TOWARDS TOTAL INTRAVENOUS ANAESTHESIA (TIVA) IN GOATS

By

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Dedicated to two women, without whom my life would be hollow:

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TOTAL INTRAVENOUS ANAESTHESIA (TIVA) IN GOATS

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The objectives of the present series of studies focused on providing information that would improve the literature resource on goat anaesthesia, with more emphasis on total intravenous anaesthesia. Anaesthetic and physiologic effects of total intravenous anaesthesia techniques have been documented in humans, and to some extend in dogs and ponies (Nolan, 2004), but there are very few reports on use of the technique in goats.

Findings from a series of studies to evaluate the sedative, anaesthetic and cardiopulmonary effects of different central nervous systems depressants using 6 goats in a randomized crossover design are reported in this thesis. The benzodiazepines such as diazepam and midazolam, the phenothiazines such as acepromazine and some opioids such as butorphanol are among the drugs that are currently used for sedation in goats. In the first study reported in this thesis, midazolam alone, and combinations of butorphanol with acepromazine or midazolam administered intramuscularly reduced the dose of propofol required for induction of general anaesthesia in goats with minimal effects on cardiopulmonary function. Three of the studies reported in this thesis

evaluated the effects intravenous administration midazolam, fentanyl and propofol on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats. Midazolam, fentanyl and propofol reduced isoflurane minimum alveolar concentration in a dose-dependent manner with no adverse effects on cardiovacular function. The last study reported in this thesis evaluated and compared the efficacy of propofol combined with fentanyl, or midazolam for total intravenous anesthesia in goats. The results of last reported study indicate that total intravenous anaesthesia achieved by co-administration of propofol and either fentanyl or midazolam for induction and maintenance of anaesthesia in spontaneously-breathing, oxygen-supplemented goats is satisfactory, although caution must be exercised with the fentanyl-propofol combination as recovery from anaesthesia was rough on some occasions.

The findings from the present studies provide specific data on anaesthetic and physiologic effects of several drugs and drug-combinations in anaesthetised goats. The information will be a valuable reference source for general practice veterinarians, veterinary anaesthetists and other biomedical scientists. The information should also help improve general welfare of goats undergoing anaesthetic procedures.

Keywords: goat, general anaesthesia, sedation, total intravenous anaesthesia, acepromazine, midazolam, butorphanol, fentanyl, isoflurane, minimum alveolar concentration, cardiovascular effects.

Chapter 1

General Introduction, Literature Review, Scope of the Study

1.1 INTRODUCTION

Goats (*Capra hircus*) have been used as biomedical research models since many years ago, particularly in the fields of orthopaedic, chemotherapeutic, cardiovascular, cerebro-vascular and respiratory research (Antognini & Eisele 1993; Fulton et al. 1994; Larenza et al. 2005). They are considered by some to be companion animals (Carroll et al. 1999). In most communal parts of Africa, goats have an important economic and cultural role (Selebi & McCrindle 2003).

Major surgeries and long diagnostic procedures in goats are usually performed under inhalation anaesthesia, with injectable anaesthesia only used for induction and to facilitate endotracheal intubation (Reid 1993). General anaesthesia is used to produce unconsciousness, analgesia and muscle relaxation, but might also suppress autonomic reflex activities and consequently lead to inadequate function of vital physiological systems such as the cardiovascular and respiratory system (Rees & Gray 1950; Antognini & Carstens 2002). Use of inhalant anaesthetics to maintain general anaesthesia is associated with dose-dependent depression of the cardiopulmonary systems (Antognini & Eisele 1993; Hall et al. 2001; Hikasa et al. 2002). Balanced anaesthesia, a technique in which several drugs are combined at reduced dosages to decrease adverse effects of each drug, is used to limit cardiopulmonary depression associated with use of inhalant anaesthetics on their own to maintain general anaesthesia (Toner 2005; Solano et al. 2006). When applying balanced anaesthesia techniques, it is important to define the purpose of each drug used. Ideally, the effect of a selected drug and the dose or plasma concentration at which the effect occurs should be known (Mama 2006).

The are situations when total intravenous anesthesia (TIVA) might be the only possible means of achieving general anaesthesia in goats, for example when performing surgical procedures on the farm or in remote settings (Dundee & McMurray 1984; Carroll et al. 1997) or when performing specific diagnostic procedures like magnetic resonance imaging (Larenza et al. 2005). Furthermore use of TIVA has several other advantages, which include low costs when compared to inhalation anaesthesia and elimination of the hazards of occupational health and atmospheric pollution (Dundee & McMurray 1984; Hasley 1991; Nolan 2004).

The major drawback to use of injectable anaesthetic agents for induction and maintenance of anaesthesia has been reported to be the high likelihood of hypoxaemia (Carroll et al. 1997; Hall et al. 2001). Development of profound hypoxaemia in goats and sheep has been documented especially with anaesthetic protocols that include α_2 adrenoceptor agonists such as xylazine, romifidine, detomidine and medetomidine (Kumar & Thurmon 1979; Celly et al. 1997; Mogoa et al. 2000). Some authors still recommend cautious use of medetomidine, but only in young healthy goats (Carroll 2005).

Recently, advances in intravenous anaesthetic techniques have arisen from availability of new drugs with more appropriate pharmacokinetic profiles and lack of organ toxicity or significant adverse side effects (Sear 1991). In veterinary medicine, one must give consideration to the species in which the technique is to be applied as both the pharmcokineticprofiles and plasma concentrations at which specific effects occur might differ between species (Mama 2006). Total intravenous anaesthetic techniques have been developed to a greater degree in some species like dogs and horses (Nolan 2004), while very little information is available to support use of most of the TIVA drugs in goats (Carroll et al. 1999). Intravenous drugs that have previously been used for continuous rate intravenous infusion anaesthesia in dogs include opioids, α_2-adrenergic agonists, benzodiazepines, ketamine, propofol and lidocaine (Hellyer et al. 2003; Muir et al. 2003; Pascoe et al. 2006; Solano et al. 2006; Steagall et al. 2006; Bufalari et al. 2007; Intelisano et al. 2008). In goats, continuous

intravenous infusion of lidocaine and ketamine to supplement isoflurane anaesthesia produced promising results (Redua et al. 2005), while satisfactory total intravenous anaesthesia was obtained with a combination of ketamine and propofol (Larenza et al. 2005). In the present studies, anaesthetic and physiologic effects of midazolam, fentanyl and propofol for total or partial intravenous anaesthesia in goats were evaluated in an effort to determine if they are suitable drugs for use in TIVA combinations in goats.

1.2 GENERAL ANAESTHESIA IN GOATS

Goats make good research subjects because they can be gentle, easy to handle and transport; and are intelligent, affectionate, friendly and clean, and they appear to be hardier than other members of the ruminant family (Fulton et al. 1994). Their small size permits them to be maintained in a relatively small area. Goats can be readily anaesthetised using small animal (dog and cat) anaesthetic equipment that is available in most clinical practices (Caulkett 2003).

Anaesthetic management in goats is usually uncomplicated with the primary notable risk being regurgitation with potentially fatal pulmonary aspiration (Hall et al. 2001). Goats have a multi-compartmental stomach with a large rumen that does not empty easily (Thurmon et al. 1999). Withholding food for 12 to 24 hours may decrease ruminal fermentation and decrease the risk of regurgitation, although there will be no significant decrease in ruminal contents. Water can be withheld for 6-12 hours (Fulton et al. 1994). To help prevent aspiration of ruminal contents, prompt placement of an endotracheal tube and inflation of the tube after induction of general anaesthesia is recommended. Due to the long, narrow oral cavity and distant laryngeal opening, an otherwise difficult endotracheal intubation may be made easier by using a long-bladed laryngoscope and a non-flexible stylet to facilitate visualisation and placement of the tube into the trachea (Caulkett 2003).

Prior to an anaesthestic procedure, a thorough physical examination should be performed with special attention given to the respiratory system. It is not uncommon for goats to develop hypoxaemia with general anaesthesia (Hall et al. 2001). Normal eructation in the goat is hampered by anesthesia and by dorsal or lateral recumbent positioning. As a result, gas accumulates in the rumen causing ruminal tympany or bloat, and the distended rumen puts pressure on the diaphragm causing the lung capacity to decrease and interfere with ventilation (Fulton et al. 1994). Pressure on the major vessels then impedes venous return to the heart. Cardiac output, blood pressure, and tissue perfusion are then compromised and can lead to possible ventilation-perfusion mismatch. Hypoxaemia and hypercarbia may result and can be life threatening. Passing a stomach tube after intubation can help resolve gaseous distension.

Supportive therapy during anesthesia should ideally include; fluids intravenous fluid administration for maintenance and replacement needs, heat conservation and supplementation, and oxygen supplementation. Fluid maintenance can be achieved with a balanced electrolyte solution like Lactated Ringers at a flow rate of 4 to 6 mL kg^{-1} $hour^{-1}$ through an intravenous catheter placed in the jugular or cephalic vein (Fulton et al. 1994).

Anaesthetic and physiologic effects of total intravenous anaesthesia techniques have been investigated to a greater degree in humans, and to some extend in dogs and ponies (Nolan 2004), but there are few reports on use of the technique in goats. Xylazine is the most commonly used sedative for ruminants (Dehghani et al. 1991; Stegmann 1999; DeRossi et al. 2003), but doubts exist about its suitability for use in goats as it has been reported to cause hypoxaemia (Kumar & Thurmon 1979). There is therefore need to investigate the effects of other premedication drugs that can be used for anaesthesia purposes in goats. The benzodiazepines like diazepam and midazolam, the phenothiazines like acepromazine and some opioids like butorphanol are among the drugs that are currently used for sedation in goats. Induction agents like propofol, ketamine and thiopentone are still the main drugs used in goats (Thurmon et al. 1996; Hall et al. 2001).

1.3 PRINCIPLES GOVERNING ASSESSMENT OF DRUG PLASMA CONCENTRATIONS AND CLINICAL EFFECTS OF DRUGS

Three of the studies in the present series of studies aimed at determining the effects of midazolam, fentanyl and propofol on isoflurane minimum alveolar concentration (MAC) respectively. Inevitably, this required measurements of drug concentrations within body fluids. Design of dosing schemes for continuous rate infusion of intravenous anaesthetic agents can be either empirical or based on pharmacokinetics (Pypendop 2006). The empirical approach, which relies on selection of an initial dose and titration of subsequent doses based on response (Dundee & McMurray 1984; Pypendop 2006), was used in the present series of studies. The pharmacokinetic approach, on the other hand, is based on knowledge of the drug concentration producing desired effect and on the rate at which the drug concentration changes, allowing calculation of the drug dose to be administered in order to maintain desired concentration (Pypendop 2006).

The ideal body site for measurement of a drug concentration would be the biological fluids immediately surrounding the site of drug action (i.e. the receptor or the CNS membrane), but because sampling at such sites is impossible one is left with no choice but to use sites that are less than ideal. Measurement of drug concentrations in blood or plasma for intravenously administered drugs or end tidal gas concentrations for inhaled anaesthetics can be used for this purpose; the most important principle being that the values obtained will be from a representative sample that is in equilibrium with the biological fluids at the site of action of the drug (Stanski 2000; Kaul & Bharti 2002).

For inhaled anaesthetic agents, Eger and Bahlman 1971, showed that the concentration and partial pressure of the anaesthetic agent in arterial blood may be estimated from the concentration of the anaesthetic agent in the alveoli (end-tidal) provided that there is no large difference (more than

50%) between the inspired and expired concentrations. Factors that are likely to contribute to a large inspired-to-expired concentration include high blood-gas partition coefficient and hypoventilation (Eger & Bahlman 1971). The alveolar concentration of anaesthetic in expired gas is in equilibrium with the concentration in arterial blood, which has equilibrated with the concentration of the drug in the brain (Stanski 2000). It is important to note that most of the injectable drugs used in anaesthesia decrease anaesthetic requirements, as measured by the reduction in MAC (Kaul & Bharti 2002). In addition, numerous altered physiological states affect the requirements of inhaled anaesthetics (Table 1.1).

Table 1.1 Factors that affect minimum alveolar concentration in animals

Effect on MAC	Factor
Decrease	hypothermia severe hypotension pregnancy hypoxaemia (PaO_2<40mmHg) anaemia opioids, ketamine, benzodiazepines, barbiturates, α2-adrenergic agonists, amphetamines, cholinesterase inhibitors, intravenous local anaesthetics, reserpine, α-methyldopa
Increase	hyperthermia hyperthyroidism
No effect	duration of anaesthesia sex hypercarbia, hypocarbia isovolaemic anaemia hypertension

Adapted from Stanski, 2000

1.4 LITERATURE ON DRUGS USED IN THE PRESENT STUDIES

1.4.1 MIDAZOLAM

Midazolam is a water-soluble benzodiazepine that is considered to be a fast-acting with a short elimination half-life (Cao et al. 2002). It can, unlike diazepam, be administered by the intramuscular route as well as the intravenous route (Thurmon et al. 1999). It is used as a mild sedative, a muscle relaxant or an anticonvulsant. It is frequently administered before ketamine to prevent muscle tremors and seizures (Thurmon et al. 1999).

Benzodiazepines have agonistic effects on specific benzodiazepine receptors located in the postsynaptic nerve endings within the central nervous system (Cao et al. 2002). The resultant increase in availability of the inhibitory neurotransmitter glycine leads to the anxiolytic and muscle relaxant effects. Sedation and anticonvulsant activity are mediated by gamma-aminobutyric acid (GABA) in the cerebral cortex and motor centers. Benzodiazepines are becoming increasingly popular in veterinary anaesthesia because of their mild cardiovascular and respiratory effects (Caulkett 2003). The sedative and hypnotic effects of midazolam are dose-dependent as well as route-dependent. In goats, midazolam, administered a as a sole agent intravenously has been reported to produce maximal sedative effects after 20 minutes at 0.6 mg kg^{-1} intramuscularly; hypnosis after 5 minutes at 0.6 mg kg^{-1} intravenously and even more profound central nervous system depression at 1.2 mg kg^{-1} (Stegmann & Bester 2001). At a dose rate of 0.125 mg kg^{-1} $hour^{-1}$, midazolam can be used to maintain anaesthesia, usually combined with other hypnotic agents like ketamine, propofol or isoflurane (Sear 1991).

1.4.2 ACEPROMAZINE

Acepromazine (ACP) is the most commonly used phenothiazine derivative in veterinary anaesthesia. It is not as commonly used in ruminants as it is in horses (Fulton et al. 1994). Acepromazine, at dosages of 0.05–0.1 mg kg^{-1} can be used to provide mild tranquillisation in goats

(Bertens et al. 1993). Phenothiazines have antagonistic action on dopamine receptors, as well as histamine receptors, serotonin receptors and catecholamine receptors (Doherty et al. 2002a). As a result of its effects on a wide array of neurotansmitters, ACP produces a variety of effects such as peripheral vasodilation, hypotension, hypothermia and behavioural modifications (Hall et al. 2001). Acepromazine is commonly combined with opioids since it lacks analgesic properties.

1.4.3 BUTORPHANOL

Butorphanol, a synthetic opioid is an agonist at κ- and an antagonist at μ-opioid receptors (Valverde & Gunkel 2005). There are conflicting statements on effects of butorphanol on μ-opioid receptors as some authors report it to be a partial agonist (Doherty et al. 2002a). Opioids are traditionally included in balanced anaesthesia for their analgesia and contribution to central nervous system depression (Trim 2005). Butorphanol has analgesic properties in ruminants, however it can also induce behavioural changes (Doherty et al. 2002a). It is important to highlight that conflicting conclusions have been drawn regarding the behavioural effects of butorphanol in goats (Carroll et al. 2001). Butorphanol, 0.02–0.20 mg kg^{-1} intramuscularly or intravenously increases sedation from acepromazine or benzodiazepines (Hall et al. 2001; Valverde & Gunkel 2005), with the sedatives helping diminish the behavioural effects of butorphanol (Carroll et al. 2001). The duration of effect is reported to be 45 minutes to 2 hours (Carroll et al. 2001, Trim 2005). Limited respiratory depression, minimal cardiovascular effects and a lack of gastrointestinal side effects are significant advantages over other opioids (Thurmon et al. 1999).

1.4.4 FENTANYL

Fentanyl, a synthetic μ-agonist opioid, is used for the treatment of moderate to severe pain in dogs and humans (Carroll et al. 1999). Opioids are used extensively for premedication, supplementing regional and general anesthesia, as primary anaesthetic agents, and as analgesics for postoperative pain (Stanski 2000). While many opioids are available for intravenous infusion, fentanyl

is frequently selected as it offers clinically desirable effects over a wide dose range and has a wide therapeutic margin (Mama 2006). The onset of action of fentanyl is rapid following intravenous or intramuscular administration with analgesia developing in 3 to 8 minutes. It has a short duration of action, with the peak effect lasting less than 30 minutes (Thurmon et al. 1996). In goats, fentanyl 0.01 mg kg^{-1} proved effective against thermal and mechanical stimuli in a nociceptive model study (Valverde & Gunkel 2005). A 0.05 mg $hour^{-1}$ transdermal patch was associated with unpredictable plasma concentrations in goats (Carroll et al. 1999). Studies in humans and dogs have shown that fentanyl is not a complete general anaesthetic agent, but can be combined with benzodiazepines or can also be combined with sub-anaesthetic doses of propofol to achieve satisfactory levels of general anaesthesia (Stanski 2000).

1.4.5 PROPOFOL

Propofol is one of the most useful agents used to achieve TIVA in humans and animals (Bettschart-Wolfensberger et al. 2000; Sano et al. 2003; Larenza et al. 2005). It is poorly water-soluble and is classically marketed as an aqueous emulsion containing 10 mg of propofol, 100 mg of soyabean oil, 22.5 mg of glycerol and 12 mg of egg lecithin per millimeter (Thurmon et al. 1996), but a clear propofol solution has recently been introduced (Dubey & Kumar 2005). In goats, propofol has a rapid and smooth onset of action, is cleared rapidly and is easy to titrate to a desired effect level (Reid et al. 1993; Larenza et al. 2005). Propofol is easy to titrate because its total body clearance is rapid, even exceeding hepatic blood flow, which makes propofol a suitable agent for TIVA (Pablo et al. 1997). Propofol causes a dose-related decrease in blood pressure due to peripheral vasodilation and myocardial depression (Sear 1991). Propofol may also cause bradycardia, epileptiform seizures and true convulsions (Bettschart-Wolfensberger et al. 2000). Propofol, 4-7 mg kg^{-1} intravenously, in unpremedicated goats and sheep, will induce sufficient anaesthesia for endotracheal intubation (Reid et al. 1993; Pablo et al. 1997) while 3 mg kg^{-1} is sufficient in premedicated goats (Bertens et al. 1993; Prassinos et al. 2005). One pharmacokinetic study of propofol in goats showed the half-life of

propofol to be 15.5 minutes with initial blood propofol concentrations of about 7 µg mL^{-1} immediately after injection of a 4 mg kg^{-1} bolus rapidly dropping in a bi-exponential pattern to 0.1 µg mL^{-1} after 60 minutes of administration (Reid et al. 1993). The continuous intravenous infusion rate to maintain anaesthesia is within the range of 0.3 to 0.6 mg kg^{-1} $minute^{-1}$. The use of propofol as the sole agent for TIVA is generally unsatisfactory, since the concentration levels required to eliminate responses to surgery cause cardiovascular and respiratory depression (Nolan 2004). Combination of propofol with ketamine is an alternative technique for TIVA that has been successfully tried in goats (Larenza 2005). Induction was achieved with propofol 1 mg kg^{-1} and ketamine 3 mg kg^{-1}. Anaesthesia was maintained with a combined infusion of propofol 0.3 mg kg^{-1} $minute^{-1}$ and ketamine 0.03 mg kg^{-1} $minute^{-1}$ and 100% inspired oxygen. Caution should be exercised when using propofol in TIVA protocols because it can only be safely mixed in one syringe with 5% glucose or lidocaine (Bettschart-Wolfensberger et al. 2000).

1.4.6 ISOFLURANE

Isoflurane is a commonly used inhalant anaesthetic, which has short induction and recovery times because of its low blood-gas solubility coefficient (McEwen et al. 2000). It can be used for induction as well as maintenance of anaesthesia in goats (Antognini & Eisele 1993). Isoflurane requirement in goats, as defined by MAC, has been reported to be within the range of 1.3 to 1.5% (Hikasa et al. 1998; Antognini et al. 2000c; Doherty et al. 2002b). To measure depth of anaesthesia, one must apply an appropriate form of stimulus to the CNS and then observe the clinical response. The stimulus should be measurable and reproducible, or supramaximal, and the time between initiation of stimulation and the occurrence of a peak response must be known reasonably well. For determination of MAC in animals, the standard stimulus has been the application of a surgical clamp to the base of the tail. Tail clamping represents a highly noxious stimulus that is clinically reproducible and not excessively traumatic (Stanski 2000).

Mask induction can be achieved using isoflurane in oxygen. Initially, the goat should be allowed to breathe 100% oxygen at a flow rate of 4 to 6 L/min for several minutes to achieve denitrification. The inhalant should be slowly increased by 0.5% increments every 30 seconds until a 3 to 3.5% vaporizer setting is reached. The goats usually struggle during the first 2 minutes, becoming recumbent, and then losing corneal and pinch withdrawal reflex in 5–10 minutes (Antognini et al. 2000c). Intubation is likely to be possible in 10 minutes using a circle patient-breathing system (Fulton et al. 1994). Oxygen flow rate can then be decreased to 1 to 2 L/min and the isofurane vaporizer setting can be maintained at a level of 1-2%. Isoflurane, like most other inhalant anaesthetic agents, causes a dose-dependent respiratory depression as well as hypotension and reduced cardiac output (Antognini & Eisele 1993; Hall et al. 2001).

1.5 PROBLEM STATEMENT

Total intravenous anaesthesia is becoming an emerging field in general anesthesia and has been developed and used in some species, notably dogs and horses. Information on TIVA protocols for goats is very scant at the moment, yet there are situations [field anesthesia, anaesthesia for magnetic resonance emerging (MRI), research] when TIVA might be the preferred way to achieve general anaesthesia in goats. The need for knowledge on effects of intravenous anaesthetics in goats becomes even more important when one considers the fact that xylazine, which has historically been the main sedative in goats has been shown to be associated with profound hypoxaemia in some goats. There is therefore need to study effects of some TIVA protocols in goats with the aim of determining the best combination of sedative, analgesic and/or hypnotic drugs that can be used.

