The impala were observed for 24 hours following recovery from general anaesthesia for signs of renarcotisation or other untoward effects.

Table 2 Scoring system used for quality of recovery following antagonist administration

Early Recovery		Late Recovery	
Score	Description	Score	Description
1	Calm transition to alertness	1	1 or 2 coordinated efforts to stand; no ataxia; normal gait
2	Generally quiet, may startle	2	1 or 2 coordinated efforts to stand; mild ataxia once standing; gait largely normal
3	Uncoordinated whole body movements; paddling; startles	3	Multiple attempts to stand; considerable ataxia; moving with unsteady gait
4	Emergence delirium, thrashing	4	Unable to stand > 30 minutes after administration of opioid anatagonists

Rescue interventions

For the duration of the trial, impala health and safety was a priority. The following potential concerns and rescue interventions were identified to create a protocol for emergency assistance when required.

Potential concerns:

1. Apnoea defined as no breathing attempt for longer than 40 seconds.
2. Hypotension defined as a mean arterial blood pressure of less than 60 mmHg for 120 seconds.
3. Cardiovascular collapse defined as asystole.
4. Respiratory compromise ($ETCO_2$ > 60 mmHg or SPO_2 < 90%)
5. Bloat

Rescue intervention for each of these concerns:

1. Assessment of potential cause; if deemed to result from thiafentanil, administration of 2 mg butorphanol (1:1 thiafentanil) to partially reverse the thiafentanil. If continued or cause unknown, tracheal intubation followed by intermittent positive pressure ventilation with an adult ambu bag attached to the ET tube.
2. Administer a balanced isotonic crystalloid fluid bolus at 30 mL kg^{-1} over 10 minutes until mean arterial blood pressure increases and/or lighten the plane of anaesthesia by decreasing the propofol infusion rate by 20%.
3. Stop anaesthesia; administer reversal agents; begin standard cardiopulmonary resuscitation (Fletcher et al. 2012).
4. Start intermittent positive pressure ventilation at 4 to 6 breaths per minute. Where hypoventilation was suspected to have resulted from thiafentanil-induced respiratory depression, administration of 2 mg butorphanol (1:1 thiafentanil) to partially reverse the thiafentanil.
5. Pass stomach tube to deflate; perform percutaneous trocharisation (16 gauge needle) if stomach tube technique fails.

Statistics

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; Minitab Inc, State College; PA, USA and IBM SPSS Statistics Version 22; International Business Machines Corp.; NY, USA) and significance determined at $P < 0.05$.

Results

All impala were deemed to be healthy based on visual inspection prior to darting, immobilised clinical examination, complete blood count and serum biochemistry. All ten impala completed the trial.

Immobilisation phase

All impala were successfully immobilised after darting. The thiafentanil-medetomidine combination produced a predictable immobilisation characterised by stiffness, ataxia, initial increase in locomotory activity, extension of the neck dorsally and finally recumbency, often as a result of tripping or falling. The time frame from darting to recumbency was relatively rapid (Table 3) and only one impala required a follow up dart before becoming recumbent. The immobilisation scores were either 1 (n = 5) or 2 (n = 5) for all animals.

Intubation was difficult in all impala because of jaw stiffness. Therefore, propofol boluses were required to achieve intubation in all animals. All impala scored 3 for intubation.

Table 3 Time intervals and drug dosages reported as median (IQR) for ten impala (*Aepyceros melampus*) undergoing immobilisation followed by propofol-medetomidine-ketamine TIVA of 120 minute duration.