1.6 BENEFITS AND SIGNIFICANCE OF THE PRESENT STUDIES

Findings of the present studies will provide specific data on anaesthetic and physiological effects of several anaesthetic drugs and drug combinations in anaesthetised goats. The data will be a valuable reference source for large animal practitioners, veterinary anaesthetist and other biomedical scientists. The information should also help improve general welfare of goats undergoing anaesthetic procedures.

1.7 OBJECTIVES OF THE PRESENT STUDIES

A series of investigations into effects of various intravenous anaesthetic agents on goats was planned. The dosages of the drugs used in the investigation were based on references cited in the literature review section of this document. The specific objectives of the five studies carried out were:

a) Investigation of the sedative, propofol-sparing and cardiopulmonary effects of acepromazine, midazolam, butorphanol and combinations of butorphanol with acepromazine or midazolam in goats.
b) Evaluation of the effects of different dosages of midazolam on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
c) Evaluation of the effects of different dosages of fentanyl on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
d) Evaluation of the effects of different dosages of propofol on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
e) Evaluation and comparison of the efficacy of propofol and fentanyl, and propofol and midazolam for total intravenous anesthesia in goats.

All the studies were approved by the Faculty's Research Committee as well as the Animal Use and Care Committee under one propotocol number (Protocol V045/06).

Sedative and cardio-pulmonary effects of acepromazine, midazolam, butorphanol, acepromazine-butorphanol and midazolam-butorphanol on propofol anaesthesia in goats

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2.1 ABSTRACT

Objective To evaluate the sedative, propofol-sparing and cardiopulmonary effects of acepromazine, midazolam, butorphanol and combinations of butorphanol with acepromazine or midazolam in goats.

Study Design Prospective, randomized, crossover experimental design

Animals Six healty goats; 3 does and 3 ewes

Methods The 6 goats were assigned to treatments, in a randomised cross-over design with a 3-week interval between treatments as follows: saline (Treatment SAL), acepromazine (Treatment ACE), midazolam (Treatment MID), butorphanol (Treatment BUT), acepromazine and butorphanol (Treatment ACEBUT) and midazolam and butorphanol (Treatment MIDBUT) as premedication agents intramuscularly. The degree of sedation was assessed twenty minutes after administration of the premedication agents. Thirty minutes after premedication, the dose of propofol required for induction of anaesthesia adequate to allow placement of an endotracheal tube was determined. Cardiovascular, respiratory and arterial blood-gas parameters were assessed up to thirty minutes after induction of general anaesthesia.

Results Acepromazine and midazolam produced significant sedation when administered alone, but premedication regimens incorporating butorphanol produced inconsistent results. The dose of propofol required for induction of anaesthesia was significantly reduced in goats that received midazolam alone, or midazolam combined with either acepromazine or butorphanol. The quality of induction of anaesthesia was good following all treatments including the control (Treatment SAL). Cardiovascular and respiratory and blood-gas parameters were within normal limits following all treatments and not significantly different between or within treatments.

Conclusion Sedation with midazolam alone, or midazolam combined with either acepromazine or butorphanol significantly reduces the induction dose of propofol with minimal cardiopulmonary effects in goats.

Keywords goat, acepromazine, midazolam, butorphanol, sedation, propofol anaesthesia

2.2 INTRODUCTION

The concept of administering sedatives before an injectable anaesthetic induction agent is well accepted in veterinary practice. Sedatives are used pre-operatively to induce sedation, improve the quality of induction of anesthesia and more importantly, may result in fewer drug-related adverse effects by reducing the amount of injectable or inhalation anaesthetics required to induce and maintain general anesthesia (Kojima et al. 2002; Sano et al. 2003; Lemke 2007).

Sedatives used for calming small ruminants include α_2 adrenoceptor agonists like xylazine, phenothiazines like acepromazine, benzodiazepines like diazepam and midazolam, and opioids like butorphanol (Riebold 2007).

Xylazine is a commonly used sedative in ruminants (Dehghani et al. 1991; Stegmann 1999; DeRossi et al. 2003) but there are concerns about the threat of hypoxaemia associated with its use in small ruminants (Kumar & Thurmon 1979; Celly et al. 1997; Bacon et al. 1998). Development of profound hypoxaemia in goats and sheep probably as a result of pulmonary oedema and extravasation of red blood cells into the alveoli has been documented especially with α_2 adrenoceptor agonists such as xylazine, romifidine, detomidine and medetomidine (Kumar & Thurmon 1979; Celly et al. 1997; Bacon et al. 1998; Mogoa et al. 2000). This development of hypoxaemia has been documented in sheep following administration of all four α_2 adrenoceptor agonists listed above (Doherty et al. 1986; Nolan et al. 1986; Celly et al. 1997), but in goats, has only been documented following administration of xylazine (Kumar & Thurmon 1979). Some authors still recommend cautious use of medetomidine, but only in young healthy goats (Carroll et al. 2005). This study did not include assessment of the α_2 adrenoceptor agonists due to the uncertainty about their safety when used in goats.

Acepromazine (ACP) is the most commonly used phenothiazine derivative for mild tranquillisation in veterinary practice (Bertens et al. 1993; Hall et al. 2001). Phenothiazines have antagonistic action on dopamine receptors, as well as on histamine receptors, serotonin receptors and catecholamine receptors (Doherty et al. 2002a; Lemke 2007). Acepromazine produces numerous effects such as peripheral vasodilation, hypotension, hypothermia and behavioural modifications (Hall et al. 2001). Acepromazine is commonly combined with opioids as it lacks analgesic properties (Hall et al. 2001; Lemke 2007).

Midazolam is a water-soluble benzodiazepine that is considered to be fast-acting with a short elimination half-life (Cao et al. 2002; Lemke 2007). It can, unlike diazepam, be administered by the intramuscular route as well as the intravenous route (Lemke 2007). Midazolam has mild cardiovascular and respiratory effects and is commonly used as a mild tranquilliser, a muscle relaxant and anticonvulsant (Lemke 2007). Benzodiazepines have agonistic effects on specific benzodiazepine receptors located in the postsynaptic nerve endings within the central nervous system (Cao et al. 2002; Mehlisch 2002). The resultant increase in availability of the inhibitory neurotransmitter glycine leads to the anxiolytic and muscle relaxant effects. Sedation and anticonvulsant activity are mediated by gamma-aminobutyric acid (GABA) in the cerebral cortex and motor centers (Young et al. 2005). The sedative and hypnotic effects of midazolam are dose-dependent as well as dependent on route of administration. Midazolam can produce maximal sedative effects in 20 minutes after intramuscular administration of 0.6 mg kg^{-1} (Stegmann & Bester 2001).

Butorphanol, a synthetic opioid, is an agonist at κ-opioid receptors and an antagonist at μ-opioid receptors (Carroll et al. 1997; Valverde & Gunkel 2005; Lamont & Mathews 2007). There are conflicting statements in the literature on effects of butorphanol on μ-opioid receptors as some authors claim it is a partial agonist (Doherty et al. 2002a). Opioids are traditionally included in balanced anaesthesia protocols for their analgesic effects, but they also have sedative effects (Lemke

2007). Butorphanol has analgesic properties in ruminants, however it can also induce excitatory behavioural changes (Carroll et al. 2001; Doherty et al. 2002a). Butorphanol 0.02–0.50 mg kg^{-1}, administered intramuscularly or intravenously, increases sedation from acepromazine or benzodiazepines (Hall et al. 2001; Valverde & Gunkel 2005; Riebold 2007), while at the same time the sedatives (acepromazine and benzodiazepines) would help diminish the behavioural effects of butorphanol (Carroll et al. 2001).

Propofol (2,6-diisopropyl-phenol) is one of the induction agents commonly used in goats. It has a rapid and smooth onset of action and is cleared rapidly from the tissues (Reid et al. 1993; Larenza et al. 2005; Grossherr et al. 2006). Besides metabolism by the liver, extrahepatic sites of metabolism, most prominently the lung, have been claimed for propofol (Grossherr et al. 2006). Propofol causes a dose-related decrease in blood pressure due to peripheral vasodilation and myocardial depression, bradycardia, epileptiform seizures and true convulsions (Sear 1991; Bettschart-Wolfensberger et al. 2002). When administered at a dose of 4-7 mg kg^{-1} intravenously in unpremedicated goats and sheep, propofol will induce sufficient anaesthesia for endotracheal intubation (Reid 1993; Pablo et al. 1997), while 3 mg kg^{-1} was shown to be sufficient for endotracheal intubation in premedicated goats (Bertens et al. 1993). One pharmacokinetic study of propofol in goats showed the half-life of propofol to be 15.5 minutes (Reid et al. 1993).

The purpose of this study was to determine the quality of sedation, the magnitude of reduction of induction dose of propofol, and the quality of general anaesthesia obtained after sedation of goats with acepromazine, midazolam and butorphanol and combinations of butorphanol with acepromazine or midazolam. The impact of these anaesthesia protocols on the cardiovascular and respiratory systems as assessed by such parameters like heart rate, blood pressure, respiratory rate, blood-gas partial pressures were also assessed.

2.3 MATERIALS AND METHODS

2.3.1 EXPERIMENTAL DESIGN AND INSTRUMENTATION

A day before the investigations, the goats, which were housed at the Onderstepoort Teaching Animal Unit (OTAU) were transferred to the University of Pretoria's Biomedical Centre (UPBRC) where the studies were carried out. Six clinically healthy Boer - Indigenous African crossbreed goats (3 does and 3 wethers) were used in this study (Table 2.2).

Using the Random Numbers Table, the 6 goats were assigned to treatments, in a randomised cross-over design with a 3-week interval between treatments as follows: SAL when saline (0.9% NaCl) was administered as a premedication agent, ACE when acepromazine was administered as a premedication agent, MID when midazolam was administered as a premedication agent, BUT when butorphanol was administered as a premedication agent, ACEBUT when acepromazine and butorphanol were administered as premedication agents and MIDBUT when midazolam and butorphanol were administered as premedication agents.

Food and water were withheld overnight, approximating 18–24 hours before anaesthesia. A clinical examination of the goats was done a day before anesthesia. Venous blood samples in ethylenediaminetetraacetic acid (EDTA) tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for a complete blood count and in serum tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for serum protein analysis were collected soon after completion of physical examination of the goats.

The goats were weighed using an electronic scale (Jadever® Richter Scale, Jadever, Scale Co. Ltd, Taipei, Taiwan) the morning of treatment day. The goats were placed on a custom-made sling-cum-table (Figure 2.1) to make restraint easier throughout the duration of the investigations.

Figure 2.1 Photograph of a goat placed on custom-made sling-cum-table.

The auricular artery was percutanously catheterized using a 24-SWG catheter (Jelco®, Medex Medical Ltd, Rossendale, Great Britain) for measurement of arterial blood pressure and collection of arterial blood samples for blood-gas analysis. A transducer (DTX Plus ™ disposable transducer, BD Medical, Johannesburg, South Africa) and a multi-parameter monitor (Model BSM-4103K, Nihon Kohden Corporation, Tokyo, Japan) calibrated according to manufacturer recommendations were used for blood pressure measurements. For transducer calibration to atmospheric pressure, the scapulo-humeral joint or the point of the sternum were used as zero reference points in sternally-recumbent or laterally-recumbent goats, respectively. Electrocardiography (ECG) electrodes were attached so that an electrocardiogram could be monitored. An 18-SWG catheter (Introcan® Safety, B. Braun Medical Incorporated, Bethlehem, United States of America) was introduced into the cephalic vein for administration of intravenous propofol and fluids.

The goats were then premedicated by the intramuscular route with 1 mL of saline (Intramed Sodium Chloride 0.9%® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) (Treatment SAL), acepromazine maleate (Aceprom 2®, Bayer Pty Ltd, Isando, South Africa) at 0.05 mg kg^{-1} (Treatment ACE), midazolam (Dormicum®, Roche Products Pty Ltd, Isando, South Africa) at 0.3 mg kg^{-1} (Treatment MID), butorphanol tartrate (Torbugesic®, Fort Dodge Animal Health, Fort Dodge, USA) at 0.1 mg kg^{-1} (Treatment BUT), acepromazine maleate at 0.05 mg kg^{-1} and butorphanol tartrate at 0.1 mg kg^{-1} (Treatment ACEBUT), or midazolam 0.3 mg kg^{-1} and butorphanol 0.1 mg kg^{-1} (Treatment MIDBUT). The degree of sedation was scored 20 minutes after administration of the premedication agent (Table 2.1), as it is most probable that the sedative effects were optimum at this time.

Table 2.1 Scoring system used to evaluate sedation, quality of induction, quality of recovery from anaesthesia in this study where preanaesthetic administration of saline 1.0 mL (SAL Treatment), acepromazine 0.05 mg kg^{-1} (ACE Treatment), midazolam 0.3 mg kg^{-1} (MID Treatment), butorphanol 0.1 mg kg^{-1} (BUT Treatment), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT Treatment), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT Treatment) was followed administration of propofol for induction of general anaesthesia in goats.

Score	Sedation (0-3)	Anaesthesia Induction (0-2)	Anaesthesia Recovery (0-2)
0	No sedation	Poor (excitement, jumps or attempt to stand after becoming recumbent, unable to place endotracheal tube)	Poor (restlessness or excitement)
1	Mild Sedation (mild sensory or motor deficits)	Fair (slightly prolonged (> 2 minutes) induction time or mild excitation)	Fair (minimal restlessness or excitement)
2	Moderate sedation (may assume sternal recumbency)	Good (no excitement, endotracheal intubation easy)	Good (no restlessness or excitement)
3	Deep sedation (failing to maintain sternal recumbency, but may raise the head without holding it up)		

Thirty minutes after administration of the premedication agents, propofol (Propofol 1%® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) was administered intravenously to induce a level of anaesthesia adequate to allow placement of an endotracheal tube. A quarter (2.5 mg kg^{-1}) of the estimated total dose (10 mg kg^{-1}) of propofol necessary to induce anaesthesia was administered within 30 seconds. A tenth (1 mg kg^{-1}) of the estimated total dose of propofol was then administered intermittently, checking depth of anaesthesia between administrations, until the jaws were adequately relaxed to allow intubation. Placement of the endotracheal tube (silicone tube, internal diameter 7.5mm) was done with the goats in sternal recumbency using a laryngoscope to facilitate the process. Immediately after intubation, the goats were placed in left lateral recumbency and the total dose of propofol required for induction was recorded. Quality of induction was scored at this point (Table 2.1).

The goats were allowed recover after intubation and no other anaesthetic agents were administered from thereon. They were allowed to breathe spontaneously on room air. Ringer Lactate solution (Intramed Ringer-Lactate® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) was administered at a rate of 4 mL kg^{-1} hr^{-1} intravenously up to 30 minutes after induction of general anaesthesia. The endotracheal tube was removed after the goats regained the swallowing reflex. Time to extubation and sternal position were recorded. It was noted whether the goats were able to walk voluntarily by the time they were taken off the working table (i.e. at 30 minutes after induction of general anaesthesia). Times to extubation and sternal position were determined as the interval between the time the last dose of propofol was administered and the time a particular event happened. Quality of recovery was scored once the goats were able to walk voluntarily based on th scoring system on Table 2.1.

Cardiopulmonary parameters like systolic, diastolic and mean arterial pressures, heart rate and respiratory rate as well as body temperature were recorded at the following times: prior to administration of the premedication agents (baseline), 20 minutes after administration of the premedication agents, at induction and at 10, 20 and 30 minutes after induction. Arterial blood

samples for blood-gas analysis were collected in pre-heparinised (Heparin sodium–Fresenius 1000 IU mL^{-1}, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) plastic syringes (Omnifix® 1ml, B.Braun, Melsungen, Germany) at the following times: prior to premedication, 20 minutes after premedication and 20 minutes after intubation. The syringes containing arterial blood samples were sealed and placed in ice water for blood-gas analysis within one hour of collection. From these blood samples, arterial oxygen tension (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), arterial hydrogen ion concentration (pHa), arterial bicarbonate ion ($[HCO_3^-]$) concentration and arterial oxygen tension (SaO_2) were measured and recorded. Blood gas analysis was done using a pre-calibrated machine (Rapidlab ™ 348 pH/Blood Gas and Electrolyte Analyser, Siemens Medical Solutions Diagnostics, Midrand, South Africa).

2.3.2 STATISTICAL ANALYSIS

Data were analysed using the R® Statistical Software, Version 2.7.2 (The R Foundation for Statistical Computing, Vienna, Austria). All data were assumed to be non-parametric because of the small sample size and are expressed as median and inter-quartile ranges.

Data on propofol induction dose, reduction in propofol induction dose, sedation score, induction scores, time to extubation, time to sternal position, time to standing, and recovery scores were tested for statistically significant differences between groups using the Friedman test. If statistically significant differences were found between treatments, post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted to identify which groups were different.

Medians of repeatedly measured variables (systolic, diastolic and mean arterial pressures, heart rate, respiratory rate, PaO_2, $PaCO_2$, pH, bicarbonate concentration, SaO_2 and body temperature) were tested for statistically significant differences between and within treatments using repeated

measures analysis of variance (ANOVA) by ranks. If statistically significant differences were found, a post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted. A value of $P < 0.05$ was considered significant.

2.4 RESULTS

There were no statistically significant differences in terms of age and weight nor between pre-anaesthetic total serum protein, haematocrit, white cell count and body temperature between the treatments (Table 2.2).

Statistically significant differences were present between the treatments in terms of sedation score ($P = 0.013$), propofol induction dose ($P = 0.006$), extubation time ($P = 0.005$) and sternal position attainment time ($P = 0.005$), but no statistically significant difference between the treatments were noted in score for quality of recovery from anaesthesia (Table 2.3, Figure 2.2). Pair-wise comparisons showed that the induction doses for MID treatment ($P = 0.032$), ACEBUT treatment ($P = 0.032$) and MIDBUT treatment ($P = 0.032$) were significantly lower than the induction dose for the control (SAL treatment). The sedation scores were significantly higher for ACE treatment ($P = 0.035$) and MID treatment ($P = 0.033$) in comparison to the control. Pair-wise comparison of induction scores, extubation times and sternal position attainment times did not show any statistically significant differences between the control and any of the treatments.

Cardiovascular system variables did not show any statistically significant differences between the control and any of the treatments as well as between the baseline reading and any other time point reading within a treatment (Table 2.4). The ECG did not show any arrhythymias for any of the sedative regimes.

Respiratory system variables were not statistically significantly different both within treatments and when treatments were compared to the control with the exception of three data

points. For MID treatment respiratory rate was significantly lower at 1 minute (P = 0.0003) after induction of anaesthesia when compared to the baseline reading and for the ACEBUT treatment respiratory rate was significantly lower at 1 minute (P = 0.000006) and at 10 minutes (P = 0.003) after induction of anaesthesia when compared to the baseline reading (Table 2.5).

Of the adverse effects observed; clonic-tonic convulsions and/or opisthotonus at induction were observed in 6 goats (1 SAL treatment, 2 ACE treatment, 1 MID treatment and 2 BUT treatment). One goat showed excitement (characterized by vocalization and falling around) at recovery following SAL treatment. One goat had induction apnoea lasting more than 30 seconds at induction following ACE treatment and MIDBUT treatment and 1 goat had ruminal bloat at recovery from anaesthesia following ACEBUT treatment.

At all times the goats were able to stand and walk voluntarily after 30 minutes from the time of induction of anaesthesia.

2.4.1 TABLES

Table 2.2 Profile of the goats [median (inter-quartile range)] used in a study where preanaesthetic administration of saline 1.0 mL (SAL treatment), acepromazine 0.05 mg kg^{-1} (ACE treatment p), midazolam 0.3 mg kg^{-1} (MID treatment, butorphanol 0.1 mg kg^{-1} (BUT treatment), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT treatment), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT treatment) was followed by intravenous administration of propofol for induction of general anaesthesia in goats.

Treatment	Variable						
	Size	Age (months)	Weight (kg)	TSP (g L^{-1})	Haematocrit (Litres $Litre^{-1}$)	White Cell Count ($x10^9$ L^{-1})	Temperature (ºC)
SAL	6 (3 M, 3 F)	5.5 (5.0-6.8)	21.8 (21.5-23.5)	62.1 (60.3-63.5)	0.33 (0.32-0.36)	13.25 (12.4-15.1)	39.0 (38.9-39.2)
ACE	6 (3 M, 3 F)	6.0 (5.3-7.5)	21.0 (19.7-23.3)	62.8 (55.6-65.1)	0.31 (0.29-0.0.33)	14.4 (13.5-15.2)	39.20 (39.0-39.3)
MID	6 (3 M, 3 F)	6.5 (4.5-7.8)	21.9 (20.2-24.5)	61.7 (60.34-63.0)	0.34 (0.28-0.35)	12.7 (12.4-13.9)	39.0 (38.9-39.4)
BUT	6 (3 M, 3 F)	8.0 (7.0-9.0)	21.7 (21.1-23.1)	63.1 (61.4-65.7)	0.32 (0.27-0.33)	13.1 (12.8-14.1)	39.2 (38.6-39.4)
ACEBUT	6 (3 M, 3 F)	8.0 (7.3-8.8)	21.9 (20.7-22.9)	63.0 (57.9-68.2)	0.38 (0.33-0.42)	17.8 (12.5-22.1)	39.0 (38.8-39.5)
MIDBUT	6 (3 M, 3 F)	6.5 (5.3-7.0)	22.0 (20.3-23.5)	62.4 (62.2-62.7)	0.33 (0.31-0.34)	14.8 (13.9-15.8)	38.9 (38.8-39.1)

NS: No statistically significant differences (P<0.05) between the six treatments.
M: male; F: female.

Table 2.3 General anesthesia induction dose, extubation time, sternal position time [median (inter-quartile range)], percentage reduction in induction dose, and sedation score, induction score, recovery score {median(range)} following preanaesthetic administration of saline 1.0 mL (SAL treatment), acepromazine 0.05 mg kg^{-1} (ACE treatment), midazolam 0.3 mg kg^{-1} (MID treatment), butorphanol 0.1 mg kg^{-1} (BUT treatment), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT treatment), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT treatment) and then intravenous administration of propofol for induction of general anaesthesia in goats.

Treatment	Variable						
	Induction Dose Propofol (mg kg^{-1})	Reduction (%) Induction Dose	Sedation Score	Induction Score	Extubation Time (min)	Sternal Position Time (min)	Recovery Score
SAL	5.05 (4.68-5.58)	Not Applicable	0(0-0)	1(1-1.75	3.0 (1.5-3.0)	4.0 (4.0-4.8)	2(1-2)
ACE	4.53 (3.42-4.72)	20.8	1.5(1-2) *	2(2-2)	4.0 (3.0-6.5)	6.5 (5.0-9.5)	2(2-2)
MID	3.13 (3.04-3.34)*	39.7*	2(1.25-2) *	2(2-2)	6.5 (5.3-7.0)	9.0 (8.0-10.0)	2(1-2)
BUT	3.94 (3.82-4.42)	22.1	1(1-1)	2(1.25-2)	3.5 (3.0-4.8)	6.0 (5.0-9.3)	2(2-2)
ACEBUT	3.94 (3.82-3.99)*	27.8*	1.5(1-2)	2(2-2)	5.5 (5.0-9.0)	9.0 (7.3-10.0)	2(2-2)
MIDBUT	3.27 (3.19-3.57)*	38.1*	1(1-1.75)	2(2-2)	9.0 (8.3-9.8)	13.5 (10.8-17.0)	2(2-2)
	#	#	#	#	#	#	NS

#: Statistically significant differences (P<0.05) between treatments,
NS: No statistically significant differences (P<0.05) between the treatments,
*: Statistically significantly different (P<0.05) from SAL (control) treatment

Table 2.4 Cardiovascular variables and body temperature [median (inter-quartile range)] following preanaesthetic administration of saline 1.0 mL (SAL treatment), acepromazine 0.05 mg kg^{-1} (ACE treatment), midazolam 0.3 mg kg^{-1} (MID treatment), butorphanol 0.1 mg kg^{-1} (BUT treatment), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT treatment), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT treatment) and then intravenous administration of propofol for induction of general anaesthesia in goats.

Variable	Treatment	Baseline	20 minutes Post-sedation	Time after induction (minutes)			
				1	10	20	30
Heart Rate (beats $minute^{-1}$)	SAL	82 (72-95)	84 (79-88)	130 (113-130)	104 (97-130)	104 (96-108)	99 (93-106)
	ACE	106 (86-120)	104 (101-106)	115 (109-120)	102 (96-116)	100 (94-109)	98 (97-106)
	MID	94 (86-102)	87 (70-98)	110 (100-120)	100 (100-115)	97 (95-109)	99 (88-115)
	BUT	82 (76-88)	73 (64-89)	120 (114-120)	101 (100-104)	98 (74-103)	86 (71-92)
	ACEBUT	92 (86-113)	94 (84-106)	112 (103-116)	94 (92-102)	92 (85-119)	93 (87-114)
	MIDBUT	80 (80-89)	88 (82-93)	117 (96-138)	105 (100-118)	94 (89-105)	90 (88-94)
Systolic Blood Pressure (mmHg)	SAL	119 (114-124)	117 (115-123)	124 (117-137)	106 (1049-108)	107 (105-108)	
	ACE	108 (100-127)	98 (93-104)	94 (92-95)	101 (98-103)	96 (93-101)	
	MID	121 (114-130)	109 (103-115)	112 (100-129)	113 (107-129)	107 (103-120)	
	BUT	113 (103-119)	98 (98-109)	114 (86-118)	103 (97-111)	98 (94-101)	
	ACEBUT	121 (115-126)	108 (102-110)	92 (91-97)	105 (97-110)	103 (99-108)	
	MIDBUT	114 (113-116)	101 (92-103)	96 (94-107)	105 (97-120)	103 (96-112)	
Diastolic Blood Pressure (mmHg)	SAL	81 (79-86)	82 (79-85)	92 (81-98)	80 (69-90)	80 (74-82)	
	ACE	71 (64-83)	56 (51-63)	57 (53-64)	66 (59-77)	65 (58-70)	
	MID	81 (78-83)	74 (71-84)	77 (61-95)	89 (67-113)	83 (68-90)	
	BUT	72 (72-78)	74 (65-74)	86 (60-93)	86 (73-88)	74 (67-78)	
	ACEBUT	79 (78-83)	69 (63-72)	57 (53-65)	79 (64-89)	74 (60-81)	
	MIDBUT	82 (77-91)	69 (60-71)	71 (59-81)	68 (62-92)	73 (63-84)	
Mean Arterial Blood Pressure (mmHg)	SAL	101 (96-105)	101 (98-106)	102 (97-108)	91 (84-94)	93 (84-96)	
	ACE	89 (77-107)	75 (69-81)	78 (68-86)	81 (75-87)	78 (72-81)	
	MID	98 (96-100)	89 (85-100)	99 (72-114)	100 (83-118)	93 (83-102)	
	BUT	88 (88-97)	87 (87-89)	100 (72-104)	95 (86-98)	87 (86-87)	
	ACEBUT	100 (99-101)	89 (82-91)	72 (67-75)	90 (76-99)	88 (83-91)	
	MIDBUT	100 (99-103)	80 (73-86)	78 (70-91)	78 (74-102)	83 (75-97)	
Body Temperature (ºC)	SAL	39.0 (38.9-39.2)	39.5 (39.3-39.5)	39.3 (39.1-39.4)	39.3 (39.0-39.4)	39.2 (39.0-39.3)	39.2 (39.0-39.3)
	ACE	39.2 (39.0-39.3)	39.0 (38.7-39.3)	38.8 (38.7-39.1)	38.6 (38.4-38.9)	38.4 (38.2-39.0)	38.4 (38.2-39.1)
	MID	39.0 (38.9-39.4)	39.0 (38.8-39.3)	38.9 (38.6-39.2)	38.7 (38.6-39.0)	38.7 (38.6-39.0)	38.7 (38.6-39.1)
	BUT	39.2 (38.6-39.4)	39.2 (38.7-39.2)	39.0 (38.6-39.2)	38.7 (38.6-38.9)	38.6 (38.4-38.8)	38.6 (38.4-38.7)
	ACEBUT	39.0 (38.8-39.5)	39.1 (38.8-39.3)	38.7 (38.5-38.8)	38.7 (38.3-38.7)	38.5 (38.0-38.6)	38.3 (38.1-38.6)
	MIDBUT	38.9 (38.8-39.1)	39.1 (38.8-39.3)	38.9 (38.3-39.0)	38.6 (38.3-38.8)	38.3 (38.2-38.5)	38.4 (38.2-38.6)

Note: No statistically significant differences ($P<0.05$) were observed between the six groups or between baseline mean and any point within a group.

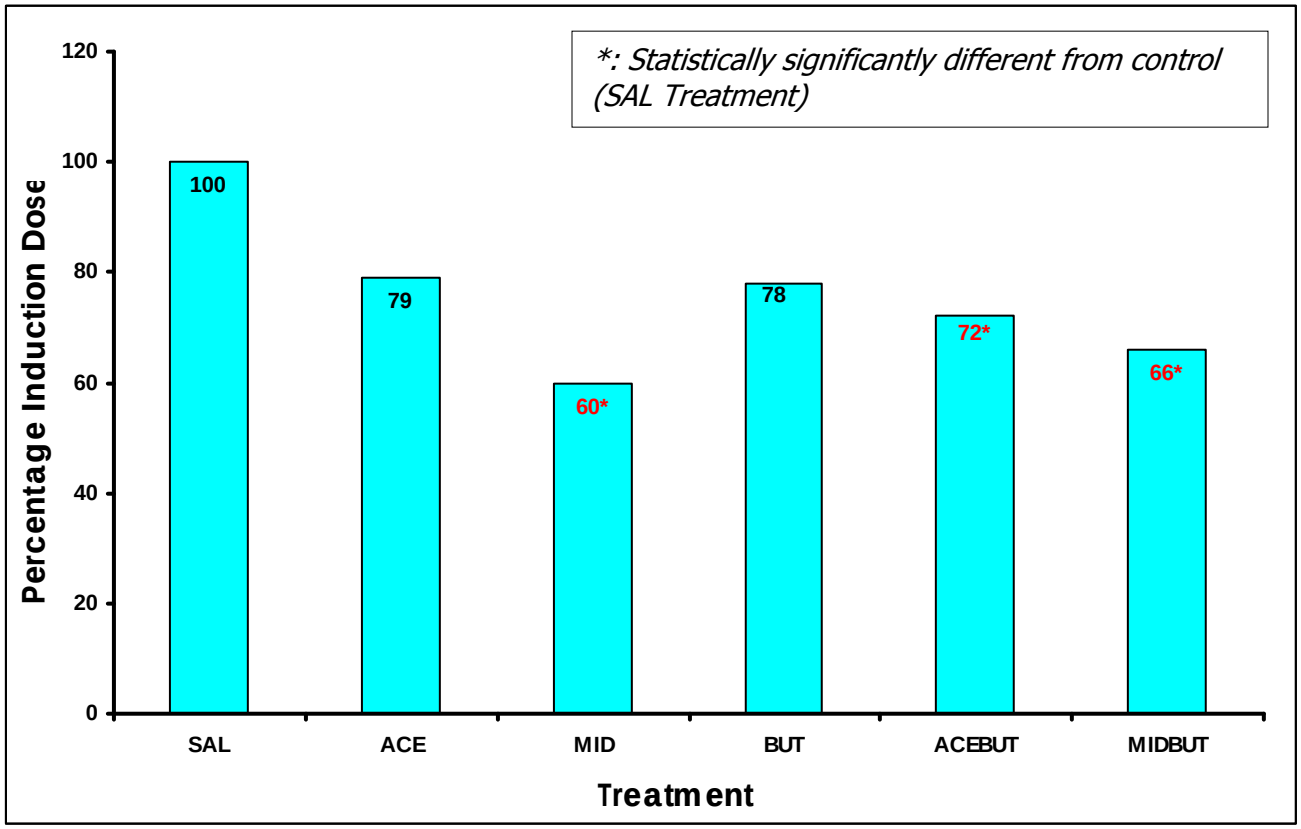
Table 2.5 Respiratory variables [median (inter-quartile range)] following preanaesthetic administration of saline 1.0 mL (SAL treatment), acepromazine 0.05 mg kg^{-1} (ACE treatment)), midazolam 0.3 mg kg^{-1} (MID treatment)), butorphanol 0.1 mg kg^{-1} (BUT treatment)), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT treatment)), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT treatment)) and then intravenous administration of propofol for induction of general anaesthesia in goats.

Variable	Treatment	Baseline	20 minutes post sedation	Time after induction (minutes)			
				1	10	20	30
f_R (breath $minute^{-1}$)	SAL	26 (21-28)	20 (17-23)	13 (9-15)	16 (16-19)	24 (18-27)	22 (20-24)
	ACE	24 (15-27)	18 (12-27)	8 (8-8) §	18 (13-20)	18 (13-20)	22 (17-24)
	MID	24 (24-30)	14 (12-19)	11 (9-12) §	13 (12-16)	20 (13-24)	20 (17-23)
	BUT	28 (28-31)	18 (16-29)	18 (13-21)	16 (16-16)	18 (16-23)	20 (20-20)
	ACEBUT	28 (28-31)	28 (19-30)	8 (6-11) §	12 (9-12) §	18 (13-20)	16 (13-19)
	MIDBUT	22 (17-30)	24 (17-31)	8 (7-11)	13 (12-15)	16 (13-19)	18 (16-20)
PaO_2 (mmHg)	SAL	85 (75-97)	80 (74-83)			74 (69-82)	
	ACE	81 (76-83)	78 (70-81)			75 (67-86)	
	MID	83 (81-85)	79 (72-81)			77 (61-80)	
	BUT	79 (78-83)	80 (72-91)			88 (85-90)	
	ACEBUT	85 (80-86)	77 (74-87)			98 (83-118)	
	MIDBUT	86 (79-89)	82 (78-87)			79 (74-91)	
$PaCO_2$ (mmHg)	SAL	33.9 (32.8-35.1)	32.6 (31.1-33.4)			33.6 (32.2-35.4)	
	ACE	33.8 (32.1-35.4)	34.4 (31.8-35.0)			33.9 (31.5-40.3)	
	MID	34.4 (33.9-35.4)	32.4 (30.7-33.7)			35.9 (34.7-38.6)	
	BUT	33.9 (31.6-36.3)	31.8 (30.9-33.3)			32.0 (28.7-32.9)	
	ACEBUT	33.6 (32.8-34.3)	34.5 (28.9-36.2)			34.0 (29.9-35.8)	
	MIDBUT	33.7 (30.7-36.2)	32.3 (29.7-36.6)			35.7 (34.6-37.8)	
pHa	SAL	7.46 (7.45-7.47)	7.48 (7.46-7.49)			7.46 (7.45-7.48)	
	ACE	7.43 (7.41-7.46)	7.45 (7.43-7.47)			7.41 (7.40-7.44)	
	MID	7.46 (7.44-7.47)	7.42 (7.40-7.43)			7.40 (7.38-7.41)	
	BUT	7.48 (7.47-7.48)	7.45 (7.44-7.48)			7.44 (7.40-7.46)	
	ACEBUT	7.44 (7.43-7.45)	7.41 (7.40-7.44)			7.42 (7.40-7.44)	
	MIDBUT	7.46 (7.45-7.47)	7.47 (7.45-7.47)			7.42 (7.39-7.43)	
$[HCO_3^-]$ (mol/litre)	SAL	23.6 (22.9-24.1)	23.6 (21.4-24.3)			23.5 (21.5-24.5)	
	ACE	21.5 (19.9-22.5)	22.1 (20.2-24.3)			22.5 (20.6-26.0)	
	MID	24.6 (22.2-25.3)	19.8 (18.5-22.8)			21.7 (20.6-23.2)	
	BUT	22.9 (22.4-26.0)	21.0 (20.7-22.5)			19.9 (16.6-21.0)	
	ACEBUT	22.7 (22.4-23.1)	21.1 (20.2-22.4)			22.7 (19.2-24.0)	
	MIDBUT	23.9 (21.2-25.6)	23.2 (20.4-25.7)			23.0 (20.7-24.7)	
SaO_2 (%)	SAL	96 (95-97)	97 (96-97)			96 (94-97)	
	ACE	96 (95-97)	96 (95-97)			95 (94-97)	
	MID	97 (96-97)	96 (95-97)			95 (90-96)	
	BUT	97 (96-97)	97 (95-97)			97 (97-97)	
	ACEBUT	97 (97-97)	96 (95-97)			97 (97-98)	
	MIDBUT	97 (96-97)	97 (96-97)			96 (95-97)	

§: Significantly different ($P<0.05$) from baseline reading
f_R - respiratory rate, PaO_2 - arterial oxygen partial pressure, $PaCO_2$ - arterial carbon dioxide partial pressure, pHa - arterial hydrogen ion concentration negative logarithm, $[HCO_3^-]$ - arterial bicarbonate ion concentration, SaO_2 - arterial oxygen saturation

2.4.2 FIGURES

Figure 2.2 Propofol dose as a percentage of dose required in the control group (SAL treatment) after administration of acepromazine 0.05 mg kg^{-1} (ACE treatment, midazolam 0.3 mg kg^{-1} (MID treatment), butorphanol 0.1 mg kg^{-1} (BUT treatment), acepromazine 0.05 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (ACEBUT treatment), and midazolam 0.3 mg kg^{-1} with butorphanol 0.1 mg kg^{-1} (MIDBUT treatment) in goats.



2.5 DISCUSSION

Uniformity of distribution of the descriptive data of the goats among the groups is supported by the fact that there were no statistically significant differences in the signalment (age, weight) as well as preanaesthetic total serum protein, haematocrit, white cell count and body temperature. This was largely expected since a randomized crossover design was used in allocation of the goats to the groups.

Acepromazine and midazolam, in agreement with currently available literature, produced a significant degree of sedation when administered alone (Bertens 1993; Stegmann & Bester 2001), but when combined with butorphanol the sedation score did not show any significant difference when compared to the unpremedicated goats. Butorphanol administered alone did not show a consistent significant degree of sedation. Lack of improvement in degree of sedation when butorphanol is combined with either acepromazine or midazolam is in disagreement with some studies in which a positive interaction was observed (Hall et al. 2001; Valverde & Gunkel 2005). This could be due to numerous reasons, such as that the sample size and the scale used to access sedation were not large enough to show differences or that the dose of butorphanol administered was not high enough. The sample size, the sedation scoring scale and the dose of butorphanol used in this study are based on those normally reported in similar studies (Carroll et al. 2001; Doherty et al. 2002a; Carroll et al. 2005). The sedative effects of butorphanol in goats have also previously been reported as variable and unpredictable (Doherty et al. 2002a). This unreability in degree of sedation could be related to butorphanol's behavioural effects associated with central nervous system excitement (Carroll 2001; Doherty et al. 2002a). Butorphanol has been reported to cause restlessness and vocalization (Carroll 2001). In the study reported here, excitement of the goats in any way was not observed when butorphanol was administered for sedation. Instead of accessing degree of sedation only at 20 minutes

after premedication as done in this study, the assessment could have been done at many time intervals as sedative effects could have varied with time for the different sedative regimes.

The dose of propofol for induction of general anaesthesia in unpremedicated goats of 5.05 mg kg^{-1} observed in the present study is similar to doses of 5.1 mg kg^{-1} (Pablo et al. 1997) and 5.6 mg kg^{-1} (Amarpal et al. 2002) reported in the literature. Significant decreases in propofol induction dose requirements were observed in goats that received midazolam alone (39.7%), midazolam combined with butorphanol (38.1%) and acepromazine combined with butorphanol (27.8%). These outcomes largely concur with previous studies which indicated that premedication of goats would decrease the dose of propofol for intubation to 3-4 mg kg^{-1} (Bertens et al. 1993, Carroll et al. 1998, Hall et al. 2001). At dosages used in the present study, premedication regimes incorporating midazolam seemed superior in terms of effect on reduction of propofol induction dose when compared to those incorporating acepromazine. This could be because midazolam has muscle relaxing properties in addition to central nervous system depressing effects or that the dose of midazolam used in this study is relatively more potent that the dose of acepromazine used. The quality of general anesthesia obtained with propofol in goats as assessed by induction score, time to extubation, time to sternal recumbency, time to standing and recovery score did not show any particular premedication regime to be superior to the control group. This could be due to the fact that goats, by nature, have a good temperament and are not easily excitable (Riebold 2007). These findings on quality and duration of anaesthesia after propofol induction with or without premedication are similar to findings from other studies in goats (Reid et al. 1993; Pablo et al. 1997; Carroll et al. 1998; Bettschart-Wolfensberger et al. 2000; Prassinos et al. 2005). Propofol anaesthesia is associated with rapid and excitement-free recoveries, with extubation time less than 10 minutes and time to standing less than 30 minutes in both premedicated and unpremedicated goats as this study as well as other related studies attest to (Reid et al. 1993; Pablo et al. 1997; Carroll et al. 1998; Bettschart-Wolfensberger et al. 2000; Prassinos et al. 2005). Occurrence of myoclonic activity (clonic-tonic convulsions and/or opisthotonus) on administration of propofol in a

number of species (Hall et al. 2001), which has been reported in other studies, was observed in few individual goats in this study. Premedication agents did not seem to influence occurrence of the myoclonic activities as their distribution between groups seemed random. These myoclonic activities may have been observed as a manifestation of light plane anaesthesia since all the goats in this study were anaesthetised just deep enough to allow placement of an endotracheal tube (Pablo et al. 1997). Some authors disagree with the speculation that higher doses of propofol would ameliorate development of myoclonic activity (Prassinos et al. 2005).

The cardiovascular data compared both within and between groups did not show any significant differences and were all within normal physiological ranges. This shows that the premedication regimes used for propofol general anaesthesia in dosages in this study minimally affected cardiovascular system function. The respiratory system was largely unaffected by the anaesthetic regimes used in this study as indicated by the obtained mean $PaCO_2$ and SaO_2 values, although the respiratory rate decreased considerably soon after administration of propofol for induction of anaesthesia. The fact that propofol is known to cause induction apnoea in most species may well explain why respiratory rate decreased soon after its administration to goats in this study (Bettschart-Wolfensberger et al. 2000, Kojima et al. 2002; Prassinos et al. 2005). Arterial blood-gas parameters, which are largely dependent on adequate function of both the respiratory and cardiovascular system in anaesthetized animals (Sobiech et al. 2005), were not affected by the type of premedication regime used in this study and were within normal limits. The findings of the effects of midazolam on arterial blood-gas and acid-base variables are similar to those of another study which showed that midazolam had minimal effects while xylazine caused hypoxaemia and respiratory acidosis in goats (Stegmann 1999). In retrospect, it might have been wise to collect arterial blood gas samples within the first 5 minutes of induction of general anaesthesia, and not after 20 minutes as done in this study, as the respiratory system depressant effects of propofol might have been more pronounced at this early time.

It is concluded that sedation of goats with midazolam alone, and combinations of butorphanol with acepromazine or midazolam reduces the dose of propofol required for induction of general anaesthesia in goats with minimal effects on cardiopulmonary function.

Chapter 3

Effects of midazolam on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats

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3.1 ABSTRACT

Objective To evaluate the effects of midazolam, a commonly used adjunct to general anaesthesia, on the MAC of isoflurane and cardiovascular function in artificially-ventilated goats.

Study Design Prospective, randomized, crossover experimental study.

Animals Six healthy goats, 3 does and 3 wethers.

Methods General anaesthesia was induced with isoflurane. Endotracheal intubation was performed after which anaesthesia was maintained with isoflurane while the goats were artificially ventilated. The baseline isoflurane minimum alveolar concentration (MAC) in response to clamping a claw with a Vulsellum forceps was determined. The goats then received midazolam treatments: bolus dose of 0.1 mg kg^{-1} followed by a maintenance dose of 0.1 mg kg^{-1} $hour^{-1}$ (Treatment LMID), bolus dose of 0.3 mg kg^{-1} followed by a maintenance dose of 0.3 mg kg^{-1} $hour^{-1}$ (Treatment MMID), bolus dose of 0.9 mg kg^{-1} followed by a maintenance dose of 0.9 mg kg^{-1} $hour^{-1}$ (Treatment HMID) intravenously. Isoflurane MAC was then re-determined for each treatment. Response to midazolam treatment was determined as the difference between the baseline and post-treatment isoflurane MAC. Vital cardiopulmonary parameters were monitored throughout the anaesthetic period. Quality of recovery was scored. The Friedman test was used to test for differences between isoflurane MACs. Medians of repeatedly-measured cardiovascular parameters were tested for differences between and within treatments using repeated measures analysis of variance (ANOVA) by ranks ($P < 0.05$ for statistical significance).