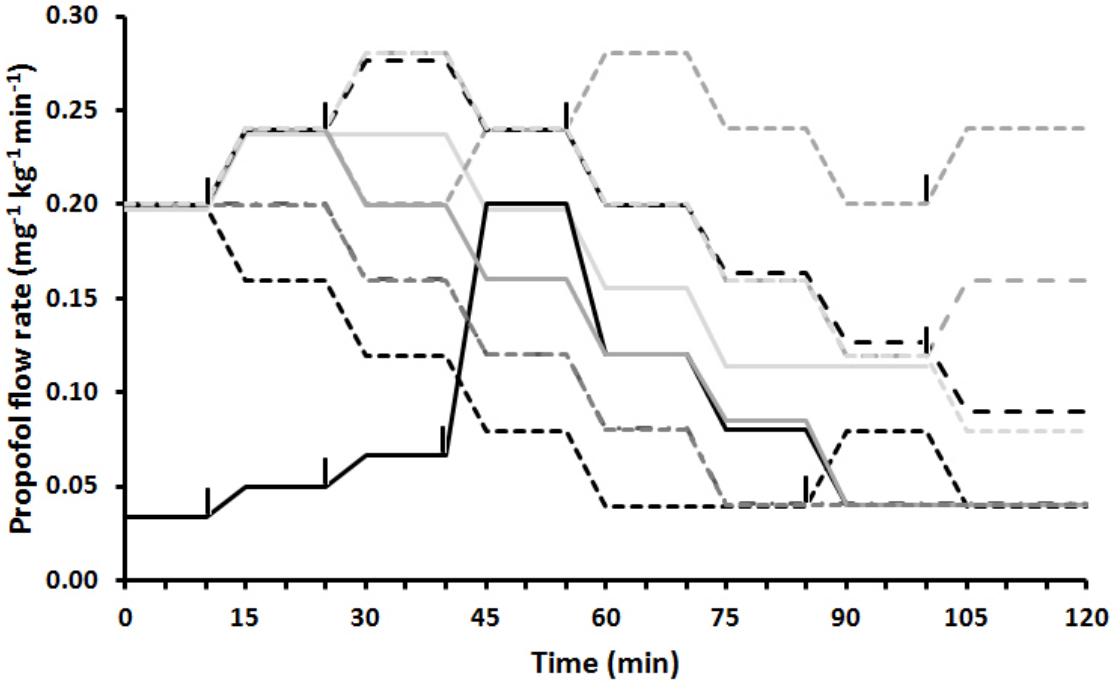
Parameter	Unit	Median (IQR)
Immobilisation		
Ataxia time	min	3.8 (3.4-4.4)
Recumbency time	min	9.6 (7.2-14.4)
Medetomidine dose	mg kg^{-1}	0.058 (0.053-0.062)
Thiafentanil dose	mg kg^{-1}	0.053 (0.048-0.057)
Induction		
Propofol dose	mg kg^{-1}	2.7 (1.9-3.3)
Maintenance and rescue intervention		
Time from darting to start of infusion	min	35.5 (29.3-39.0)
Butorphanol*	mg kg^{-1}	0.12 (0.09-0.15)
Recovery		
Atipamezole dose	mg kg^{-1}	0.34 (0.32-0.36)
Naltrexone dose	mg kg^{-1}	0.50 (0.48-0.54)
Interval from injection of atipamezole to standing	min	9.4 (8.2-10.6)
Interval from injection of naltrexone to standing	min	4.4 (3.2-5.6)

*9 out of the 10 impala required butorphanol administration (with 2 boluses n = 4, 3 boluses n = 3 and 4 boluses n = 1.

Maintenance phase

The propofol-medetomidine-ketamine combination successfully kept all the impala anaesthetised for the 120 minute maintenance period. One animal was recovered early (after 100 minutes) due to a disruption in the oxygen supply with subsequent development of severe hypoxaemia. The propofol titration generally showed an erratic downward trend (Figure 1). The first animal was inadvertently started on a very low propofol infusion rate (Figure 1, solid black line); however, the error was corrected at the 40 minute data collection time. Minimum infusion rate for propofol could not be calculated in this study.

Figure 1 Spaghetti plots of the propofol infusion rate (mg kg^{-1} min^{-1}) over time in ten impala (*Aepyceros melampus)* undergoing a propofol-medetomidine-ketamine TIVA infusion of 120 minute duration. Infusion rate was either increased or decreased by a fixed 20% of the initial rate (0.2 mg kg^{-1} min^{-1}) in a stepwise manner in accordance to a positive or negative deep pain response test, respectively, done at 15 minute intervals. Solid black vertical bars indicate a positive deep pain response.



The heart rate correlated strongly (P <0.01; correlation coefficient 0.642) and mean arterial blood pressure weakly (P <0.05; correlation coefficient 0.145) with the propofol infusion rate over time, while the respiratory rate demonstrated no correlation (P = 0.585; correlation coefficient -0.035; Figure 2).

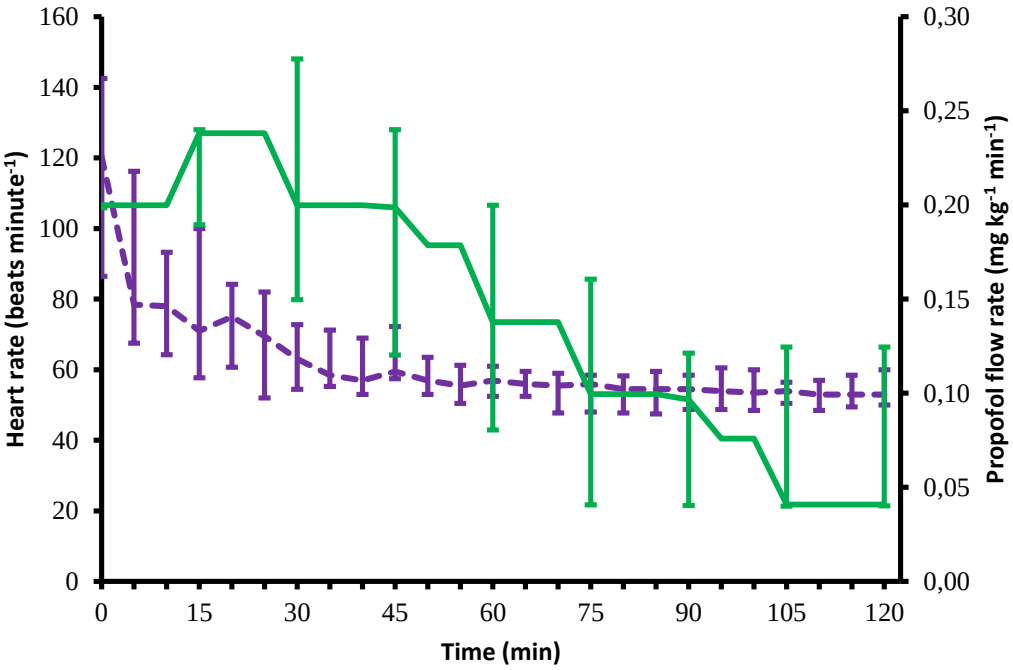
During the first hour following darting, respiration was often characterised by bouts of thoracic, abdominal and limb stiffness leading to exaggerated breathing attempts against the pronounced muscle rigidity. Sporadic breath holding and apnoea were also noticed in all impala.