Results The baseline [median (interquartile range)] isoflurane MAC in goats was 1.40 (1.38-1.41)%. Baseline isoflurane MAC was statistically significantly reduced following Treatment LMID, Treatment MMID and Treatment HMID by 16.8%, 35.1% and 54.7% respectively. Cardiovascular function was minimally affected for all three midazolam treatments. Quality of recovery from anaesthesia was good for all three groups.

Conclusions and clinical relevance Midazolam, administered by constant rate infusion, significantly reduces isoflurane requirements for maintenance of general anaesthesia without significantly affecting cardiovascular function in goats.

Keywords goat, midazolam, isoflurane, minimum alveolar concentration, sedative

3.2 INTRODUCTION

Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazol(1,5-α)(1,4)-benzodiazepine), a commonly used adjunct to general anaesthesia, has been shown to decrease anaesthetic requirements of volatile anaesthetic agents after intravenous administration in both humans and different animal species (Taira et al. 2000; Lemke 2007; Hendrickx et al. 2008). Determination of the degree of reduction of the MAC of isoflurane following administration of different dosages of midazolam may be useful in assessment of the relationship between the dosage of midazolam administered and the expected degree of reduction in isoflurane MAC.

Midazolam is a water-soluble imidazobenzodiazepine-receptor agonist (Cao et al. 2002; Lemke 2007), which has a rapid onset of action after intravenous or intramuscular administration due to its high lipophilicity at body pH (Clutton 1998; Plumb 2005). Benzodiazepines are becoming increasingly popular in veterinary anaesthesia because of their mild cardiovascular and respiratory effects (Mehlisch 2002). Midazolam occupies the benzodiazepine receptor on the benzodiazepine-GABA-Cl channel complex and therefore facilitates the inhibitory action of GABA (γ-aminobutyric acid), the major inhibitory neurotransmitter of the central nervous system (Tatsuo et al. 1999; Cao et al. 2002; Ong et al. 2004; Young et al. 2005). The sedative and hypnotic effects of midazolam are dose-dependent as well as dependent on route of administration. Midazolam produces maximal sedative effects in 20 minutes after intramuscular administration of 0.6 mg kg^{-1}; hypnosis is achieved after 5 minutes when administered at 0.6 mg kg^{-1} intravenously and an even more profound central nervous system depression is achieved when administered at 1.2 mg kg^{-1} intravenously (Stegmann & Bester 2001). Midazolam lacks systemic analgesic effects when administered intravenously or intramuscularly and is therefore usually combined with opioids when used for premedication of surgical patients (Tatsuo et al. 1999; Mehlisch 2002; Nishiyama 2006).

Isoflurane is a commonly used inhalant general anaesthetic, which has short induction and recovery times because of its low blood:gas solubility coefficient (McEwen et al. 2000; Hall et al. 2001). The mechanism by which isoflurane produces unconciousness and immobility is still not yet full understood, although the proposed sites of action are the brain and spinal cord (Antognini & Carstens 1999; Jinks et al. 1999; Antognini et al. 2000a; Antognini et al. 2000b; Antognini & Carstens 2002; Carstens & Antognini 2005; Hendrickx et al. 2008). The most likely mechanism by which isoflurane produces anaesthetic effects is by potentiating the GABA (γ-aminobutyric acid) receptor-channel complex (Larsen et al. 1998). Isoflurane can be used for induction as well as maintenance of

anaesthesia in goats (Antognini & Eisele 1993). Isoflurane is mostly exaled unchanged, with only 0.2% of the inhaled proportion requiring hepatic metabolism (Njoku et al. 1997). Isoflurane, like most other inhalant anaesthetic agents, causes respiratory depression, hypotension and reduced cardiac output in a dose-dependent pattern (Antognini & Eisele 1993; Hall et al. 2001; Hikasa et al. 2002).

In the present experimental study we assessed the effects of midazolam on isoflurane MAC. We defined isoflurane MAC according to Merkel and Eger 1963, as the lowest isoflurane alveolar (end-tidal) concentration required by an individual goat to prevent gross purposeful movement in response to a supramaximal stimulus, which in this study was claw-clamping using a Vulsellum forceps. Isoflurane requirement for general anaesthesia in goats, as defined by MAC, was determined in previous studies to be 1.3 to 1.5% (Antognini & Eisele 1993; Hikasa et al. 1998; Antognini et al. 2000; Hikasa et al. 2002; McEwen et al. 2000; Doherty et al. 2004). By combining isoflurane with anaesthetic-sparing drugs like midazolam, potentially less isoflurane will be required to maintain general anesthesia, and therefore the adverse cardiopulmonary effects associated with high doses of isoflurane may be minimized. We tested the null hypothesis that midazolam does not affect isoflurane MAC against alternative hypothesis that midazolam reduces isoflurane MAC in goats.

3.3 MATERIALS AND METHODS

3.3.1 EXPERIMENTAL DESIGN AND INSTRUMENTATION

Six clinically healthy goats (3 does and 3 wethers) were used in this study. The goats were assigned to three groups with order of treatments randomized in a cross-over pattern with a four-week washout period between treatments. General anaesthesia was achieved initially with isoflurane only and then later with isoflurane combined with a constant rate infusion of low dose midazolam (Treatment LMID), moderate dose midazolam (Treatment MMID) or high dose midazolam (Treatment HMID). Health status was assessed by physical examination, a complete blood count and serum biochemical analysis; all findings were normal. The signalment of the goats is summarized in Table 3.1.

Food and water were withheld for 16–22 hours before anaesthesia. The goats were weighed 30 minutes before the experiment. Baseline rectal temperature measured by a digital thermometer, heart rate measured by thoracic auscultation and respiratory rate were recorded before the goats were placed on a custom-made sling-cum-table for easier restraint. The auricular artery on the right ear was catheterized using a 24-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) which was

then connected to a calibrated transducer (DTX Plus transducer, BD Medical, Johannesburg, South Africa) for measurement of systolic, diastolic and mean arterial blood pressures. The blood pressure readings were obtained from a calibrated electronic strain gauge transducer connected to a multi-parameter monitor (Cardiocap/5, Datex-Ohmeda Corporation, Helsinki, Finland), which had been calibrated against a mercury column within 2 month of commencement of the study. For transducer calibration to atmospheric pressure, the scapulo-humeral joint or the point of the sternum were used as zero reference points in sternally-recumbent or laterally-recumbent goats, respectively. An 18-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) was introduced into the right cephalic vein for administration of intravenous fluids and midazolam. Another 18-SWG catheter was placed in the right jugular vein for collection of venous blood samples for determination of midazolam plasma concentration.

Mask induction of the goats with isoflurane (Forane® Liquid, Abbott Laboratories Pty Ltd, Constantia Kloof, South Africa) delivered in oxygen from a circle anaesthetic breathing system with a calibrated Tec 3 out-of-circle vaporiser (Fluotec 3®, BOC Health Care, West Yorkshire, England) was achieved with the goats restrained in sternal position. A tight-fitting facemask was used to limit dead space and gas leaks around the mask (Figure 3.1). Each goat was accustomed to the mask by initially being allowed to breathe 100% volume oxygen at 6 L minute^{-1} for at least one minute before isoflurane administration rate was slowly begun with 0.5% volume increments every 15 seconds until a 3.5% volume vaporizer setting was reached. This vaporizer setting was then maintained until the jaw was relaxed enough to allow intubation. Placement of the endotracheal tube (silicone tube, internal diameter 7.5 mm) was done with the goats in sternal recumbency using a laryngoscope to facilitate the process. If intubation was not successful due to poor relaxation of the jaws, isoflurane delivery by facemask was continued before attempting again. The cuff of the endotracheal tube was inflated to prevent leakage of gases from the breathing circuit to a pressure of 20 cmH_20.

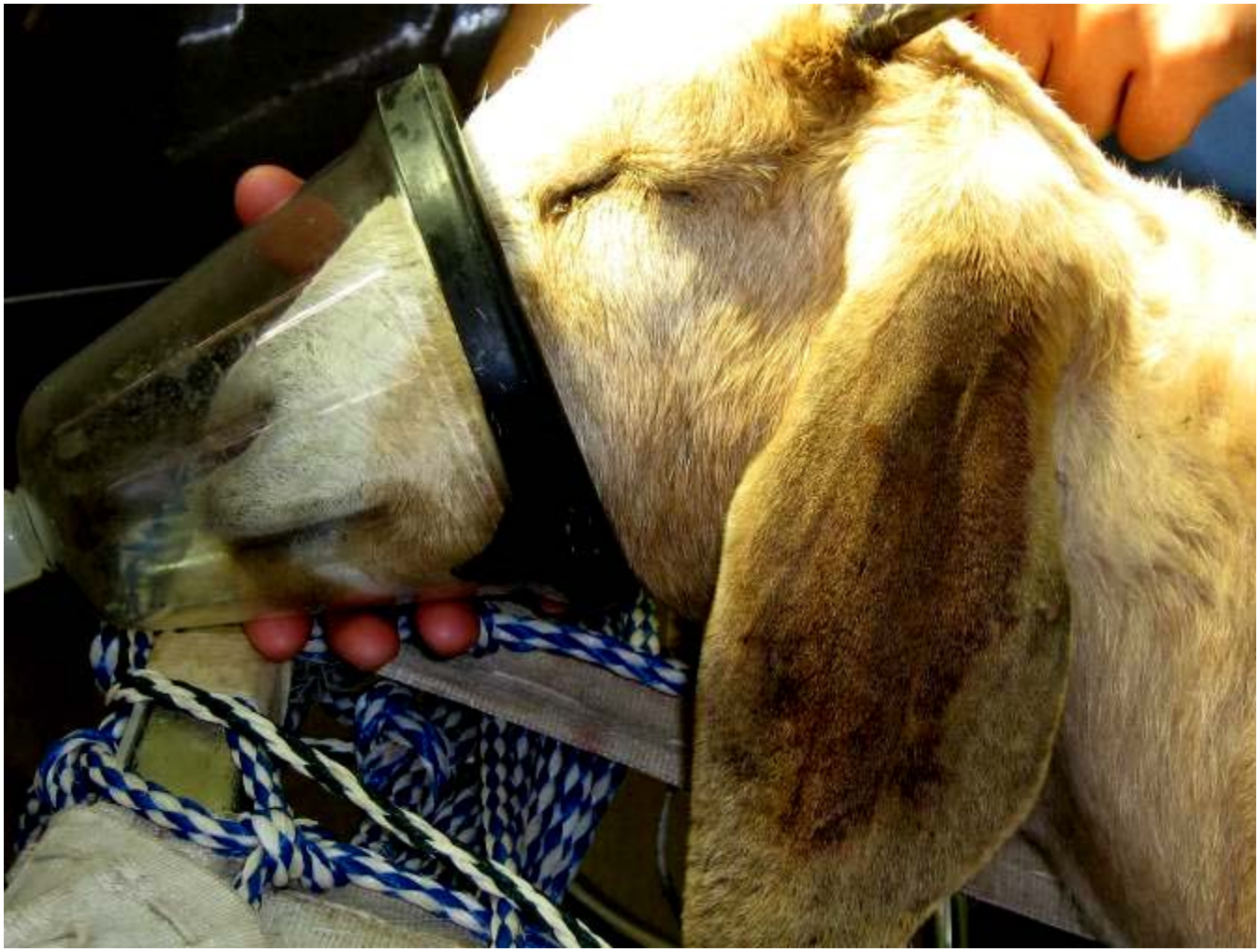


Figure 3.1 Mask induction of general anaesthesia with isoflurane in oxygen using a transparent, tight-fitting face mask.

Immediately after intubation, the goats were placed in left lateral recumbency with fresh oxygen flow set at 2 L minute^{-1} and initial end-tidal (expired) isoflurane concentration targeted to be 1.6% volume. Intermittent positive pressure ventilation (Ohmeda 7000 Ventilator, Ohmeda, Madison, Wisconsin, USA) was used to maintain end-tidal carbon dioxide within the range of 35-45 mmHg throughout the procedure. Ringer Lactate solution (Intramed Ringer-Lactate® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) was administered by a pump (Infusomat, BBraun, Melsungen, Germany) at a rate of 4 mL kg^{-1} hour^{-1} intravenously.

Instrumentation for recording of physiological parameters was set up using a multi-parameter monitor (Cardiocarp/5, Datex-Ohmeda Corporation, Helsinki, Finland). Three electrocardiography (ECG) electrodes were placed on shaven areas (on the middle of the left shoulder, on the midline 2 cm in front of the point of the sternum and on the midline 2 cm cranial to the tip of the xiphoid) to provide a lead II ECG tracing. Haemoglobin oxygen saturation (SpO_2) was measured via a pulseoximetry infrared probe placed around the tongue, which calculated heart rate as well. Inspired and expired concentrations of isoflurane, carbon dioxide and oxygen were obtained from a flow sensor and a side-stream gas sampler placed between the endotracheal tube and the Y-piece of the breathing system. The flow rate through the gas sampling line was constant at 200 mL $minute^{-1}$. Respiratory rate was calculated from the capnogram. The gas analyzer had been calibrated with calibration gas as recommended by the manufacturer within 2 months of commencement of the studies and would automatically self-calibrate to atmospheric air at the beginning of the experiment. Temperature was measured by an oesophageal probe place as close to the base of the heart as possible. This was done by marking from outside how far the temperature probe had to be placed to reach the point of the elbow. We targeted to maintain oesophageal temperature between 37.5 and 39.5ºC using a forced warmed air blanket and ordinary blankets placed around the goats. The physiological parameters were measured continuously during the anaesthetic period, but recorded every 15 minutes.

Determination of the baseline isoflurane (control) MAC began 15 minutes after end-tidal isoflurane concentration had been held constant at 1.6%. Isoflurane MAC determination involved application of a noxious stimulus with a Vulsellum forceps clamped to the second ratchet to the claw about 1 cm below the coronary band for 60 seconds or until occurrence of purposeful movement. Purposeful movement was strictly defined as gross movement of the head or limbs, including movement of the limb to which the Vulsellum forceps was being applied. End-tidal isoflurane concentration was then adjusted according to response to noxious stimulation. If no movement occurred, the end-tidal isoflurane concentration was reduced by a tenth (approximately 10% of its value) and held constant for at least 15 minutes before application of a noxious stimulus again. If movement was noticed, the end-tidal isoflurane concentration was increased by a tenth and held constant for at least 15 minutes before application of a noxious stimulus again. The four claws on the two uppermost limbs were clamped consecutively in a clockwise fashion. Isoflurane MAC was calculated as the average of two successive concentrations; the end-tidal isoflurane concentration at which movement in response to noxious stimulation occurred and the preceeding end-tidal isoflurane concentration at which movement did not occur. The isoflurane MAC was determined in duplicate and the mean of the two MACs was taken as baseline isoflurane MAC.

Following baseline MAC determination, the goats then received a bolus dose of midazolam administered manually over a 1 minute period; at 0.1 mg kg^{-1}, 0.2 mg kg^{-1}, or 0.9 mg kg^{-1} intravenously; followed by a maintenance dose of; 0.1 mg kg^{-1} $hour^{-1}$, 0.2 mg kg^{-1} $hour^{-1}$, or 0.9 mg kg^{-1} $hour^{-1}$ for Treatment LMID, Treatment MMID and Treatment HMID, respectively. Midazolam for maintenance of general anaesthesia was prepared up to 60 mL in normal saline and was administered by CRI from a 60 mL syringe controlled by a syringe-driving pump (Perfusor Compact, BBraun, Melsungen, Germany). The midazolam syringe was connected to the right cephalic vein catheter, to which the Ringer Lactate administration line was also connected, by an extension tube via a three-way stopcock. The midazolam loading dose was administered over a period of 1 minute and administration of the maintenance dose started directly afterwards. The accuracy of delivery of midazolam by the pump was checked at the end of the experiment by calculating the expected infused amount based on infusion rates and comparing this to actual volume infused from the syringe.

Midazolam-treatment isoflurane MAC was then determined by applying a noxious stimulus with a Vulsellum forceps after every 15 minutes of end-tidal isoflurane concentration equilibration, and depending on the goat's response, adjusting the end-tidal isoflurane concentration in the same manner as described above. Response to midazolam treatment for each goat was defined as the difference between baseline and midazolam-treatment isoflurane MAC.

Since baseline isoflurane MAC was determined each time before a goat underwent one of the three midazolam treatments, the final baseline isoflurane MAC for each goat was calculated as the average of the three baseline MAC values obtained. There was no need to adjust the isoflurane end-tidal concentrations obtained to atmospheric pressure as the gas module used for measuring respiratory gas concentrations had a sensor that constantly measured atmospheric pressure and adjusted respiratory gas readings as if they were measured at one atmospheric pressure.

Venous blood samples (4.5 mL) were collected via the right jugular vein catheter in heparinised tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for determination of midazolam plasma concentration at 0, 1, 5, 15, 30 and every 30 minutes from the time of propofol bolus administration until the propofol-treatment isoflurane MAC had been determined. The blood samples were centrifuged at 2 500 revolutions per minute for 15 minutes after which plasma was collected and stored at –20ºC for midazolam concentration analysis later.

After determination of midazolam-treatment isoflurane MAC, administration of midazolam and isoflurane was discontinued and the quality of recovery from anaesthesia of the goats observed. The endotracheal tube was removed once the goats regained the swallowing reflex.

Time to extubation, sternal position and standing were recorded. All times were determined as the interval between the time of discontinuation of midazolam and isoflurane administration and the time a particular event happened.

Quality of recovery from anaesthesia was scored on a 0 – 2 scale where: 0 = restlessness, 1 = relatively smooth, with some restlessness, 2 = smooth.

3.3.2 STATISTICAL ANALYSIS

Data were analysed using the R® Statistical Software, Version 2.7.2 (The R Foundation for Statistical Computing, Vienna, Austria). All data were assumed to be non-parametric because of the small sample size and are expressed as median and inter-quartile ranges.

Data on isoflurane MAC, isoflurane MAC reduction after midazolam treatment, isoflurane MAC determination time, time to extubation, time to sternal position, time to standing, and recovery scores were tested for statistically significant differences between treatments using the Friedman test. If statistically significant differences were found between treatments, post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted.

Medians of repeatedly measured variables (heart rate, mean arterial blood pressure, SpO_2 and body temperature) were tested for statistically significant differences between and within treatments using repeated measures analysis of variance (ANOVA) by ranks. If statistically significant differences were found, a post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted. A value of $P < 0.05$ was considered significant.

3.4 RESULTS

There were no statistically significant differences in terms of median age, weight, pre-anaesthetic total serum protein, haematocrit, white cell count and body temperature between the treatments (Table 3.1).

Mask induction of anaesthesia using isoflurane was satisfactorily achieved in about 10 minutes with minimal struggling of the goats throughout the induction period. Data on observed isoflurane MAC values, changes in isoflurane MAC after treatment with midazolam and the time it took to determine isoflurane MAC are summarized in Table 3.2.

The baseline median minimum alveolar concentration (MAC) for isoflurane in goats was 1.40 (1.38-1.41)% volume. This baseline median MAC was statistically significantly higher than the median MAC values obtained after LMID treatment (P = 0.003), MMID treatment (P = 0.013), and HMID treatment (P = 0.013). The differences in median isoflurane MAC value between the four groups (control and three midazolam treatments) were so obvious that each of the four groups had a median isoflurane MAC that was statistically significantly different from each of the other three groups (Figure 3.2).

The percentage reductions in isoflurane MAC after LMID treatment, MMID treatment and HMID treatment were 16.8 (12.7-19.1)%, 35.1 (29.9-40.4)% and 54.7 (48.6-56.3)% respectively. The MAC reduction percentage values were all statistically significantly different from each other (Figure 3.3).

The time it took to determine baseline MAC was 70.0 (70.0-73.8) minutes. Following midazolam treatment, time taken to determine MAC ranged from 67.5 minutes to 90.0 minutes for the treatment groups, and was dependent on the dose of midazolam used (Table 3.2). The time taken to determine MAC following administration of the highest midazolam dose was statistically significantly higher than the time it took to determine to determine MAC following administration of the lowest midazolam dose (P = 0.019).

Midazolam plasma concentrations could not be determined due to failure to obtain the analytical standards (100% midazolam powder) that is essential for the analysis.

The medians of cardiovascular system and respiratory system variables did not show any statistically significant differences between treatments or between the baseline reading and any subsequent points within a treatment. At all times, mean arterial blood pressure was above 70 mmHg and SpO2 above 90%. The end-tidal carbon dioxide concentration was successfully maintained by mechanical ventilation within the normal range of 35-45 mmHg. The body temperature of the goats was maintained between 37.1–39.3ºC (Table 3.3). Body temperature decreased gradually with time despite all the measures that were taken to conserve and supplement body heat in the goats. The body temperature readings observed in 2 groups onwards of 15 minutes from commencement of midazolam treatment were statistically significantly lower than baseline observations, but were still within physiologically acceptable limits.

The quality of recovery from anaesthesia was good for all the three treatments. The variables used to assess quality of recovery from anaesthesia did not show any statistically significant differences between groups (Table 3.4).

3.4.1 TABLES

Table 3.1 Profile of the goats [median (inter-quartile range)] used in a study in which the effects of intravenously administered midazolam: 0.1 mg kg^{-1} bolus followed by continuous infusion at 0.1 mg $kg^{-1}hr^{-1}$ (LMID Treatment), 0.3 mg $kg^{-1}g$ bolus followed by continuous infusion at 0.3 mg $kg^{-1}hr^{-1}$ (MMID Treatment), or 0.9 mg kg^{-1} bolus followed by continuous infusion at 0.9 mg $kg^{-1}hr^{-1}$ (HMID Treatment) on the minimum alveolar concentration of isoflurane were investigated.

	Parameter					
Treatment	Age (months)	Weight (kg)	Total Serum Protein (g/l)	Haematocrit (litres $litre^{-1}$)	White Cell Count ($x10^9$ $litre^{-1}$)	Rectal Temp (ºC)
LMID	12.0 (11.3-12.8)	29.7 (24.1-33.6)	65.2 (63.3-67.7)	0.37 (0.32-0.40)	12.72 (12.3-14.30)	39.2 (38.9-39.3)
MMID	12.00 (12.0-12.8)	27.3 (24.9-30.3)	68.6 (61.9-69.9)	0.36 (0.33-0.37)	13.2 (11.7-14.9)	39.0 (38.5-39.3)
HMID	12.2 (11.3-12.8)	28.7 (23.5-31.6)	67.4 (63.9-70.9)	0.36 (0.34-0.39)	12.4 (11.7-13.1)	39.0 (38.9-39.1)

NS: No statistically significant differences (P<0.05) between treatments

Table 3.2 Effect [median (inter-quartile range)] of intravenously administered midazolam: 0.1 mg kg^{-1} bolus followed by continuous infusion at 0.1mg/kg/hr (LMID Treatment), 0.3 mg kg^{-1} bolus followed by continuous infusion at 0.3 mg $kg^{-1}hr^{-1}$ (MMID Treatment), or 0.9 mg kg^{-1} bolus followed by continuous infusion at 0.9 mg $kg^{-1}hr^{-1}$ (HMID Treatment) on the minimum alveolar concentration (MAC) of isoflurane in goats.

Treatment	Isoflurane MAC (%vol)	Change post-treatment (%)	MAC determination time (minutes)
Control	1.40 (1.38-1.41) *	Not applicable	70.0 (70.0-73.8)
LMID	1.18 (1.15-1.20)*	-16.8 (12.7-19.1)*	67.5 (60.0-75.0)
MMID	0.91 (0.85-0.95)*	-35.07 (29.9-40.4)*	90.0 (75.0-105.0)
HMID	0.65 (0.63-0.68)*	-54.7 (48.6-56.3)*	90.0 (90.0-90.0)#

*: statistically significantly different (P <0.05) from all other treatments
#: statistically significantly different (P <0.05) from LMID treatment

Table 3.3 Physiological parameters [median (inter-quartile range)] observed following intravenous administration of midazolam: 0.1 mg kg^{-1} bolus followed by continuous infusion at 0.1 mg $kg^{-1}hr^{-1}$ (LMID Treatment), 0.3 mg kg^{-1} bolus followed by continuous infusion at 0.3 mg $kg^{-1}hr^{-1}$ (MMID Treatment), or 0. 9 mg kg^{-1} bolus followed by continuous infusion at 0.9 mg $kg^{-1}hr^{-1}$ (HMID Treatment) in isoflurane-anaesthetised goats.

Variable	Unit	Treatment	Time	Period of Baseline Isoflurane MAC Determination (minutes)				Period of Midazolam-treatment Isoflurane MAC Determination (minutes)				
			Baseline	2	15	30	45	2	15	30	45	60
Heart Rate	beats min^{-1}	LPROP	84 (77-106)	101 (89-110)	93 (86-107)	91 (86-105)	94 (85-103)	100 (90-110)	99 (86-111)	103 (87-111)	106 (88-114)	101 (87-112)
		MPROP	84 (73-101)	91 (80-102)	91 (83-98)	86 (77-97)	92 (79-95)	72 (67-85)	71 (68-80)	74 (71-83)	81 (74-90)	89 (77-99)
		HPROP	74 (68-80)	86 (82-96)	89 (80-98)	90 (77-102)	91 (75-108)	70 (67-93)	70 (67-80)	75 (72-89)	77 (69-89)	93 (86-98)
SAP	mmHg	LPROP	134 (131-144)	121 (109-126)	115 (99-128)	120 (103-131)	124 (112-127)	116 (108-122)	111 (106-128)	111 (100-120)	110 (108-114)	109 (106-118)
		MPROP	117 (106-123)	107 (101-120)	105 (99-112)	96 (93-104)	100 (98-100)	92 (89-96)	94 (87-98)	94 (85-103)	94 (89-103)	105 (99-109)
		HPROP	116 (113-123)	102 (100-111)	100 (96-108)	105 (102-106)	103 (100-111)	100 (92-104)	96 (81-101)	107 (101-113)	120 (106-128)	120 (114-124)
DAP	mmHg	LPROP	94 (90-96)	89 (86-95)	81 (63-99)	86 (74-104)	97 (80-101)	92 (90-94)	90 (84-98)	88 (70-93)	82 (80-84)	87 (84-89)
		MPROP	79 (73-86)	74 (71-86)	72 (64-78)	70 (64-78)	71 (69-75)	57 (51-71)	63 (53-68)	68 (57-76)	72 (64-84)	84 (78-91)
		HPROP	81 (66-92)	78 (69-82)	75 (71-77)	81 (79-82)	82 (80-87)	73 (66-78)	67 (55-72)	79 (69-93)	96 (82-99)	92 (88-98)
MAP	mmHg	LPROP	115 (113-116)	106 (96-110)	96 (80-111)	100 (88-116)	111 (95-112)	104 (93-109)	99 (94-113)	98 (84-106)	97 (95-98)	98 (93-103)
		MPROP	94 (88-105)	84 (72-99)	83 (78-90)	80 (78-88)	83 (81-87)	70 (63-79)	74 (66-77)	78 (68-87)	82 (78-92)	93 (87-100)
		HPROP	100 (88-109)	89 (83-95)	86 (81-89)	92 (89-94)	94 (91-98)	83 (78-91)	79 (66-8665)	91 (83-102)	106 (87-112)	105 (100-110)
SpO_2	%	LPROP	-	98 (98-99)	98 (97-99)	98 (96-98)	97 (97-98)	98 (97-98)	98 (97-98)	98 (97-99)	97 (96-98)	98 (96-98)
		MPROP	-	99 (98-99)	99 (98-99)	98 (96-99)	99 (98-99)	98 (97-98)	99 (98-99)	98 (97-99)	98 (96-98)	97 (96-98)
		HPROP	-	98 (97-98)	98 (98-98)	98 (97-98)	98 (97-98)	97 (96-98)	99 (96-99)	98 (96-99)	97 (95-98)	95 (94-97)
$PE´CO_2$	mmHg	LPROP	-	38.1 (33.8-34.5)	35.2 (33.9-37.1)	35.3 (34.1-36.4)	34.9 (33.9-39.8)	35.3 (34.7-37.5)	36.0 (33.9-40.9)	36.4 (34.7-39.2)	37.5 (36.2-41.1)	36.8 (36.2-39.0)
		MPROP	-	38.0 (34.6-43.7)	37.6 (32.7-41.4)	36.9 (35.0-39.3)	35.7 (35.2-38.6)	36.1 (35.0-37.8)	35.0 (35.0-35.0)	35.0 (35.0-40.1)	34.6 (34.2-35.5)	35.3 (35.0-36.3)
		HPROP	-	35.7 (35.2-40.9)	35.0 (34.4-37.2)	36.5 (34.6-37.8)	35.3 (35.0-35.7)	36.5 (35.8-41.2)	35.7 (35.2-36.9)	35.0 (34.4-35.5)	34.2 (33.6-40.5)	39.9 (38.0-43.5)
Temp	(ºC)	LPROP	39.2 (38.9-39.3)	38.6 (38.4-38.9)	38.3 (38.2-38.4)	38.1 (37.9-38.4)	38.1 (37.8-38.2)	38.0 (37.9-38.1)	38.0 (37.9-38.0) *	37.8 (37.6-37.9) *	37.8 (37.5-37.8) *	37.7 (37.4-37.9) *
		MPROP	39.0 (38.8-39.3)	38.3 (38.2-38.5)	38.3 (38.2-38.5)	38.2 (38.0-38.3)	38.2 (38.1-38.6)	38.2 (37.6-38.5)	38.1 (37.8-38.4)	38.1 (37.7-38.5)	38.1 (37.6-38.4)	37.4 (37.5-38.3)
		HPROP	39.0 (38.9-39.1)	38.3 (38.2-38.6)	38.4 (38.2-38.5)	38.0 (37.7-38.2)	37.9 (37.7-37.9)	37.7 (37.6-37.9)	37.6 (37.4-37.8) *	37.3 (37.1-37.7) *	37.2 (37.1-37.7) *	38.3 (37.2-37.9) *

*: statistically significantly different (P<0.05) from baseline reading within group.
SAP- systolic arterial pressure; DAP- diastolic arterial pressure; MAP- mean arterial pressure; SpO_2- saturation of haemoglobin with oxygen in peripheral blood; $PE´CO_2$- end-tidal carbon dioxide partial pressure; Temp- body temperature.

Table 3.4 Quality of recovery from anaesthesia [median (inter-quartile range)] observed in a study where the effects of intravenously administered midazolam: 0.1 mg kg^{-1} bolus followed by continuous infusion at 0.1 mg $kg^{-1}hr^{-1}$ (LMID Treatment), 0.3 mg kg^{-1} bolus followed by continuous infusion at 0.3 mg $kg^{-1}hr^{-1}$ (MMID Treatment), or 0.9 mg kg^{-1} bolus followed by continuous infusion at 0.9 mg $kg^{-1}hr^{-1}$ (HMID Treatment) on the minimum alveolar concentration of isoflurane in goats were investigated.

Treatment	Time to Extubation (minutes)	Time to Sternal Position (minutes)	Time to Standing (minutes)	Recovery Score
LMID	3.0 (2.3-3.0)	4.0 (1.5-5.0)	12.5 (10.0-15.0)	2 (2-2)
MMID	3.0 (2.3-4.5)	3.0 (1.5-4.5)	13.5 (10.5-18.8)	2 (2-2)
HMID	5.0 (2.8-5.0)	5.0 (1.5-7.0)	26.0 (20.5-33.8)	2 (2-2)

Note: No statistically significant differences ($P<0.05$) between any groups

3.4.2 FIGURES

Figure 3.2 Box-and-Whiskers plot of median isoflurane MAC (% volume) observed in isoflurane anaesthetised goats (Control) and after intravenous administration of midazolam (LMID Treatment, MMID Treatment, or HMID Treatment) to isoflurane-anaesthetised goats. Each box represents data from the 25th to the 75th percentiles, the bold line represents the median value, and the whiskers represent the range of scores, while the small dots outside the box represent outliers. Also highlighted are the *P*-values obtained when the isoflurane MAC were compared statistically between treatments.

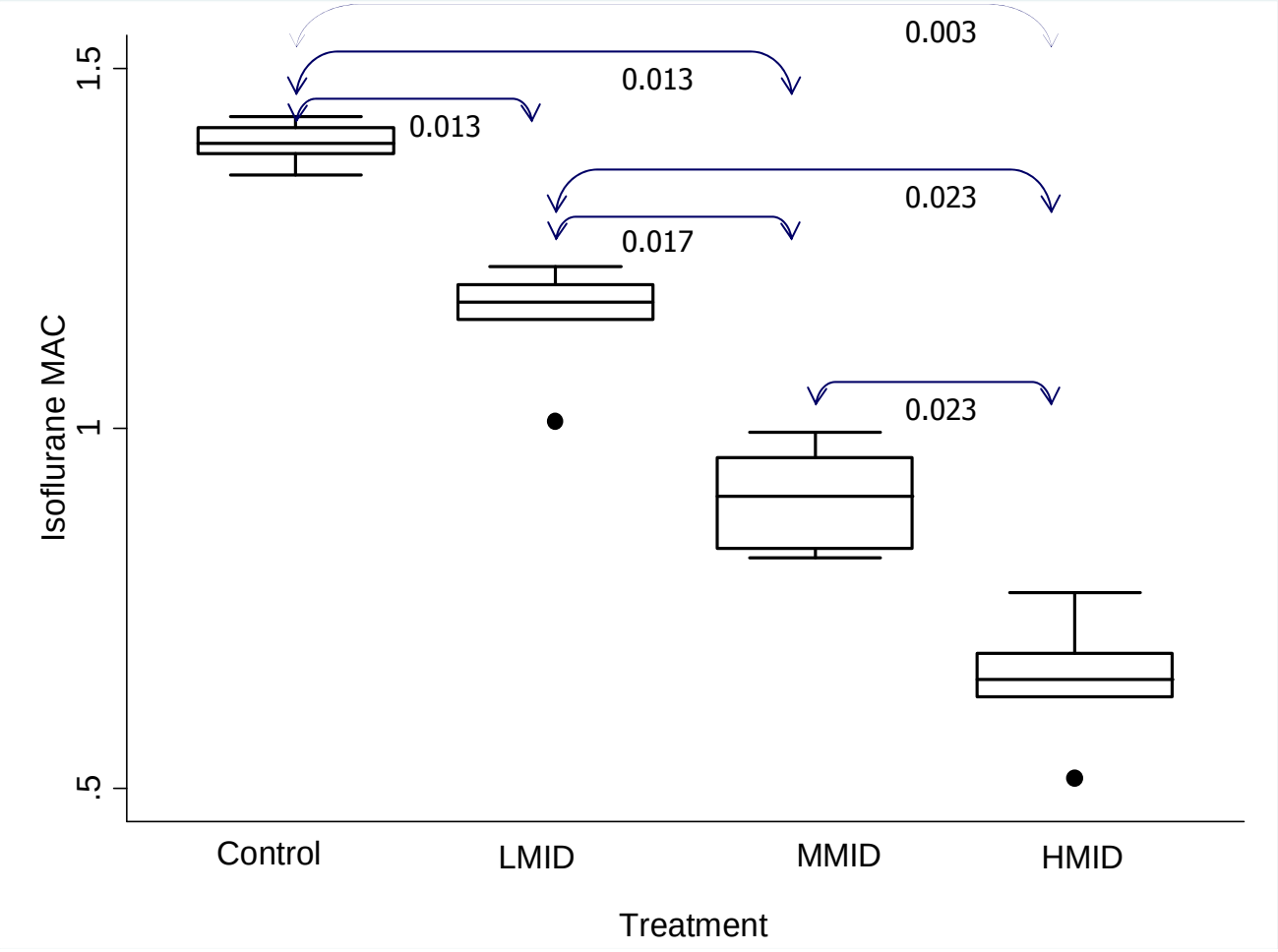
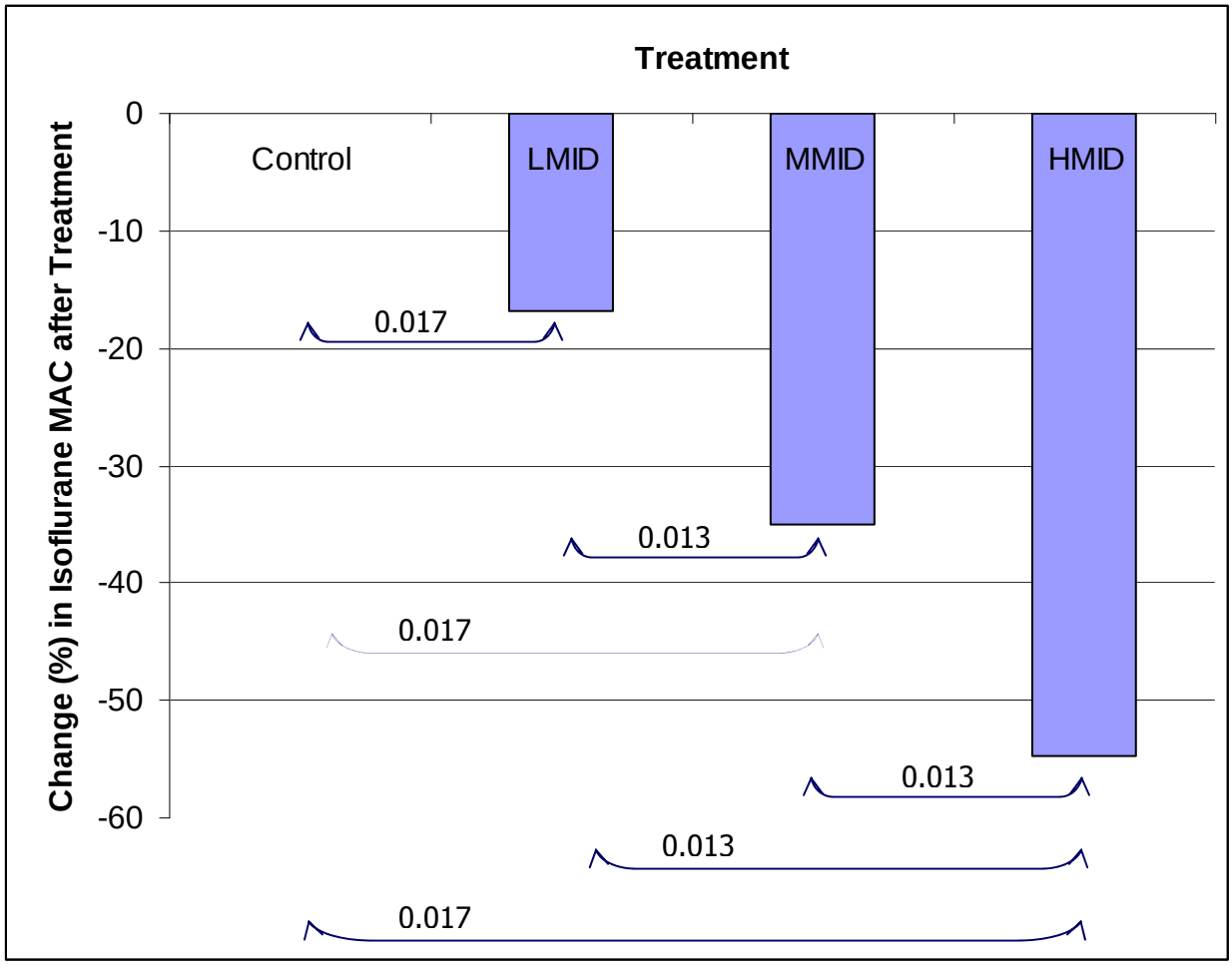


Figure 3.3 Percentage change in isoflurane MAC observed in isoflurane anaesthetised goats (Control) and after intravenous administration of midazolam (LMID Treatment, MMID Treatment, or HMID Treatment) to isoflurane-anaesthetised goats. Also highlighted are the *P*-values obtained when the change in isoflurane MAC following midazolam treatement were compared statistically between treatments.



3.5 DISCUSSION

The observed median isoflurane MAC of 1.40% volume in the present study is comparable to observations from other studies in which clamping the claws or the tail was used as the noxious stimuli (Hikasa et al. 1998; Antognini et al. 2000c; McEwen et al. 2000; Hikasa et al. 2002; Doherty et al. 2002a). One study reported the MAC of isoflurane in goats as 1.50% volume (Antognini and Eisele 1993). Two other studies reported isoflurane MAC as 1.40% volume (Hikasa et al. 2002; Doherty et al. 2004), while others have reported isoflurane MACs of 1.23-1.29% volume (Hikasa et al. 1998; Doherty et al. 2002a; Doherty et al. 2002b).

The median percentage reductions in isoflurane MAC of 16.8%, 35.1% and 54.7% following administration of Treatment LMID, Treatment MMID and Treatment HMID respectively, show a substantial and dose-dependent effect. There is no previous study focusing specifically on the effects of midazolam on isoflurane MAC in goats or other animal species; but, in humans, administration of low dose midazolam (bolus at 0.1 mg kg^{-1} followed by 0.06 mg kg^{-1} $hour^{-1}$ maintenance dose), moderate dose midazolam (bolus at 0.2 mg kg^{-1} followed by 0.12 mg kg^{-1} $hour^{-1}$ maintenance dose), high dose midazolam (bolus at 0.4 mg kg^{-1} followed by 0.24 mg kg^{-1} $hour^{-1}$ maintenance dose) reduced halothane MAC by 40%, 50% and 70% respectively (Inagaki et al. 1993). This comparison shows that midazolam produces marked reductions in inhalation anaesthetic requirements for general anaesthesia in both humans and goats, with a more pronounced reduction in humans. A 2001 study by Stegmann & Bester in which midazolam was administered intravenously to conscious goats at different dosages concluded that the sedative and hypnotic effects of midazolam were dose-dependent. The magnitude of reduction of isoflurane MAC following midazolam administration shows that midazolam has a significant role to play as an adjunct to balanced anaesthesia in goats. The reduction in isoflurane requirements for maintaining general anaesthesia is important as use of less isoflurane will obtund isoflurane-related adverse effects (Hall et al. 2001; Dzikiti et al. 2003). The common adverse effects associated with isoflurane include respiratory depression, hypotension and reduced cardiac output (Antognini & Eisele 1993; Hall et al. 2001; Hikasa et al. 2002). Using less isoflurane also has the advantage of reducing the hazard of atmospheric pollution, and thus reducing environmental exposure to isoflurane and its metabolic products (Joubert 1999).

The results on impact of midazolam administration on cardiovascular parameters show that midazolam is able to substantially spare isoflurane required to maintain general anesthesia in goats without causing substantial negative effects on cardiovascular function. The mean arterial blood

pressure and SpO_2 obtained even after administration of the highest dose of midazolam in this study were within normal physiological limits and were similar to baseline readings within a group. It is well documented in literature that midazolam, especially when used alone does not cause significant changes on cardiopulmonary function (Mehlisch 2002; Plumb 2005; Lemke 2007; Dzikiti et al. 2009). It is important to maintain body temperature, blood pressure and tissue oxygenation within normal physiological limits as was done in the present study because hypothermia, severe hypotension and hypoxaemia are all known to reduce inhalations anaesthetic requirements (Stanski 2000; Eger II 2002). Although the body temperature of the goats in this study steadily decreased to a lowest reading of 37.2ºC with time, this value still falls within normal ranges reported in healthy, non-anaesthetised goats in which values as low as 37.2ºC have been reported (Ayo et al. 1998)

The quality of recovery from anaesthesia was good in all groups. Administration of midazolam as an adjunct to isoflurane for maintenance of general anaesthesia did not substantially prolong time to removal of the endotracheal tube, time to attainment of sternal position or time to standing. The short recovery periods observed in this study suggest that the half-life of midazolam following intravenous administration in goats is short, although data on pharmacokinetics of midazolam in goats in literature is lacking. The short recovery periods also show that the interaction between midazolam and isoflurane does not significantly prolong recovery time.

In conclusion, intravenously administered midazolam decreased isoflurane MAC in a dose-dependent manner. Cardiovascular function was not substantially affected by administration of midazolam. This study shows that intravenous administration of midazolam significantly spares isoflurane requirements for maintenance of general anaesthesia without significantly affecting cardiovascular function in goats.