All but one impala required butorphanol as a rescue intervention for the apnoea, with most requiring more than one bolus dose (2 boluses n = 4, 3 boluses n = 3 and 4 boluses n = 1). A

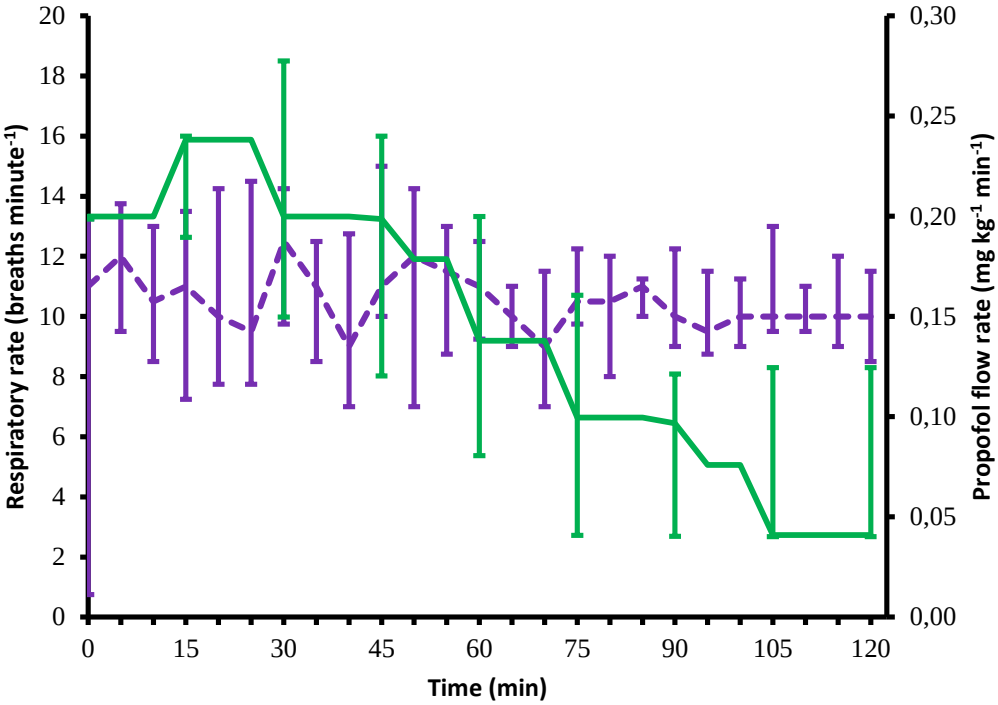
reduction in rigidity in the chest and limbs and improved ventilation was noted after butorphanol administration. Despite the general visual appearance of inadequate ventilation all impala maintained adequate measured minute ventilation for an anaesthetised animal with adequate tidal volumes of 26 (19-32) mL kg^{-1} and respiratory rates of 10 (9-12) breaths $minute^{-1}$.

Figure 2 (a). Heart rate (beats min^{-1}), (b). respiratory rate (breaths min^{-1}) and (c). mean arterial blood pressure (mmHg) plotted against propofol infusion rate (mg kg^{-1} min^{-1}; solid green line) over time. Median (IQR) values are reported at 5 minute intervals for the entire 120 minute propofol-medetomidine-ketamine TIVA infusion in ten impala (*Aepyceros melampus*).