Effects of fentanyl on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats

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4.1 ABSTRACT

Objective To evaluate the effects of fentanyl, a short-acting μ-agonistic opioid analgesic agent, on MAC of isoflurane and cardiovascular function in artificially-ventilated goats.

Study Design Prospective, randomized, crossover experimental study.

Animals Six healthy goats, 3 does and 3 wethers.

Methods Following induction of general anaesthesia with isoflurane, endotracheal intubation was performed and anaesthesia was maintained with isoflurane in oxygen while the lungs of the goats were artificially ventilated. Baseline isoflurane MAC in response to clamping a claw with a Vulsellum forceps was determined. The goats then received three fentanyl treatments: bolus of 0.005 mg kg^{-1} followed by a maintenance dose of 0.005 mg kg^{-1} $hour^{-1}$ (Treatment LFENT), bolus of of fentanyl at 0.015 mg kg^{-1} followed by a maintenance dose of 0.015 mg kg^{-1} $hour^{-1}$ (Treatment MFENT), bolus of 0.03 mg kg^{-1} followed by a maintenance dose of 0.03 mg kg^{-1} $hour^{-1}$ (Treatment HFENT), intravenously. Isoflurane MAC was re-determined following fentanyl treatment. Response to fentanyl treatment was determined as the difference between the baseline and post-treatment mean isoflurane MAC. Cardiopulmonary parameters were monitored throughout the anaesthetic period. Quality of recovery was scored. The Friedman test was used to test for differences between isoflurane MACs. Medians of repeatedly cardiovascular parameters test for differences between and within treatments using repeated measures analysis of variance (ANOVA) by ranks. ($P < 0.05$ for statistical significance).

Results The baseline [median (interquartile range)] isoflurane MAC in the goats was 1.32 (1.29-1.36)% volume. Administration of fentanyl treatments as described reduced the median isoflurane MAC to 0.98 (0.92-1.01)% volume, 0.75 (0.69-0.79)% volume and 0.58 (0.51-0.65)% volume following LFENT treatment, MFENT treatment and the HFENT treatment respectively. Cardiovascular function was not adversely affected by any of the three fentanyl treatments. Quality of recovery from general anaesthesia was satisfactory following all fentanyl treatements.

Conclusions and clinical relevance Fentanyl reduces isoflurane MAC in a dose-dependent manner with minimal adverse cardiovascular effects.

Keywords goat, fentanyl, analgesia, isoflurane, minimum alveolar concentration

4.2 INTRODUCTION

The level of anaesthesia during surgery is determined by the interaction of hypnotic drugs, analgesic drugs, and the intensity of noxious stimulation (Bouillon et al. 2004). General anaesthetic agents, like isoflurane, primarily contribute to anaesthesia through their hypnotic effects, while opioids, like fentanyl, contribute through analgesic effects. The dose-response curve of opioid analgesics for reducing hypnotic requirements is steepest for blocking autonomic responses following noxious stimulation, somewhat less steep for blocking movement (motor) responses and least steep for inducing unconsciousness (Antognini & Carstens 2002). The interaction of hypnotic agents and opioid analgesics during general anaesthesia can be schematically described by a hierarchical model, in which, initially a noxious stimuli processed at sub-cortical levels of the central nervous system (CNS) has its nociceptive signal attenuated by opioids and subsequently, when the attenuated signal is projected to the cortical levels, arousal is suppressed by hypnotic agents (Bouillon et al. 2004).

Fentanyl, a synthetic μ opioid agonist, is used for the treatment of moderate to severe pain (Carroll et al. 1999; Plumb 2005; Lamont & Mathews 2007). Opioids are used extensively for premedication, for analgesic supplementation during regional and general anesthesia, as primary anaesthetic agents and as analgesics for postoperative pain (Clutton 1998; Stanski 2000). While many opioids are suitable for intravenous infusion, fentanyl is frequently selected as the drug of choice as it offers clinically desirable effects over a wide dose range and has a wide therapeutic margin (Mama 2006). The onset of action of fentanyl is rapid following intravenous (Lamont & Mathews 2007) or intramuscular administration with analgesia, sedation, ataxia, respiratory depression and hyperaesthesia developing in 3 to 8 minutes (Carroll et al. 1999). It has a short duration of action, with the peak effect lasting less than 30 minutes (Carroll et al. 1999; Lee et al. 2000). A potential disadvantage of short-acting analgesics could be the less than satisfactory pain-relief in the postoperative period (Dunn et al. 2006). In goats, fentanyl administered intravenously at 0.01 mg kg^{-1} proved effective against thermal and mechanical stimuli in a nociceptive model study (Valverde & Gunkel 2005). In another study, fentanyl was used intravenously in goats at 0.002 mg kg^{-1} hr^{-1} to satisfactorily supplement nitrous oxide and isoflurane for maintenance of general anaesthesia (Andel et al. 2000). Studies in humans and dogs have shown that fentanyl alone does not result in complete general anaesthesia, but can be combined with benzodiazepines or sub-anaesthetic doses of propofol to achieve satisfactory levels of general anaesthesia (Carroll et al. 1999; Stanski 2000).

Isoflurane is a commonly used inhalant general anaesthetic, which has short induction and recovery times because of its low blood:gas solubility coefficient (Hall et al. 2001; McEwen et al. 2000). The mechanism by which isoflurane produces unconciousness and immobility is still not yet fully understood, although it is assumed that the sites of action are the brain and spinal cord (Antognini & Carstens 2002). The most likely mechanism by which isoflurane produces anaesthetic effects is potentiatiation of the GABA (γ-aminobutyric acid) receptor-channel complex (Larsen et al. 1998). Isoflurane can be used for induction as well as maintenance of anaesthesia in goats (Antognini & Eisele 1993). Isoflurane, like most other inhalant anaesthetic agents, causes respiratory depression, hypotension and reduced cardiac output in a dose-dependent pattern (Antognini & Eisele 1993; Hall et al. 2001; Hikasa et al. 2002).

In the present experimental study we assessed the effects of fentanyl on isoflurane MAC in artificially ventilated goats. We defined isoflurane MAC according to Merkel and Eger 1963, as the lowest isoflurane alveolar (end-tidal) concentration required by an individual goat to prevent gross purposeful movement in response to a supramaximal stimulus, which in this study was claw-clamping using a Vulsellum forceps. Isoflurane requirement for general anaesthesia in goats, as defined by MAC, was determined in previous studies to be 1.3 to 1.5% (Antognini & Eisele 1993; Hikasa et al. 1998; Antognini et al. 2000c; Doherty et al. 2002a; Hikasa et al. 2002; McEwen et al. 2000: Doherty et al. 2004). Combining isoflurane with analgesic drugs like fentanyl potentially reduces the dose of isoflurane required to maintain general anesthesia and consequently reduces occurrence of adverse dose-dependent cardiopulmonary effects normally associated with isoflurane anaesthesia. We tested the null hypothesis that fentanyl does not affect isoflurane MAC against alternative hypothesis that fentanyl reduces isoflurane MAC in goats.

4.3 MATERIALS AND METHODS

4.3.1 EXPERIMENTAL DESIGN AND INSTRUMENTATION

Six clinically healthy goats (3 does and 3 wethers) were used in this study. The goats were assigned to three treatments, with order of treatments randomized in a cross-over pattern, with a four-week washout period between treatments. General anaesthesia was achieved initially with isoflurane only and then later with isoflurane combined with a constant rate infusion of low dose fentanyl (Treatment LFENT), moderate dose fentanyl (Treatment MFENT) or high dose fentanyl (Treatment

HFENT). Health status was assessed by physical examination, a complete blood count and serum biochemical analysis; all findings were normal. The signalment of the goats is summarized in Table 4.1.

Food and water were withheld for 16–22 hours before anaesthesia. The goats were weighed 30 minutes before the experiment. Baseline rectal temperature measured by a digital thermometer, heart rate measured by thoracic auscultation and respiratory rate were recorded before the goats were placed on a custom-made sling-cum-table for easier restraint. The auricular artery on the right ear was catheterized using a 24-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) which was then connected to a calibrated transducer (DTX Plus transducer, BD Medical, Johannesburg, South Africa) for measurement of systolic, diastolic and mean arterial blood pressures. The blood pressure readings were obtained from a calibrated electronic strain gauge transducer connected to a multi-parameter monitor (Cardiocarp/5, Datex-Ohmeda Corporation, Helsinki, Finland), which had been calibrated against a mercury column within 1 month of commencement of the study. For transducer calibration to atmospheric pressure, the scapulo-humeral joint or the point of the sternum were used as zero reference points in sternally-recumbent or laterally-recumbent goats, respectively. An 18-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) was introduced into the right cephalic vein for administration of intravenous fluids and fentanyl. Another 18-SWG catheter was placed in the right jugular vein for collection of venous blood samples for determination of fentanyl plasma concentration.

Mask induction of the goats with isoflurane (Forane® Liquid, Abbott Laboratories Pty Ltd, Constantia Kloof, South Africa) delivered in oxygen from a circle anaesthetic breathing system with a calibrated Tec 3 out-of-circle vaporiser (Fluotec 3®, BOC Health Care, West Yorkshire, England) was achieved with the goats restrained in sternal position. A tight-fitting facemask was used to limit dead space and gas leaks around the mask. Each goat was accustomed to the mask by initially being allowed to breathe 100% volume oxygen at 6 L minute^{-1} for at least one minute before isoflurane administration rate was slowly begun with 0.5% volume increments every 15 seconds until a 3.5% volume vaporizer setting was reached. This vaporizer setting was then maintained until the jaw was relaxed enough to allow intubation. Placement of the endotracheal tube (silicone tube, internal diameter 7.5 mm) was done with the goats in sternal recumbency using a laryngoscope to facilitate the process. If intubation was not successful due to poor relaxation of the jaws, isoflurane delivery by facemask was continued before attempting again. The cuff of the endotracheal tube was inflated to prevent leakage of gases from the breathing circuit to a pressure of 20 cmH_20.

Immediately after intubation, the goats were placed in left lateral recumbency with fresh oxygen flow set at 2 L minute^{-1} and initial end-tidal isoflurane concentration targeted to be 1.6% volume. Intermittent positive pressure ventilation (Ohmeda 7000 Ventilator, Ohmeda, Madison, Wisconsin, USA) was used to maintain end-tidal carbon dioxide between 35–45 mmHg (4.7 and 6.0 kPa) throughout the procedure. Ringer Lactate solution (Intramed Ringer-Lactate® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) was administered by a pump (Infusomat, BBraun, Melsungen, Germany) at a rate of 4 mL kg^{-1} hour^{-1} intravenously.

Instrumentation for recording of physiological parameters was set up using a multi-parameter monitor (Cardiocap/5, Datex-Ohmeda Corporation, Helsinki, Finland). Three electrocardiography (ECG) electrodes were placed on shaven areas (on the middle of the left shoulder, on the midline 2 cm in front of the point of the sternum and on the midline 2 cm cranial to the tip of the xiphoid) to provide a lead II ECG tracing. Haemoglobin oxygen saturation (SpO_2) was measured via a pulseoximetry infrared probe placed around the tongue, which calculated heart rate as well. Inspired and expired concentrations of isoflurane, carbon dioxide and oxygen were obtained from a flow sensor and a side-stream gas sampler placed between the endotracheal tube and the Y-piece of the breathing system. The flow rate through the gas sampling line was constant at 200 mL minute^{-1}. Respiratory rate was calculated from the capnogram. The gas analyzer had been calibrated with calibration gas as recommended by the manufacturer within 1 month of commencement of the studies and would automatically self-calibrate to atmospheric air at the beginning of the experiment. Temperature was measured by an oesophageal probe place as close to the base of the heart as possible. This was done by marking from outside how far the temperature probe had to be placed to reach the point of the elbow. We targeted to maintain oesophageal temperature between 37.5 and 39.5ºC using a forced warmed air blanket and ordinary blankets placed around the goats. The physiological parameters were measured continuously during the anaesthetic period, but recorded every 15 minutes.

Determination of the baseline isoflurane (control) MAC began 15 minutes after end-tidal isoflurane concentration had been held constant at 1.6%. Isoflurane MAC determination involved application of a noxious stimulus with a Vulsellum forceps clamped to the second ratchet to the claw about 1 cm below the coronary band for 60 seconds or until occurrence of purposeful movement (Figure 4.1). Purposeful movement was strictly defined as gross movement of the head or limbs, including movement of the limb to which the Vulsellum forceps was being applied. End-tidal isoflurane concentration was then adjusted according to response to noxious stimulation. If no movement occurred, the end-tidal isoflurane concentration was reduced by a tenth (approximately 10% of its

value) and held constant for at least 15 minutes before application of a noxious stimulus again. If movement was noticed, the end-tidal isoflurane concentration was increased by a tenth and held constant for at least 15 minutes before application of a noxious stimulus again. The four claws on the two uppermost limbs were clamped consecutively in a clockwise fashion. Isoflurane MAC was calculated as the average of two successive concentrations; the end-tidal isoflurane concentration at which movement in response to noxious stimulation occurred and the preceeding end-tidal isoflurane concentration at which movement did not occur. The isoflurane MAC was determined in duplicate and the mean of the two MACs was taken as baseline isoflurane MAC.

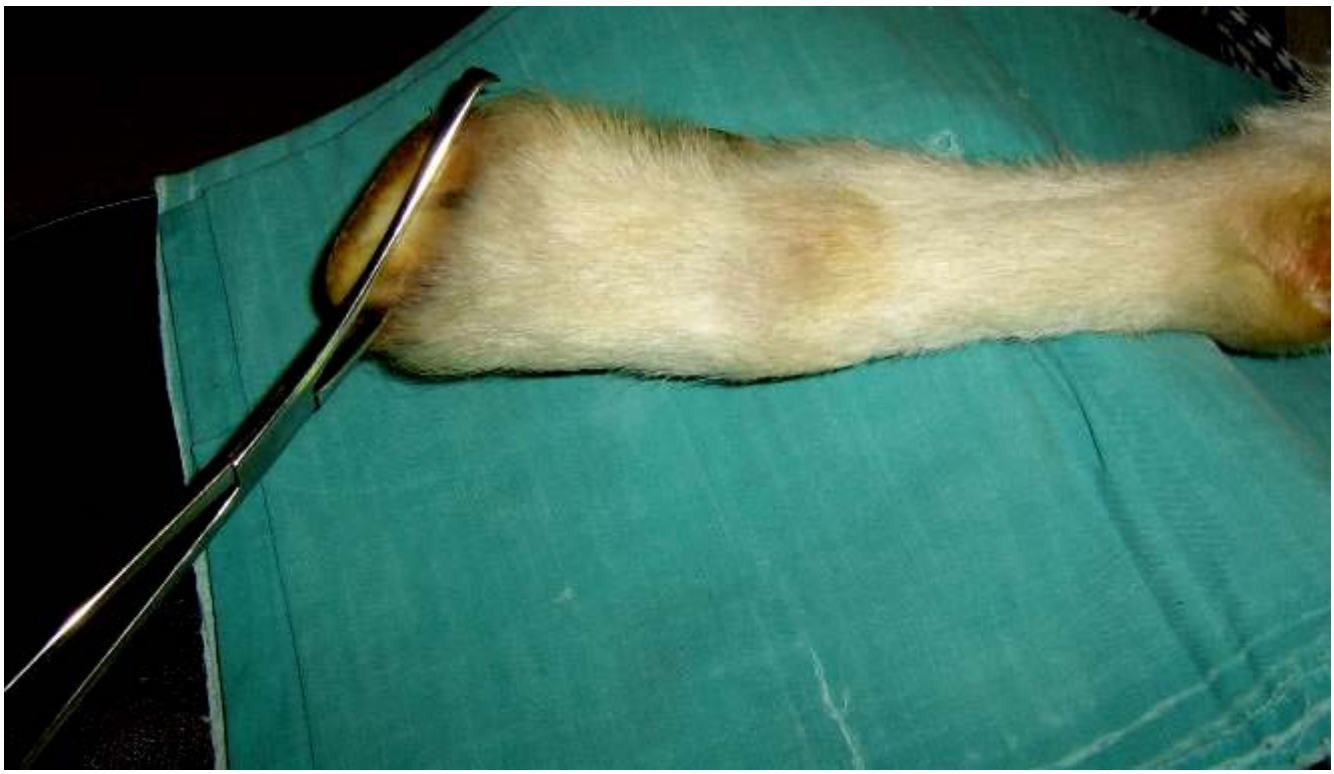


Figure 4.1 Vulsellum forceps clamped to the claw for noxious stimulation.

Following baseline MAC determination, the goats then received a bolus dose of fentanyl administered manually over a 1 minute period; at 0.005 mg kg^{-1}, 0.015 mg kg^{-1}, or 0.030 mg kg^{-1} intravenously; followed by a maintenance dose of 0.005 mg kg^{-1} $hour^{-1}$, 0.015 mg kg^{-1} $hour^{-1}$, or 0.030 mg kg^{-1} $hour^{-1}$ as Treatment LFENT, Treatment MFENT and Treatment HFENT, respectively. Fentanyl for maintenance of general anaesthesia was prepared up to 60 mL in normal saline and was administered by CRI from a 60 mL syringe controlled by a syringe-driving pump (Perfusor Compact, BBraun, Melsungen, Germany). The fentanyl syringe was connected to the right cephalic vein catheter, to which the Ringer Lactate administration line was also connected, by an extension tube via a three-

way stopcock. The fentanyl loading dose was administered over a period of 1 minute and administration of the maintenance dose started immediately afterwards. The accuracy of delivery of fentanyl by the pump was checked at the end of the experiment by calculating the expected infused amount based on infusion rates and comparing this to actual volume infused from the syringe.

Fentanyl-treatment isoflurane MAC was then determined by applying a noxious stimulus with a Vulsellum forceps after every 15 minutes of end-tidal isoflurane concentration equilibration, and depending on the goat's response, adjusting the end-tidal isoflurane concentration in the same manner as described above. Response to fentanyl treatment for each goat was defined as the difference between baseline and fentanyl-treatment isoflurane MAC.

Since baseline isoflurane MAC was determined each time before a goat underwent one of the three fentanyl treatments, the final baseline isoflurane MAC for each goat was calculated as the average of the three baseline MAC values obtained. There was no need to adjust the isoflurane end-tidal concentrations obtained to atmospheric pressure as the gas module used for measuring respiratory gas concentrations had a sensor that constantly measured atmospheric pressure and adjusted respiratory gas readings as if they were measured at one atmospheric pressure.

Venous blood samples (4.5 mL) were collected via the right jugular vein catheter in heparinised tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for determination of fentanyl plasma concentration at 0, 1, 5, 15, 30 and every 30 minutes from the time of propofol bolus administration until the propofol-treatment isoflurane MAC had been determined. The blood samples were centrifuged at 2 500 revolutions per minute for 15 minutes after which plasma was collected and stored at –20ºC for fentanyl concentration analysis later.

After determination of fentanyl-treatment isoflurane MAC, administration of fentanyl and isoflurane was discontinued and the quality of recovery from anaesthesia of the goats observed. The endotracheal tube was removed once the goats regained the swallowing reflex.

Time to extubation, sternal position and standing were recorded. All times were determined as the interval between the time of discontinuation of fentanyl and isoflurane administration and the time a particular event happened.

Quality of recovery from anaesthesia was scored on a 0 – 2 scale where: 0 = restlessness, 1 = relatively smooth, with some restlessness, 2 = smooth.

4.3.2 STATISTICAL ANALYSIS

Data were analysed using the R® Statistical Software, Version 2.7.2 (The R Foundation for Statistical Computing, Vienna, Austria). All data were assumed to be non-parametric because of the small sample size and are expressed as median and inter-quartile ranges.

Data on isoflurane MAC, isoflurane MAC reduction after fentanyl treatment, isoflurane MAC determination time, time to extubation, time to sternal position, time to standing and recovery scores were tested for statistically significant differences between treatments using the Friedman test. If statistically significant differences were found between treatments, post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted.

Medians of repeatedly measured variables (heart rate, mean arterial blood pressure, SpO_2 and body temperature) were tested for statistically significant differences between and within groups using repeated measures analysis of variance (ANOVA) by ranks. If statistically significant differences were found, a post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted. A value of $P < 0.05$ was considered significant.

4.4 RESULTS

There were no statistically significant differences in goat profile data, i.e. median age, weight, pre-anaesthetic total serum protein, haematocrit, white cell count and body temperature between treatments (Table 4.1).

Mask induction of anaesthesia using isoflurane was satisfactorily achieved in about 10 minutes with minimal struggling of the goats throughout the induction period. Data on observed isoflurane MACs, changes in isoflurane MAC after treatment with fentanyl and the time it took to determine isoflurane MAC are summarized in Table 4.2.

The observed median baseline MAC of isoflurane in goats of 1.32 (1.29-1.36)% volume was statistically significantly higher than the median MACs obtained after intravenous administration of all fentanyl treatments (P =0.013 all round). The median isoflurane MAC following LFENT treatment was statistically significantly higher than those observed following MFENT treatment (P = 0.013) and HFENT treatment (P = 0.013). See Figure 4.2 for comprehensive information.

The percentage reductions in isoflurane MAC after intravenous administration of fentanyl were 27.6%, 40.7% and 56.6% in the LFENT group, MFENT group and the HFENT group respectively. The differences in MAC reduction were all statistically significantly different from each other (Figure 4.3). The magnitude of MAC reduction following fentanyl administration was dose-related.

Fentanyl plasma concentrations could not be determined due to failure to obtain the analytical standards (100% fentanyl powder) that is essential for the analysis.

The time it took to determine baseline isoflurane MAC was 67.5 minutes. Following fentanyl treatment, mean time required to determine isoflurane MAC ranged from 75.0 minutes to 120.0 minutes for the three treatments, and was dependent on the dose of fentanyl used. The time it took to determine isoflurane MAC for HFENT treatment and MFENT treatment was significantly higher than than the time it took to determine baseline isoflurane MAC (P = 0.027 for both) and the the time it took to determine isoflurane MAC following LFENT treatment (P = 0.024 and 0.047 respectively).

The data obtained for cardiovascular system and respiratory system variables did not show any statistically significant differences between groups or between the baseline reading and any subsequent points within a group (Table 4.3). At all time points, mean arterial blood pressure was above 60 mmHg and SpO_2 above 90%. The end-tidal carbon dioxide concentration was successfully maintained within the normal range of 35-45 mmHg by adjusting respiratory rate and intrathoracic pressure settings on the mechanical ventilator. The body temperature of the goats was maintained between 37.5–39.3ºC.