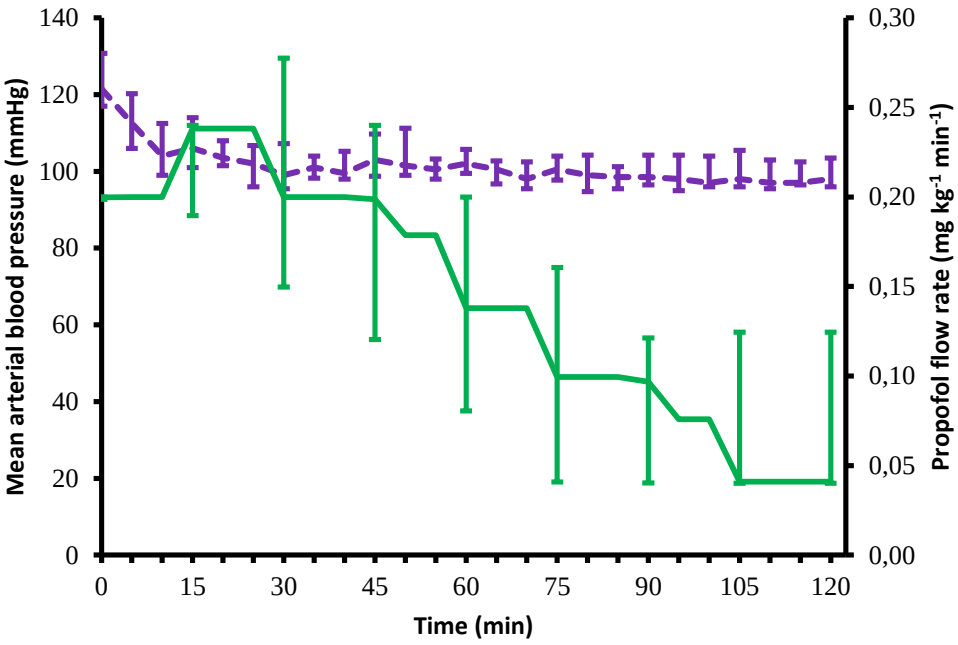
(a)



(b)



(c)



All impala were hypoxaemic prior to the initiation of oxygen supplementation, with a median PaO_2 of 45 (42-51) mmHg (Table 4). The PaO_2 significantly (P = 0.005) improved from 30 minutes onwards compared to initial readings. SpO_2 had a strong correlation with PaO_2 (P < 0.001; r^2 = 0.645).

$PaCO_2$ significantly (P = 0.001) increased throughout anaesthetic maintenance; despite a normal end-tidal carbon dioxide tension ($EtCO_2$) throughout the anaesthetic maintenance period. Arterial pH indicated a mild compensated respiratory acidosis that gradually worsened over the maintenance time. The lactate level significantly decreased over time from the initial (Time 0) sample (P <0.001).

All impala regurgitated multiple times during the maintenance period. To manage the regurgitation, the impala's head was kept lowered during transport to facilitate drainage of any ingesta from the mouth and the ET tube cuff was only partially deflated prior to extubation. No adverse effects of the regurgitation were observed during or after the study. Very mild bloat was observed in one animal only; no treatment was required.

Despite that the propofol-medetomidine-ketamine infusion maintained adequate general anaesthesia, the cardiopulmonary derangements, highlighted by hypoxaemia and hypercapnia, were sufficient evidence to reject the null hypothesis of the study.

Recovery phase

Recovery was uneventful and all animals scored a 1 for early recovery and all scored either 1 (n=8) or 2 (n=2) for late recovery, which was calm and rapid with no to mild ataxia.

Table 4 Physiological and calculated parameters of ten impala (*Aepyceros melampus*) undergoing propofol-medetomidine-ketamine TIVA. Parameters reported as median (IQR) at 30 minute intervals for 120 minute duration of infusion.

		Time (minutes) from induction of anaesthesia					
Variable	Unit	0[#]	30	60	90	120	P value[‡]
Peripheral haemoglobin oxygen saturation (SpO_2)	%	69 (58-75)	98* (96-99)	96* (95-97)	96* (95-97)	97* (94-98)	<0.001
Rectal temperature	^oC	38.6 (37.9-39.3)	37.9* (37.1-38.5)	37.6* (37.0-37.8)	37.2* (36.7-37.5)	36.8* (36.6-37.3)	<0.001
Respiratory gases and spirometry							
Fractional inspired oxygen concentration (FiO_2)		0.21	0.49* (0.46-0.53)	0.49* (0.44-0.52)	0.52* (0.49-0.54)	0.49* (0.44-0.53)	0.002
End-tidal carbon dioxide tension ($EtCO_2$)	mmHg kPa	41.2 (37.1-43.1) 5.5 (4.9-5.7)	35.8 (34.1-41.8) 4.7 (4.5-5.6)	41.8 (36.6-44.5) 5.6 (4.9-5.9)	44.2 (41.8-47.5) 5.9 (5.6-6.3)	45.8 (41.7-51.9) 6.1 (5.6-6.9)	0.016
Expiratory tidal volume	mL kg^{-1}	39 (29-49)	25 (18-29)	25 (12-29)	25 (9-26)	27 (15-31)	0.118
Arterial blood gas							
Arterial oxygen tension (PaO_2)	mmHg kPa	45.0 (42.3-51.1) 6.0 (5.6-6.8)	98.8 (65.4-107.2) 13.2 (8.7-14.3)	87.2 (77.8-97.9) 11.6 (10.3-13.1)	95.5* (83.5-104.6) 12.7* (11.1-13.9)	91.5* (87.9-115.6) 12.2* (11.7-15.4)	0.005
Arterial carbon dioxide tension ($PaCO_2$)	mmHg kPa	51.9 (47.4-54.7) 6.9 (6.3-7.3)	57.0 (47.2-67.8) 7.6 (6.3-9.0)	66.4* (58.7-71.6) 8.8* (7.8-9.6)	68.7* (58.1-78.3) 9.2* (7.7-10.4)	69.5* (60.2-76.3) 9.3* (8.0-10.2)	0.001
pH		7.35 (7.33-7.38)	7.37 (7.30-7.42)	7.33 (7.29-7.36)	7.32 (7.28-7.34)	7.30 (7.26-7.34)	0.049
Arterial bicarbonate ion concentration (HCO_3^-)	mmol L^{-1}	28.6 (26.9-30.9)	31.8 (30.5-34.9)	33.5* (31.9-36.7)	34.4* (32.9-35.7)	32.9* (32.2-38.3)	0.001
Base excess		3.2 (2.3–5.1)	5.9 (4.7-10.1)	7.6 (5.7-10.3)	7.6 (6.1-9.4)	6.8 (5.4-11.6)	<0.001
Blood lactate	mmol L^{-1}	2.1 (1.8-4.0)	0.6* (0.6-0.7)	0.4* (0.4-0.5)	0.4* (0.3-0.5)	0.3* (0.3-0.4)	<0.001
Oxygenation and ventilation indices							
$PaO_2 : F_iO_2$	mmHg	214 (201-243)	203 (143-204)	178 (172-189)	185 (169-193)	187 (199-217)	0.938
Dead-space to tidal volume ratio (Vd/Vt)[†]	%	20 (11-28)	29* (24-38)	35 (33-44)	33 (29-36)	33 (32-39)	0.003

[#] Initial "baseline" samples taken after immobilisation but before oxygen administration or initiation of maintenance protocol; [†]Normal values for Vd/Vt ratio are 20-40%; [‡]Based on Friedman tests. *Medians are significantly different ($P < 0.05$) to baseline using Wilcoxon signed rank tests and Bonferroni correction of P values.

Discussion

The propofol-medetomidine-ketamine total intravenous infusion provided reliable maintenance of anaesthesia. Use of this protocol in the field may be feasible if it is titrated appropriately to clinical effect and if oxygen is supplemented. Hypoxemia and inadequate oxygenation occurred despite adequate measured ventilation, indicating that other mechanisms than hypoventilation are responsible for it. Regurgitation was also a clinically significant concern with use of this protocol.

The propofol was successfully titrated in the impala to maintain a state of surgical anaesthesia based on immobility, sluggish-to-absent palpebral reflex and lack of response to noxious stimuli in the form of clamping the claw. The titration of propofol showed an erratic, generally downward trend reaching a nadir of 0.05 mg kg^{-1} min^{-1}. Interestingly, even an animal that was accidentally started on 0.05 mg kg^{-1} min^{-1} propofol infusion also remained recumbent although it did respond to noxious stimuli until the error was corrected. Thereafter the propofol infusion rate was corrected and titrated back to 0.05 mg kg^{-1} min^{-1} by the end of the maintenance period (with no response to noxious stimuli).

For the purpose of developing a practical protocol for TIVA in wild ungulates undergoing surgery, ketamine and medetomidine were included to provide analgesia, reduce the requirements of propofol, and improve the cardiovascular stability of the anaesthetic. Ketamine is known to be metabolised to the active metabolite norketamine (Kästner 2007), and it is entirely possible that this may have accumulated and played a role in the decreased propofol requirements. As a result of the erratic responses, the effects of other drugs in the combination, plus a lack of serum concentration determination, a minimum infusion rate for propofol could not be determined in

this study. However, for practical use, we recommend that the combination is used with an initial propofol infusion rate of 0.2 mg kg^{-1} min^{-1} and titrated according to depth of anaesthesia and response to surgical stimulation.

The protocol maintained stable cardiovascular function throughout the anaesthetic period, as indicated by heart rate and mean arterial blood pressure. Propofol has been demonstrated to cause dose-dependent hypotension brought about by decreased systemic vascular resistance (Clarke et al. 2014). It is likely that the indirect sympathomimetic action of ketamine is able to counter the vasodilation caused by propofol, as suggested by Larenza et al. (2005), who showed that a combination of propofol and ketamine maintained blood pressure better than sevoflurane in anaesthetised domestic goats. The immobilisation combination also likely played a role in maintenance of blood pressure. Opioids are known to preserve cardiovascular stability, and there is even well documented evidence of potent opioids causing hypertension (Lance & Kenny 2012). In addition, medetomidine, an α_2-adrenoreceptor agonist which maintains vascular tone and thus blood pressure, was included in both the dart and the infusion at relatively large doses (Clarke et al. 2014).

However, despite apparently normal cardiopulmonary function based on physiological parameters (respiratory rate, tidal volume and arterial blood pressure), the protocol caused clinically significant cardiopulmonary aberrations. All animals were severely hypoxaemic (Haskins 2013) at the initial blood gas sample taken prior to oxygen supplementation or propofol administration. During the maintenance period, where oxygen was supplemented throughout, arterial oxygen tension improved to levels considered to be acceptable for the altitude following initiation of oxygen support, however, values measured were still lower than expected for the amount of oxygen inhaled (expected PaO_2 > 215 mmHg for 50% inspired oxygen).