The quality of recovery from anaesthesia was good for all the three treatment groups. The variables used to assess quality of recovery from anaesthesia did not show any statistically significant differences between treatments, except the time taken for the goats to stand, which showed significant

differences between treatments ($P = 0.04$), but did not show any two specific groups to be different when pair-wise comparisons were performed (Table 4.4). Although the recovery from anaesthesia was smooth following all three fentanyl treatments, it was noticed that 4 out of 6 times, the goats showed exaggerated intermittent tail-waging at recovery following MFENT and HFENT treatment.

4.4.1 TABLES

Table 4.1 Profile of the goats [median (inter-quartile range)] used in a study in which the effects of intravenously administered fentanyl: 0.005 mg kg^{-1} bolus followed by continuous infusion at 0.005 mg $kg^{-1}hr^{-1}$ (LFENT Treatment), 0.015 mg kg^{-1} bolus followed by continuous infusion at 0.015 mg $kg^{-1}hr^{-1}$ (MFENT Treatment), or 0.03 mg kg^{-1} bolus followed by continuous infusion at 0.03 mg $kg^{-1}hr^{-1}$ (HFENT Treatment) on the MAC of isoflurane were investigated.

	Parameter					
Treatment	Age (months)	Weight (kg)	TSP (g L^{-1})	Haematocrit (Litres $Litre^{-1}$)	White Cell Count ($x10^9$ L^{-1})	Rectal Temp (ºC)
LFENT	15.5 (14.3-16.0)	36.7 (29.6-40.7)	65.7 (64.1-66.1)	0.39 (0.38-0.41)	13.5 (12.7-14.3)	39.1 (39.0-39.2)
MFENT	15.0 (15.0-15.8)	36.9 (33.6-38.6)	62.9 (61.2-66.7)	0.37 (0.34-0.40)	14.5 (13.6-15.4)	39.10 (38.8-39.3)
HFENT	14.5 (14.0-15.8)	35.9 (31.1-37.6)	65.5 (63.1-68.4)	0.37 (0.36-0.40)	14.69 (13.7-15.8)	39.1 (39.0-39.2)

NS: No statistically significant differences (P <0.05) between the three treatments

Table 4.2 Effect [median (inter-quartile range)] of intravenously administered fentanyl: 0.005 mg kg^{-1} bolus followed by continuous infusion at 0.005 mg $kg^{-1}hr^{-1}$ (LFENT Treatment), 0.015 mg kg^{-1} bolus followed by continuous infusion at 0.015 mg $kg^{-1}hr^{-1}$ (MFENT Treatment), or 0.03 mg kg^{-1} bolus followed by continuous infusion at 0.03 mg $kg^{-1}hr^{-1}$ (HFENT Treatment) on the MAC of isoflurane in goats.

Treatment	Isoflurane MAC (%vol)	Change post-treatment (%)	MAC determination time (minutes)
Control	1.32 (1.29-1.36) *	Not applicable	67.5 (65.0-70.0)
LFENT	0.98 (0.92-1.01) *	-27.6 (24.9-29.3)*	75.0 (75.0-75.0)
MFENT	0.75 (0.69-0.79) #	-40.7 (40.0-47.7)*	97.5 (90.0-116.3)#
HFENT	0.58 (0.51-0.65) #	-56.6 (51.9-60.8)*	120.0 (108.8-120.0)#

*: statistically significantly different (P <0.05) from all other groups
#: statistically significantly different (P <0.05) from Control treatment and LFENT treatment

Table 4.3 Physiological parameters [median (inter-quartile range)] observed following intravenous administration of fentanyl: 0.005 mg kg^{-1} bolus followed by continuous infusion at 0.005 mg $kg^{-1}hr^{-1}$ (LFENT Group), 0.015 mg kg^{-1}bolus followed by continuous infusion at 0.015 mg $kg^{-1}hr^{-1}$ (MFENT Group), or 0.03 mg kg^{-1} bolus followed by continuous infusion at 0.03 mg $kg^{-1}hr^{-1}$ (HFENT Group) in isoflurane-anaesthesthetised goats.

Variable	Unit	Treatment	Time	Period of Baseline Isoflurane MAC Determination (minutes)				Period of Fentanyl-treatment Isoflurane MAC Determination (minutes)				
			Baseline	2	15	30	45	2	15	30	45	60
Heart Rate	beats min^{-1}	LPROP	86 (81-88)	89 (78-91)	88 (85-94)	90 (85-98)	90 (88-95)	76 (67-83)	77 (71-82)	75 (68-83)	76 (65-84)	86 (73-87)
		MPROP	90 (82-99)	86 (82-93)	87 (85-90)	89 (85-97)	88 (85-98)	68 (65-73)	74 (68-76)	67 (61-75)	69 (61-74)	69 (62-74)
		HPROP	90 (76-11)	88 (75-99)	87 (73-104)	88 (73-103)	88 (76-111)	62 (47-72)	69 (56-81)	71 (55-78)	71 (54-82)	71 (54-76)
SAP	mmHg	LPROP	120 (106-133)	86 (85-109)	98 (94-105)	101 (94-103)	98 (96-101)	95 (88-100)	97 (92-99)	96 (90-98)	95 (90-100)	96 (87-103)
		MPROP	110 (104-115)	103 (88-111)	102 (90-109)	104 (89-106)	103 (96-109)	88 (71-100)	94 (89-97)	100 (93-103)	103 (99-106)	96 (97-106)
		HPROP	127 (113-135)	102 (90-120)	102 (94-117)	102 (100-105)	99 (95-103)	78 (70-81)	97 (93-108)	98 (97-104)	96 (90-113)	98 (87-119)
DAP	mmHg	LPROP	74 (68-77)	56 (51-81)	63 (60-73)	70 (61-76)	78 (65-82)	63 (62-73	70 (64-75)	71 (67-76)	75 (65-80)	71 (66-79)
		MPROP	77 (73-80)	64 (50-76)	74 (57-80)	79 (63-84)	80 (65-85)	58 (54-65)	70 (67-74)	74 (73-75)	76 (73-79)	75 (72-79)
		HPROP	79 (77-90)	71 (62-87)	71 (63-83)	74 (72-76)	70 (64-76)	54 (51-57)	69 (68-80)	71 (68-77)	66 (64-80)	65 (64-85)
MAP	mmHg	LPROP	98 (90-104)	70 (64-90)	77 (72-85)	85 (74-90)	88 (79-92)	78 (71-84)	82 (75-88)	81 (76-86)	84 (77-88)	82 (74-89)
		MPROP	96 (90-98.8)	90 (69-94)	88 (70-91)	92 (72-94)	91 (79-95)	71 (62-77)	80 (79-82)	85 (83-87)	88 (83-91)	84 (81-92)
		HPROP	109 (97-115)	83 (74-102)	84 (75-98)	87 (86-87)	83 (78-87)	63 (61-65)	82 (80-95)	84 (82-91)	79 (77-96)	80 (76-103)
SpO_2	%	LPROP	-	99 (98-99)	99 (98-99)	98 (97-98)	98 (97-98)	98 (97-98)	97 (96-98)	97 (96-99)	97 (97-98)	97 (96-98)
		MPROP	-	98 (97-99)	99 (98-100)	98 (97-99)	98 (96-98)	98 (96-98)	97 (95-99)	97 (95-98)	96 (94-98)	95 (94-97)
		HPROP	-	98 (93-98)	98 (96-98)	98 (96-98)	98 (95-98)	98 (96-98)	99 (98-99)	98 (97-98)	98 (97-98)	98 (98-99)
$PE´CO_2$	mmHg	LPROP	-	45.3 (42.2-46.9)	43.9 (42.4-44.8)	42.3 (40.3-43.5)	40.5 (38.1-43.5)	39.8 (36.9-43.1)	41.6 (37.9-44.8)	34.9 (34.5-35.3)	36.7 (34.9-38.4)	37.5 (36.0-39.6)
		MPROP	-	41.0 (36.9-44.1)	44.2 (38.0-45.6)	45.6 (44.5-45.6)	43.7 (43.3-45.2)	43.7 (40.9-44.9)	41.0 (41.0-42.2)	41.0 (39.3-43.3)	42.6 (42.0-43.7)	43.3 (42.8-43.9)
		HPROP	-	44.1 (40.5-45.4)	43.2 (41.0-43.9)	44.8 (40.7-45.6)	44.5 (37.2-45.4)	41.4 (38.0-44.8)	42.2 (37.8-45.4)	44.1 (41.0-45.4)	40.3 (35.5-44.5)	44.5 (43.3-45.6)
Temp	(ºC)	LPROP	39.1 (39.0-39.2)	38.5 (38.4-38.5)	38.3 (38.3-38.6)	38.3 (38.1-38.5)*	38.1 (38.0-38.5)*	38.1 (37.9-38.4)*	37.8 (37.7-38.3)*	37.8 (37.6-38.2)*	37.7 (37.6-38.1)*	37.6 (37.5-38.0)*
		MPROP	39.1 (38.8-39.3)	38.6 (38.2-38.8)	38.5 (38.2-38.8)	38.4 (38.1-38.6)	38.4 (38.0-38.6)	38.3 (38.1-38.5)*	38.2 (37.9-38.4)*	38.1 (37.8-38.3)*	38.0 (37.8-38.3)*	38.0 (37.9-38.3)*
		HPROP	39.1 (39.0-39.2)	38.5 (38.4-38.8)	38.5 (38.3-38.8)	38.4 (38.1-38.7)	38.3 (38.1-38.5)	38.3 (38.0-38.5)*	38.1 (37.9-38.3)*	38.1 (37.9-38.1)*	38.0 (37.9-38.0)*	38.0 (37.8-38.0)*

*: statistically significantly different ($P<0.05$) from baseline reading within treatment

SAP- systolic arterial pressure; DAP- diastolic arterial pressure; MAP- mean arterial pressure; SpO_2- saturation of haemoglobin with oxygen in peripheral blood; $PE´CO_2$- end-tidal carbon dioxide partial pressure; Temp- body temperature.

Table 4.4 Quality of recovery from anaesthesia [median (inter-quartile range)] observed in a study where the effects of intravenously administered fentanyl: 0.005 mg kg^{-1} bolus followed by continuous infusion at 0.005 mg $kg^{-1}hr^{-1}$ (LFENT Treatment), 0.015 mg kg^{-1} bolus followed by continuous infusion at 0.015 mg $kg^{-1}hr^{-1}$ (MFENT Treatment), or 0.03 mg kg^{-1} bolus followed by continuous infusion at 0.03 mg $kg^{-1}hr^{-1}$ (HFENT Treatment) on the MAC of isoflurane in goats were investigated.

Treatment	Time to Extubation (minutes)	Time to Sternal Position (minutes)	Time to Standing (minutes)	Recovery Score
LFENT	2.0 (2.0-2.8)	3.0 (3.0-4.5)	5.0 (5.0-7.3)	2 (2-2)
MFENT	3.0 (3.0-3.0)	4.0 (3.0-5.0)	9.0 (7.25-11.5)	2 (2-2)
HFENT	3.0 (3.0-3.0)	3.0 (3.0-4.5)	10.0 (8.5-10.0)	1.5 (1-2)

Note: No statistically significant differences (P <0.05) between any treatments

4.4.2 FIGURES

Figure 4.2 Box-and-Whiskers plot of median isoflurane MAC (% volume) observed in isoflurane anaesthetised goats (Control) and after intravenous administration of fentanyl (LFENT Treatment, MFENT Treatment, or HFENT Treatment) to isoflurane-anaesthetised goats. Each box represents data from the 25th to the 75th percentiles, the bold line represents the median value, and the whiskers represent the range of scores, while the small dots outside the box represent outliers. Also highlighted are the *P*-values obtained when the isoflurane MAC were compared statistically between treatments.

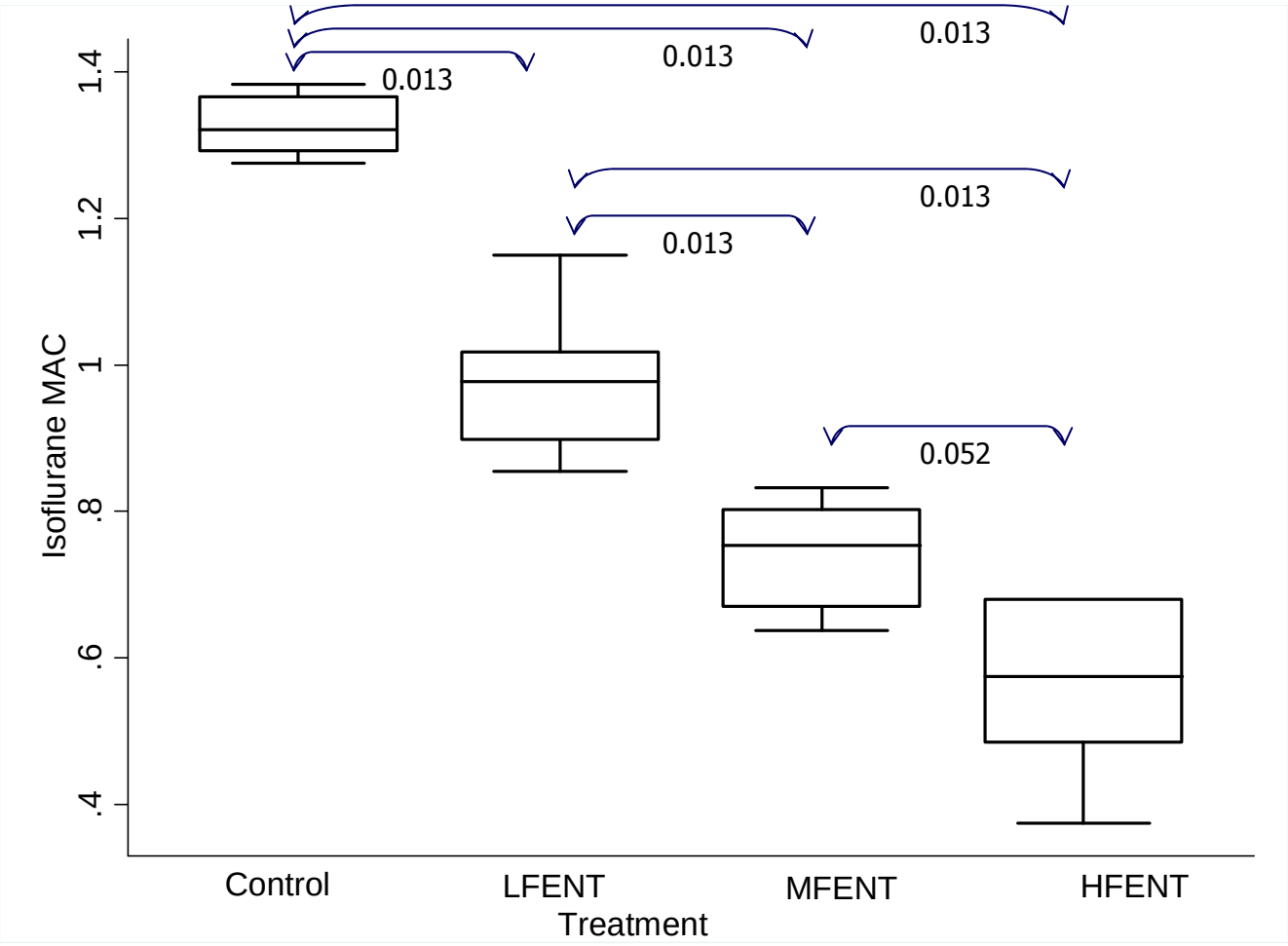
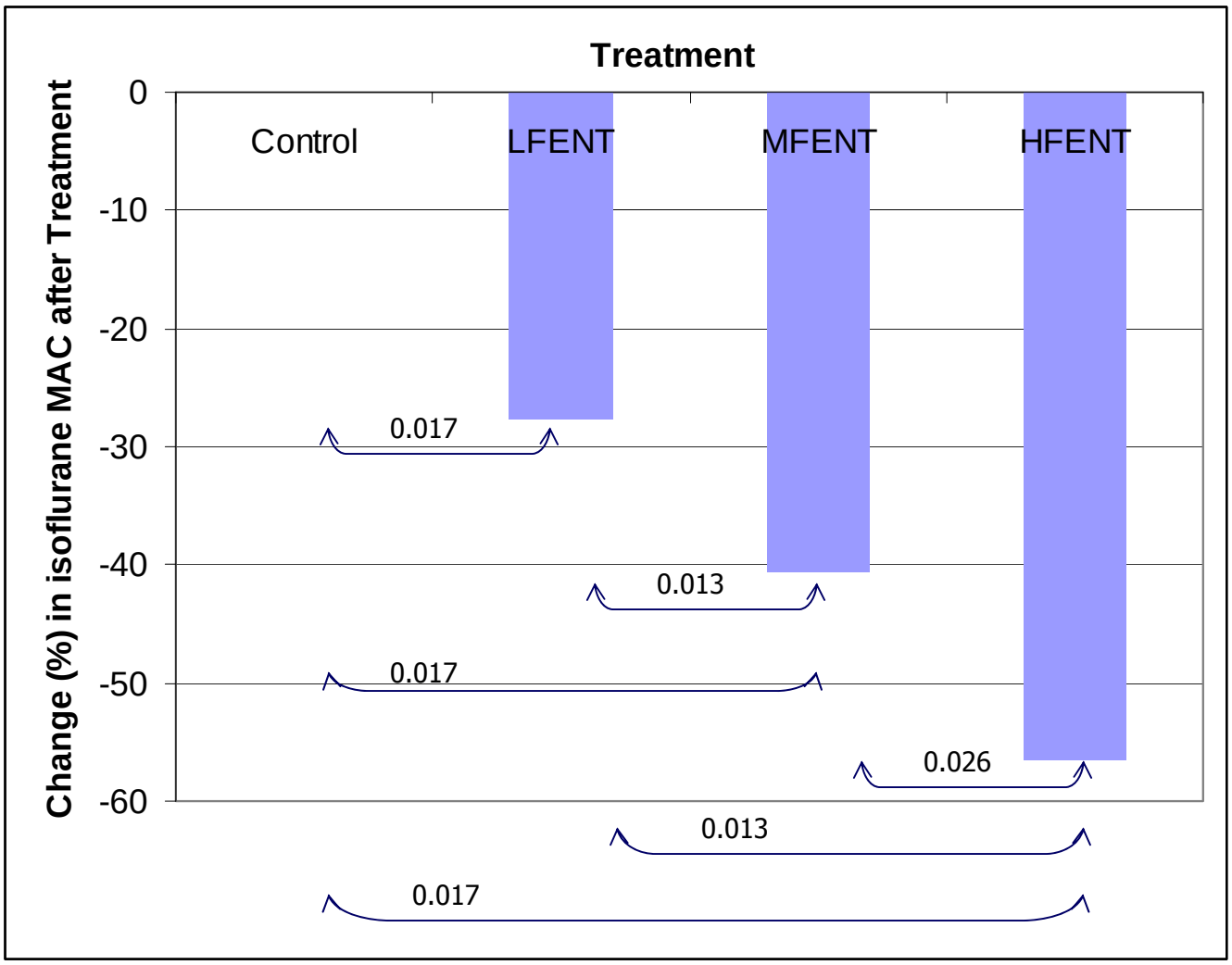


Figure 4.3 Percentage change in isoflurane MAC observed in isoflurane anaesthetised goats (Control) and after intravenous administration of midazolam (LFENT Treatment, MFENT Treatment, or HFENT Treatment) to isoflurane-anaesthetised goats. Also highlighted are the *P*-values obtained when the change in isoflurane MAC following fentanyl treatement were compared statistically between treatments.



4.5 DISCUSSION

The median baseline isoflurane MAC of 1.32 (1.29–1.36)% volume observed in the present study lies with the range reported by different literature sources. In 1993, Antognini & Eisele reported isoflurane MAC in goats as 1.5% volume, while other research teams reported isoflurane MACs in goats ranging from 1.23% volume to 1.29% volume (Doherty et al. 2002a; Doherty et al. 2002b; Hikasa et al. 1998). The similarity of isoflurane MAC in goats across the different studies is to be expected since MAC of an inhalation anaesthetic agent is expected to remain the same within any species if physiological states like body temperature, blood pressure, haematocrit and tissue perfusion are maintained within normal levels (Stanski 2000). To the eye, the median baseline isoflurane MAC of 1.32 (1.29–1.36)% obtained in the present study looks different from that obtained in an earlier study (see Chapter 3) in which a baseline isoflurane MAC of 1.40 (1.38–1.41) was obtained in the same goats, but no statistical significant difference were obtained on comparisons of the 2 data sets. Moreover, both values of baseline isoflurane MAC fall well within the range reported in goats from previous studies as cited above.

The reductions in isoflurane MAC by 27.6%, 40.7% and 56.6% observed in the present study after administration of Treatment LFENT, Treatment MFENT and Treatment HFENT respectively show that fentanyl administered intravenously reduces isoflurane MAC in a dose-dependent manner and could have a role as an analgesia adjunct in balanced anaesthesia protocols in goats. There is little information to date on the interaction of opioids with volatile anaesthestic agents in goats. Data from studies in humans, and less consistently so in animals, have shown that the interaction between opioids and potent inhaled anaesthetics result in a dose-dependent reduction of inhaled anaesthestic requirements for general anaesthesia (Criado & Gomez de Segura, 2003; Hendrickx et al. 2008). In dogs, fentanyl administered transdermally at 0.003 mg kg^{-1} $hour^{-1}$ reduced isoflurane MAC in normothermic goats by approximately 37% (Wilson et al. 2006). In the horse, fentanyl administered at 0.00469 mg kg^{-1} bolus followed by continuous infusion at about 0.0678 mg kg^{-1} $hour^{-1}$ resulted in an 18% reduction in isoflurane MAC (Thomas et al. 2006). In swine, fentanyl administered at 0.05 mg kg^{-1} $hour^{-1}$, 0.1 mg kg^{-1} $hour^{-1}$ and 0.2 mg kg^{-1} $hour^{-1}$ reduced isoflurane minimum alveolar concentration by 24.5%, 29.9% and 45.9% respectively (Moon et al. 1995). Fentanyl seems to be less potent in reducing isflurane MAC in goats than in dogs, but is more potent in reducing isoflurane MAC in goats when compared to the horse and swine. These differences in degree of isoflurane MAC reduction by fentanyl in various species show the existence of species difference in pharmacologic actions of fentanyl. The pure μ-agonist opioid, morphine, administered as a single bolus at a clinically high dose

of 2 mg kg^{-1} intravenously in goats reduced isoflurane MAC by 30% (Doherty et al. 2004). Fentanyl, being a pure μ-agonist opioid more potent than morphine, would be expected to cause more pronounced reductions in isoflurane MAC, as supported by observations from the present study.