The hypoxaemia and inadequate oxygenation demonstrated in the present study may be the result of hypoventilation, right-to-left shunting, ventilation/perfusion mismatching, diffusion deficits, depletion of oxygen reserves and body positioning (Mich et al. 2008).

Hypoventilation is a common side effect of many anaesthetic agents. Propofol is known to cause centrally-mediated respiratory depression (Clarke et al. 2014), however, the impala were hypoxaemic prior to propofol administration, and thus it is likely that the drugs in the dart played an important role. Thiafentanil is well documented as causing hypoxaemia and hypercapnia (Citino et al. 2001). Respiration following darting and even during the early maintenance period of this trial was often characterised as a few breaths in a row followed by periods of apnoea (Biot's respiratory pattern). Potent opioids are thought to decrease ventilation through a centrally mediated effect (right shift in the CO_2 response curve and depression of the rhythm generating centres) that results in decreased respiratory rate (Pattinson 2008) and tidal volume (Bush et al. 2004). Interestingly, other than bouts of apnoea following darting, respiratory rates remained within a normal range (10-20 breaths min^{-1}) throughout the maintenance period (McDonnel & Kerr 2007). Tidal volumes measured during the trial were over 25 ml kg^{-1}, which is more than double that of similar small ruminants (domestic goats) at 12 ml kg^{-1} (McDonnel & Kerr 2007). Thus a simplified explanation of hypoventilation causing hypoxaemia is unlikely.

Potent opioids have been documented to cause "wooden chest syndrome" in people (Coruh et al. 2013), whereby they cause decreased chest wall compliance and thus difficulty in maintaining spontaneous respiration (Soares et al. 2014). This is likely a similar reason for the thoracic and abdominal muscle stiffness noted in this study and muscle rigidity noted by others (Citino et al. 2001; Janssen et al. 1993). In addition to limiting muscle movement, the stiff abdomen pushing

the diaphragm cranially and ridgid thorax may decrease the functional residual capacity of the lung field and thus lessen the oxygen reserves (Sirian & Wills 2009). Butorphanol is a mixed agonist-antagonist opioid that has been shown to partly reduce opioid-induced respiratory depression (Haw et al. 2014). In this study, butorphanol administration caused relaxation of the muscles that was followed by more co-ordinated chest efforts during ventilation, a more regular breathing pattern and subsequent improvement in haemoglobin saturation.

Right-to-left shunting and ventilation-perfusion mismatching are also likely to have contributed to the development of hypoxaemia. Arterial carbon dioxide tension increased throughout the anaesthetic period, causing a pronounced hypercapnia at the end of the maintenance period. End-tidal carbon dioxide tension, however, remained within normal limits (< 45 mmHg) the entire time and did not follow the same trend as the arterial carbon dioxide tension, as is illustrated by the increasing Vd/Vt ratio (Table 4). This wide gap between the arterial to end-tidal carbon dioxide is typical of alveolar dead-space ventilation or severe right-to-left shunt formation (Yamauchi et al. 2011).

Potent opioids are known to cause intrapulmonary shunting and pulmonary hypertension and these effects of thiafentanil may have played a role during the maintenance phase of the study (Lance & Kenny 2012). In addition, α_2-adrenoreceptor agonists have been shown to cause pulmonary hypertension and oedema in sheep and other small ruminants (Celly et al. 1999). Furthermore, medetomidine is known to increase the intrapulmonary shunt fraction in sheep by 5-25% (Kästner 2006).

The PaO_2:FiO_2 ratio remained low (normal > 300 mmHg) for the duration of the anaesthetic period. These low ratios further indicate that hypoventilation was not the only cause of

hypoxaemia in the impala, and pulmonary pathology, such as oedema, shunting or ventilation-perfusion mismatching were potentially also involved. Although the calculation of FiO_2 from fractional expired oxygen may have affected the index, but the grossly abnormal PaO_2:FiO_2 ratio values indicate the severity of the co-morbidities which result in hypoxaemia.

Another factor contributing to the hypoxaemia may have been the length of time between darting and initiation of oxygen supplementation. The median time between darting and initiating oxygen was 35.5 minutes and during this time, the residual volume of the lung may have been depleted of oxygen (Sirian & Wills 2009). This depletion may have been worsened by the episodes of apnoea and poor ventilation experienced during this period.

The improved oxygenation at 30 minutes may have been due to butorphanol interventions administered during the first thirty minutes of TIVA maintenance of anaesthesia. Haw et al. (2014) recently demonstrated that a combination of butorphanol and oxygen insufflation caused less opioid-induced hypoxaemia than when either intervention was used alone in etorphine-immobilised white rhinoceros. Gas exchange may also have worsened after the 30 minute mark as a result of worsening shunts, atelectasis or other pulmonary pathology associated with the increasing length of anaesthetic and recumbency (Mich et al. 2008), which could explain why oxygen tension worsened after this time despite there being no change in fractional inspired oxygen.

Continuous oxygen supplementation while using this protocol is vital. During anaesthesia in one animal, oxygen supplies became depleted. This animal then developed severe hypoxaemia (PaO_2 45.4 mmHg: 6.05 kPa) which resulted in tachycardia and hypertension; we recovered this animal early (after 100 minutes). In addition, butorphanol has been shown to be effective in reducing

thiafentanil-induced respiratory compromise and should be available when using the former for darting. The above findings also indicate that respiratory rate alone is not an adequate measure of ventilation. Importantly, although SpO_2 did show a strong correlation ($r^2 = 0.645$) with PaO_2, it is felt that a very strong correlation ($r^2 > 0.850$) between the two is required before recommending only monitoring by pulse oximetry (Guyatt et al. 1995). Thus, although a pulse oximeter could still be used in the field as a general indicator of blood oxygenation, blood gas analysis is recommended as a more reliable and safer alternative and should be performed at regular intervals throughout anaesthesia.

Regurgitation in ruminants under anaesthesia is a well-known phenomenon, and may be associated with fatal outcomes if it is not anticipated and managed. Regurgitation during this trial seemed more frequent than expected. Hossain & Camburn (1984) suggested that regurgitation occurs during light anaesthesia via an active but uncontrolled reflex and during deep anaesthesia as a result of loss of oesophageal tone and increased intra-ruminal pressure.

The lower oesophageal sphincter is the most important barrier in the prevention of reflux of gastric contents and barrier pressure is a function of the difference between lower oesophageal sphincter pressure and intra-ruminal pressure (Benington & Severn 2007). Many drugs, including propofol and opioids, decrease lower oesophageal sphincter pressure (Benington & Severn 2007). However, studies in goats have demonstrated contrasting results related to regurgitation during propofol anaesthesia. Reid et al. (1993) demonstrated increased regurgitation in goats anaesthetised with propofol, while numerous other studies did not document regurgitation (Prassinos et al. 2005; Carroll et al. 1998 and Bettschart-Wolfensberger et al. 2000).

Increased intra-ruminal pressure may also have played a role in the process. As noted previously, the abdominal muscles of the anaesthetised impala were very rigid at times, which can translate into increased intra-abdominal pressure and thus increased intra-ruminal pressure (Benington & Severn 2007). Decreased ruminal tone may also have contributed to regurgitation, through contributions to both decreased lower oesophageal pressure and increased intra-ruminal pressure due to build-up of ingesta, fluid and gas. Medetomidine, as with the other α_2-adrenoreceptor agonists, is well documented in causing rumen atony in small ruminants (Hugar et al. 2000, Mohammad et al. 1993).

Inadequate starving is associated with an increased incidence of regurgitation (Hossain & Camburn 1983). Although the impala were starved of concentrates for 12 hours prior to anaesthesia, they still had free access to hay, lucerne and water until darting.

Regurgitation was likely a result of a combination of all these factors. A limitation to this study is that we did not auscultate rumen movements, and thus cannot differentiate whether active eructation or passive regurgitation occurred. The important clinical application of this finding is that endotracheal intubation with a cuffed tube is essential to protect the airway. In addition, positioning is important. Right lateral recumbency with the head up causes the lowest frequency of regurgitation (Hossain & Camburn 1984). Starving prior to anaesthesia, whenever possible, will also assist. However, this study wanted to simulate real-world wildlife practice in free-living conditions where starving is either unknown or impossible to achieve prior to darting. No bloating was observed in these impala, despite the prolonged period of recumbency, likely because gas was being removed with the regurgitated matter.

Unfortunately, due to the use of wild animals, initial samples and measurements could only be taken following immobilisation, and thus drugs used for darting and events during and following darting would have played a role in influencing the animals physiology during maintenance of anaesthesia. This limited the ability to completely interpret the cardiorespiratory effects of the TIVA combination. Additional limitations of the study include the small sample size and the use of female animals only. Interpretation of some of the respiratory data was further limited by the setup of the oxygen supply bypassing the pitot tube and gas sample line, which made calculating an inspired oxygen fraction from the expired oxygen fraction necessary. However, the oxygenation indices remained profoundly abnormal using either measured F_EO_2 or the calculated FiO_2.

Conclusion

Propofol-medetomidine-ketamine total intravenous infusion provided a reliable anaesthesia in thiafentanil-medetomidine immobilised impala and its use in the field may be feasible if titrated to clinical effect and certain precautions are taken. The propofol infusion should be started at 0.2 mg kg^{-1} min^{-1} and titrated according to surgical stimulation and depth of anaesthesia. Due to the risk of hypoxaemia, careful monitoring and oxygen supplementation are essential with this protocol. In addition, protection of the airway with a cuffed endotracheal tube is of critical importance.

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Addendum

Contents

Data collection form

Animal Identification

Impala number: _____ Date: _____
Species: Impala (*Aepyceros melampus*)
Sex: _____ Est. Age: _____ Trial number: _____
Body mass: _____ BCS: _____
Temperament: _____

Summary

Immobilisation drugs:

Drug	Dosage	Dose	Volume
Thiafentanil			
Medetomidine			

Partial Reversal

Drug	Dosage	Dose	Volume
Butorphanol			

Induction

Drug	Dosage	Dose	Volume
Propofol			

Maintenance/CRI

Drug	mg/kg/h	mg total	Rate
Medetomidine			
Ketamine			
Propofol			

Reversal

Drug	Dosage	Dose	Volume
Atipamezole			
Naltrexone			

Date:	**Impala Number:**

Immobilisation

Dart Preparation

Drug	Dosage	Dose	**Volume**
Thiafentanil			**mL**
Medetomidine			**mL**
			Total dart volume **mL**

Capture

Dart Time		
Number of dart attempts		
Recumbency Time		

Capture Score – Immobilisation quality

Score	**Description**
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

Partial Reversal

Butorphanol		**mL**	**Time:**

Monitoring

Approach Time					
Time	**Pulse (bpm)**	**Respiration (bpm)**	**Temperature (°C)**	**SPO_2**	**Comments**

Additional Notes and Comments

Date: **Impala Number:**

Blood Gas

Time	

Atmospheric Pressure

Intubation

Propofol			
Calculated dose	4 mg/kg	mg	**mL**
Bolus dose	0.5 mg/kg	mg	**mL**
Boluses administered		Total administered	**mL**
Intubation Time			
ET tube size			

Ability to Intubate

Score	Description
1	Orotracheal intubation easy at first attempt
2	Orotracheal intubation achieved after 2-3 attempts
3	Additional propofol required before orotracheal intubation successful
4	Orotracheal intubation unsuccessful

Additional Notes and Comments

Date:	**Impala Number:**

Maintenance

Initial Infusion Preparation

Medetomidine		Ketamine	
MK Combination			**mL/hr**
Initial propofol			**mL/min**

Fluid Rate

Sodium chloride 0.9%	5 mL/kg/hr	**mL/hr**
Bolus fluids		**mL**
Total fluids given		**mL**

Oxygen

Oxygen Flow Rate	
Start Time	

Times

Infusion start time	
Infusion stop time	
Total infusion time	
Total infusion volume	

Additional Notes and Comments

Date:	Impala Number:

Data Collection Sheet: Anaesthetic Maintenance Phase Monitoring

Anaes (min)	Actual Time	Propofol (ml/h)	MK (ml/h)	Pulse (bpm)	Syst BP (mmHg)	Diast BP (mmHg)	Mean BP (mmHg)	Resp Rate (bpm)	Tidal Vol	$EtCO_2$	SPO_2	Musc PO_2	Temp (°C)	ETO_2	Palpeb Reflex	Deep Pain		Blood Gas
																Claw	Response	
0																LF Ax		
5																		
10																		
15																LF Ab		
20																		
25																		
30																LH Ab		
35																		
40																		
45																LH Ax		
50																		
55																		
60																LF Ax		
65																		
70																		
75																LF Ab		
80																		
85																		
90																LH Ab		
95																		
100																		
105																LH Ax		
110																		
115																		
120																LF Ax		

Date:	**Impala Number:**

Recovery

Reversal Drugs

Drug	Dosage	Dose	**Volume**	**Time**
Atipamezole (IM)		mg	**mL**	
Naltrexone (IV)		mg	**mL**	

Recovery

Extubation time	
Standing time	

Recovery Score

Early Recovery		**Late Recovery**	
Score	**Description**	**Score**	**Description**
1	Easy transition to alertness	1	1 or 2 coordinated efforts to stand; minimal ataxia; normal gait
2	Generally quiet, may startle	2	1 or 2 coordinated efforts to stand; some ataxia once standing; gait largely normal
3	Uncoordinated whole body movements; paddling; startles	3	Multiple attempts to stand; considerable ataxia; moving with unsteady gait
4	Emergence delirium, thrashing	4	Unable to stand > 30 minutes after administration of opioid anatagonists

Additional Notes and Comments

Presentations and publications arising from the study

The following presentations and publications have resulted from the study:

Presentations

Event	Venue	Date	Title	Type
SAVA Wildlife Congress 2015	The Blades, Pretoria	21 March 2015	Propofol-medetomidine-ketamine total intravenous anaesthesia in impala: a novel approach to field anaesthesia	Abstract presentation

Publications

Buck RK, Meyer LCR, Stegmann GF, Kastner SBR, Kummrow M, Gerlach C, Fosgate G & Zeiler GE. Propofol-medetomidine-ketamine total intravenous anaesthesia in thiafentanil-medetomidine immobilised impala (*Aepyceros melampus*) of 120 minute duration. *Veterinary Anaesthesia and Analgesia* (undergoing review).

Animal Ethics Approval Certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	The efficacy of medetomidine-ketamine-propofol anaesthesia in impala *(Aepyceros melampus)*
PROJECT NUMBER	V042-14
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. R Buck

STUDENT NUMBER (where applicable)	043 884 02
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	Impala *(Aepyceros melampus)*	
NUMBER OF ANIMALS	15	
Approval period to use animals for research/testing purposes		July – August 2014
SUPERVISOR	Dr. G Zeiler	

KINDLY NOTE:

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

APPROVED	Date 30 June 2014
CHAIRMAN: UP Animal Ethics Committee	Signature