The results on the effects of fentanyl administration on cardiovascular parameters show that fentanyl can substantially spare isoflurane required to maintain general anesthesia in goats without causing adverse effects on cardiovascular function. The arterial blood pressure and SpO_2 obtained even after administration of the highest dose of fentanyl in this study were within normal physiological limits and were not statistically significantly different from baseline readings within a group. Fentanyl is known to cause clinically desirable effects over a wide dose range as it has a wide therapeutic margin (Mama 2006). The minimal cardiovascular effects obtain in this study confirm a widely accepted view that opioids result in haemodynamic stability if used in combination with low doses of inhalation anaesthetics for maintenance of general anaesthesia (Shibutani et al. 1999). A well known adverse effect of high doses of fentanyl is bradycardia (Criado & Gomez de Segura, 2003; Plumb 2005). In the present study, heart rate decreased in a dose-dependent manner after the beginning of fentanyl administration, but the level of decrease was not statistically significantly different from baseline heart rate readings. The lowest median heart rate across all three groups was 62 (47-72) beats per minute observed in the group that received the highest dose of fentanyl and this was still within normal acceptable heart rate range for goats. It must be taken into consideration that the goats in this study were all artificially ventilated as a way to offset extremes of tissue hypoxia and this would have minimized occurrence of respiratory depression, one of the well known adverse effects of fentanyl (Criado & Gomez de Segura 2003; Mildh et al. 1998; Plumb 2005.

The oesophageal temperature decreased gradually over the anaesthetic period in all three groups, but stayed within physiological acceptable limits with the lowest median oesophageal temperature of 37.7ºC observed in the group that received the lowest dose of fentanyl. This shows that heat conservation methods employed (covering with ordinary blankets and warming with a warm-air heating blanket) were successful in preventing excessive heat loss. The normal physiological body temperature range for goats is 37.2–39.7ºC (Ayo et al. 1998). Hypothermia is known to cause a reduction in MAC, but even biggest decrease in body temperature observed in this study of 1.4ºC would have affected isoflurane MAC only very slightly. Moreover this decrease in body temperature was consistent across the three groups.

The period of recovery from anaesthesia was short for all three groups with all goats attaining sternal recumbency within 4 minutes and standing within 10 minutes of termination of general anaesthesia. In

goats fentanyl has an extremely short duration of action due to the rapid elimination rate and clearance coupled with a large apparent volume of distribution at steady state (Carroll et al. 1999). Exaggerated tail-wagging was observed in the majority of goats that received higher doses of fentanyl, but the general behaviour of the goats was acceptable at all times during recovery. In 1999, Carroll and colleagues also reported a different form of abnormal behaviour in the form of increased vocalisation and activity during the first hour after intravenous administration of a single bolus of fentanyl in conscious goats. We did not observe this type of abnormal behaviour may be because the goats in our study were unconscious during the first hour of fentanyl administration.

In conclusion, fentanyl reduced isoflurane MAC in a dose-dependent manner with no adverse effects on cardiovascular function in goats. The results indicate that, fentanyl administered intravenously as a constant rate infusion following a bolus dose, can safely be used as an analgesic adjunct to general anesthesia in goats.

Effects of propofol on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats

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5.1 ABSTRACT

Objective To evaluate the effects of propofol, a commonly used intravenous general anaesthestic agent, on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.

Study Design Prospective, randomized, crossover experimental study.

Animals Six healthy goats, 3 does and 3 wethers.

Methods General anaesthesia was induced with isoflurane in oxygen. Endotracheal intubation was performed and anaesthesia was maintained with isoflurane in oxygen while the lungs of the goats were artificially ventilated. Baseline isoflurane MAC in response to clamping a claw with a Vulsellum forceps was determined. The goats then received three propofol treatments: bolus of 0.5 mg kg^{-1} followed by a maintenance dose of 0.05 mg kg^{-1} $minute^{-1}$ (Treatment LPROP), bolus of 1.0 mg kg^{-1} followed by a maintenance dose of 0.1 mg kg^{-1} $minute^{-1}$ (Treatment MPROP), bolus of 2.0 mg kg^{-1} followed by a maintenance dose of 0.2 mg kg^{-1} $minute^{-1}$ (Treatment HPROP), intravenously. Isoflurane MAC was re-determined following propofol treatment. Response to propofol treatment was determined as the difference between the baseline and propofol-treatment isoflurane MAC. Cardiopulmonary parameters were monitored throughout the anaesthetic period. Quality of recovery was scored. The Friedman test was used to test for differences between isoflurane MACs. Medians of repeatedly measured cardiovascular parameters were tested for differences between and within treatments using repeated measures analysis of variance (ANOVA) by ranks. ($P < 0.05$ for statistical significance).

Results From a baseline [median (interquartile range)] isoflurane MAC of 1.37 (1.36-1.37)% volume, administration of propofol treatments reduced the isoflurane MAC to 1.15 (1.08-1.15), 0.90 (0.87-0.93)

and 0.55 (0.49-0.58)% volume following LPROP, MPROP and HPROP treatment, respectively. Cardiovascular function was minimally affected by all propofol treatments. Quality of recovery from general anaesthesia was satisfactory following all propofol treatments.

Conclusions and clinical relevance Propofol reduces isoflurane MAC in a dose-dependent manner with minimal adverse cardiovascular effects in goats

Keywords goat, propofol, isoflurane, minimum alveolar concentration, MAC, anaesthesia

5.2 INTRODUCTION

Major surgery and prolonged diagnostic procedures in goats are usually performed under inhalation anaesthesia, using injectable anaesthetic agents only for induction and to facilitate endotracheal intubation (Reid et al.1993).

Propofol (2,6 diisopropylphenol) is conventionally used for induction of general anaesthesia. The exact site at which propofol acts is not yet clear, although there is mounting evidence that the $GABA_A$ (γ-aminobutyric acid) receptor modulates, at least in part, propofol's effects (Hui et al. 1995, Antognini et al. 2000d). Propofol's pharmacokinetic profile makes it one of the most useful general anaesthetic agents to use for partial intravenous anaesthesia or total intravenous anaesthesia in humans and other animals (Sebel & Lowdon 1989; Bettschart-Wolfensberger et al. 2000; Larenza et al. 2005; Dunn et al. 2006). In goats, propofol has a rapid and smooth onset of action, is cleared rapidly and is easy to titrate to a desired effect (Larenza et al. 2005; Prassinos et al. 2005).

Isoflurane is a commonly used inhalant anaesthetic agent, which has short induction and recovery times because of its low lipid solubility coefficient (Antognini & Eisele 1993). The most likely mechanism by which isoflurane produces anaesthetic effects is potentiation of the GABA (γ-aminobutyric acid) receptor-channel complex in the brain and spinal cord (Larsen et al. 1998; Antognini & Carstens 2002). Isoflurane, like most other inhalant anaesthetic agents, causes respiratory depression, hypotension and reduced cardiac output in a dose-dependent pattern (Antognini & Eisele 1993; Hikasa et al. 2002). Isoflurane requirement for general anaesthesia in goats, as defined by MAC, has been reported to range between 1.23% volume and 1.5% volume (Antognini & Eisele 1993; Hikasa et al. 1998; Antognini et al. 2000c; Hikasa et al. 2002; Doherty et al. 2002a; Doherty 2002b).

There are situations when partial or total intravenous anaesthesia might be the most suitable means of achieving general anaesthesia in goats. Anaesthesia for brain surgery might require combined use of propofol and isoflurane since high concentrations of isoflurane tend to increase cerebral blood flow (CBF), while propofol has been reported to decrease CBF in humans (Jansen et al. 1999). Partial or total intravenous anaesthesia studies might provide useful information on appropriate anaesthetic agent dosages for performing surgical procedures on the farm or for some diagnostic procedures like magnetic resonance imaging, (Carroll et al. 1997; Larenza et al. 2005).

In the present experimental study, the effects of propofol on isoflurane MAC were assessed. Isoflurane MAC was defined according to Merkel and Eger 1963, as the lowest isoflurane alveolar (end-tidal) concentration required by an individual goat to prevent gross purposeful movement in response to a supramaximal stimulus, which in this study was claw-clamping using a Vulsellum forceps. The null hypothesis that propofol does not affect isoflurane MAC was tested against the alternative hypothesis that propofol reduces isoflurane MAC in goats in a dose-dependent manner.

5.3 MATERIALS AND METHODS

5.3.1 EXPERIMENTAL DESIGN AND INSTRUMENTATION

Six clinically healthy goats (3 does and 3 wethers) were used in this study. The goats were assigned to three treatments, with order of treatment randomized in a cross-over pattern, with a four-week washout period between treatments. General anaesthesia was achieved initially with isoflurane only and then later with isoflurane combined with a constant rate infusion of low dose propofol (Treatment LPROP), moderate dose propofol (Treatment MPROP) or high dose propofol (Treatment HPROP). Median (interquartile range) age was 19.0 (17.5-19.0) months for Treatment LPROP, 18.0 (18.0-18.0) months for Treatment MPROP and 18.0 (17.3-18.8) months for Treatment HPROP while weight was 42.2 (36.6-45.5) kg for Treatment LPROP, 41.1 (37.3-43.1) kg for Treatment MPROP and

42.3 (35.2-43.6) kg for Treatment HPROP. Health status was assessed by physical examination, a complete blood count and serum biochemical analysis; all findings were normal.

Food and water were withheld for 16–22 hours before anaesthesia. The goats were weighed 30 minutes before the experiment. Baseline rectal temperature measured by a digital thermometer, heart rate measured by thoracic auscultation and respiratory rate were recorded before the goats were placed on a custom-made sling-cum-table for easier restraint. The auricular artery on the right ear was catheterized using a 24-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) which was then connected to a calibrated transducer (DTX Plus transducer, BD Medical, Johannesburg, South Africa) for measurement of systolic, diastolic and mean arterial blood pressures. The blood pressure readings were obtained from a calibrated electronic strain gauge transducer connected to a multi-parameter monitor (Cardiocap/5, Datex-Ohmeda Corporation, Helsinki, Finland), which had been calibrated against a mercury column within 2 months of commencement of the study. For transducer calibration to atmospheric pressure, the scapulo-humeral joint or the point of the sternum were used as zero reference points in sternally-recumbent or laterally-recumbent goats, respectively. An 18-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) was introduced into the right cephalic vein for administration of intravenous fluids and propofol. Another 18-SWG catheter was placed in the right jugular vein for collection of venous blood samples for determination of propofol plasma concentration.

Mask induction of the goats with isoflurane (Forane® Liquid, Abbott Laboratories Pty Ltd, Constantia Kloof, South Africa) delivered in oxygen from a circle anaesthetic breathing system with a calibrated Tec 3 out-of-circle vaporiser (Fluotec 3®, BOC Health Care, West Yorkshire, England) was achieved with the goats restrained in sternal position. A tight-fitting facemask was used to limit dead space and gas leaks around the mask. Each goat was accustomed to the mask by initially being allowed to breathe 100% volume oxygen at 6 L minute^{-1} for at least one minute before isoflurane

administration rate was slowly begun with 0.5% volume increments every 15 seconds until a 3.5% volume vaporizer setting was reached. This vaporizer setting was then maintained until the jaw was relaxed enough to allow intubation. Placement of the endotracheal tube (silicone tube, internal diameter 7.5 mm) was done with the goats in sternal recumbency using a laryngoscope to facilitate the process. If intubation was not successful due to poor relaxation of the jaws, isoflurane delivery by facemask was continued before attempting again. The cuff of the endotracheal tube was inflated to prevent leakage of gases from the breathing circuit to a pressure of 20 cmH_20.

Immediately after intubation, the goats were placed in left lateral recumbency with fresh oxygen flow set at 2 L $minute^{-1}$ and initial end-tidal isoflurane concentration targeted to be 1.6% volume. Intermittent positive pressure ventilation (Ohmeda 7000 Ventilator, Ohmeda, Madison, Wisconsin, USA) was used to maintain end-tidal carbon dioxide between 35 – 45 mmHg throughout the procedure. Ringer Lactate solution (Intramed Ringer-Lactate® Fresenius, Bodene Pty Ltd, trading as Intramed, Port Elizabeth, South Africa) was administered by a pump (Infusomat, BBraun, Melsungen, Germany) at a rate of 4 mL kg^{-1} $hour^{-1}$ intravenously.

Instrumentation for recording of physiological parameters was set up using a multi-parameter monitor (Cardiocarp/5, Datex-Ohmeda Corporation, Helsinki, Finland). Three electrocardiography (ECG) electrodes were placed on shaven areas (on the middle of the left shoulder, on the midline 2 cm in front of the point of the sternum and on the midline 2 cm cranial to the tip of the xiphoid) to provide a lead II ECG tracing. Haemoglobin oxygen saturation (SpO_2) was measured via a pulseoximetry infrared probe placed around the tongue, which calculated heart rate as well. Inspired and expired concentrations of isoflurane, carbon dioxide and oxygen were obtained from a flow sensor and a side-stream gas sampler placed between the endotracheal tube and the Y-piece of the breathing system. The flow rate through the gas sampling line was constant at 200 mL $minute^{-1}$. Respiratory rate was calculated from the capnogram. The gas analyzer had been calibrated with calibration gas as

recommended by the manufacturer within 2 months of commencement of the studies and would automatically self-calibrate to atmospheric air at the beginning of the experiment. Temperature was measured by an oesophageal probe place as close to the base of the heart as possible. This was done by marking from outside how far the temperature probe had to be placed to reach the point of the elbow. We targeted to maintain oesophageal temperature between 37.5 and 39.5ºC using a forced warmed air blanket and ordinary blankets placed around the goats. The physiological parameters were measured continuously during the anaesthetic period, but recorded every 15 minutes.

Determination of the baseline isoflurane (control) MAC began 15 minutes after end-tidal isoflurane concentration had been held constant at 1.6%. Isoflurane MAC determination involved application of a noxious stimulus with a Vulsellum forceps clamped to the second ratchet to the claw about 1 cm below the coronary band for 60 seconds or until occurrence of purposeful movement. Purposeful movement was strictly defined as gross movement of the head or limbs, including movement of the limb to which the Vulsellum forceps was being applied. End-tidal isoflurane concentration was then adjusted according to response to noxious stimulation. If no movement occurred, the end-tidal isoflurane concentration was reduced by a tenth (approximately 10% of its value) and held constant for at least 15 minutes before application of a noxious stimulus again. If movement was noticed, the end-tidal isoflurane concentration was increased by a tenth and held constant for at least 15 minutes before application of a noxious stimulus again. The four claws on the two uppermost limbs were clamped consecutively in a clockwise fashion. Isoflurane MAC was calculated as the average of two successive concentrations; the end-tidal isoflurane concentration at which movement in response to noxious stimulation occurred and the preceeding end-tidal isoflurane concentration at which movement did not occur. The isoflurane MAC was determined in duplicate and the mean of the two MACs was taken as baseline isoflurane MAC.

Following baseline MAC determination, the goats then received a bolus dose of propofol administered manually over a 1 minute period; at 0.5 mg kg^{-1}, 1.0 mg kg^{-1}, or 2.0 mg kg^{-1} intravenously; followed by a maintenance dose of; 0.05 mg kg^{-1} $minute^{-1}$, 0.1 mg kg^{-1} $minute^{-1}$, or 0.2 mg kg^{-1} $minute^{-1}$ as Treatment LPROP, MPROP and HPROP, respectively. Propofol for maintenance of general anaesthesia was administered by CRI from a 60 mL syringe controlled by a syringe-driving pump (Perfusor Compact, BBraun, Melsungen, Germany). The propofol syringe was connected to the right cephalic vein catheter, to which the Ringer Lactate administration line was also connected, by an extension tube via a three-way stopcock. The propofol loading dose was administered over a period of 1 minute and administration of the maintenance dose started directly afterwards. The accuracy of delivery of propofol by the pump was checked at the end of the experiment by calculating the expected infused amount based on infusion rates and comparing this to actual volume infused from the syringe.

Isoflurane MAC following propofol-treatment was then determined by applying a noxious stimulus with a Vulsellum forceps after every 15 minutes of end-tidal isoflurane concentration equilibration, and depending on the goat's response, adjusting the end-tidal isoflurane concentration in the same manner as described above. Response to propofol treatment for each goat was defined as the difference between baseline and propofol-treatment isoflurane MAC.

Since baseline isoflurane MAC was determined each time before a goat underwent one of the three propofol treatments, the final baseline isoflurane MAC for each goat was calculated as the average of the three baseline MAC values obtained. There was no need to adjust the isoflurane end-tidal concentrations obtained to atmospheric pressure as the gas module used for measuring respiratory gas concentrations had a sensor that constantly measured atmospheric pressure and adjusted respiratory gas readings as if they were measured at one atmospheric pressure.

Venous blood samples (4.5 mL) were collected via the right jugular vein catheter in heparinised tubes (BD Vacutainer® Systems, Plymouth, United Kingdom) for determination of propofol plasma concentration at 0, 1, 5, 15, 30 and every 30 minutes from the time of propofol bolus administration until the propofol-treatment isoflurane MAC had been determined. The blood samples were centrifuged at 2 500 revolutions per minute for 15 minutes after which plasma was collected and stored at –20 ºCelsius for propofol concentration analysis later.

After determination of propofol-treatment isoflurane MAC, administration of propofol and isoflurane was discontinued and the quality of recovery from anaesthesia of the goats observed. The endotracheal tube was removed once the goats regained the swallowing reflex. Time to extubation, sternal position and standing were recorded. All times were determined as the interval between the time of discontinuation of propofol and isoflurane administration and the time a particular event happened.

Quality of recovery from anaesthesia was scored on a 0 – 2 scale where: 0 = restlessness, 1 = relatively smooth, with some restlessness, 2 = smooth.

5.3.2 PROPOFOL PLASMA CONCENTRATION ANALYSIS

High performance liquid chromatography (HPLC) grade methanol (400 µL) containing 100 µg L^{-1} of thymol (internal standard) was added to 100 µL of thawed and centrifuged plasma. Each sample was then vortex-mixed at maximum speed for 30 seconds. The samples were then sonicated in ultrasonic bath for 10 minutes, following which the samples were again vortexed at maximum speed for 30 seconds and then centrifuged. A volume of the supernatant was transferred to auto-sampler vials from which 15 µL was drawn for analysis. Plasma propofol concentrations were determined by a HPLC flourometric method as described by Vree and colleagues (Vree et al. 1999). Separation and quantification were performed using a Shimatzu HPLC system consisting of a SIL-20AHT auto-sampler,

a LC-20AB UFLC pump with a DGU-20AS de-gasser and an RF-10AXL fluorescence detector. The analytical column was an Altech Apollo C18, 150 x 4.6mm column with a 5 µm particle size with a 4.0 x 2.0 mm Phenomenex Gemini C18 guard column run under isocratic flow of 80% HPLC gradient grade methanol at a flow rate of 1.0 mL mimute^{-1}. Calibration was performed using a range of 0.25 – 25.00 µg mL^{-1}. Linear regression was performed using Y = aX + c, where a = 0.4429666 and c = -0.3939823. The linearity was measured using a correlation coefficient which was r^2 = 0.9914810.
The propofol plasma concentration at the time of propofol-treatment isoflurane MAC determination was calculated as an average of the propofol concentration of the sample obtained immediately prior to propofol-treatment isoflurane MAC determination and that of the sample obtained immediately after propofol-treatment isoflurane MAC determination.

5.3.3 STATISTICAL ANALYSIS

Data were analysed using the R® Statistical Software, Version 2.7.2 (The R Foundation for Statistical Computing, Vienna, Austria). All data were assumed to be non-parametric because of the small sample size and are expressed as median and inter-quartile ranges.

Data on isoflurane MAC, isoflurane MAC reduction after propofol treatment, isoflurane MAC determination time, time to extubation, time to sternal position, time to standing, and recovery scores were tested for statistically significant differences between treatments using the Friedman test. If statistically significant differences were found between treatments, post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted. Correlation between isoflurane MAC and plasma propofol concentration at time of MAC determination was tested using the Spearman rank correlation test. The linear relationship between median isoflurane MAC and median propofol plasma concentrations at time of isoflurane MAC determination was determine using simple linear regression method.

Medians of repeatedly measured variables (heart rate, mean arterial blood pressure, SpO_2 and body temperature) were tested for statistically significant differences between and within treatments using repeated measures analysis of variance (ANOVA) by ranks. If statistically significant differences were found, a post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted. A value of $p < 0.05$ was considered significant.

5.4 RESULTS

Mask induction of anaesthesia using isoflurane was satisfactorily achieved in about 10 minutes with minimal struggling of the goats throughout the induction period. Data on observed isoflurane MACs, changes in isoflurane MAC after treatment with propofol and the time it took to determine isoflurane MAC are summarized in Table 5.1.

The propofol-treatment isoflurane MAC values observed in this study were statistically significantly lower than the baseline isoflurane MAC of 1.37 (1.36-1.37)% volume ($P \leq 0.03$). The propofol-treatment isoflurane MAC values observed in all groups were all statistically significantly different from each other (Figure 5.1).

The percentage reductions in isoflurane MAC after intravenous administration of propofol were 16.4 (16.1-16.4)%, 34.7 (32.3-36.3)% and 59.7 (57.4-64.3)% following Treatment LPROP, MPROP and HPROP respectively. The differences in MAC reduction were all statistically significantly different from each other (Figure 5.2).

The time taken to determine baseline isoflurane MAC, 65.0 (65.0-65.0) minutes, was statistically significantly different from that of determining propofol-treatment isoflurane MAC following

Treatment MPROP (P = 0.022) and Treatment HPROP (P = 0.025), but was not statistically significantly different from 60.0 minutes it took to determine propofol-treatment isoflurane MAC following Treatment LPROP.

The trends in plasma propofol concentrations following its intravenous administration as a bolus followed by continuous rate infusion (CRI) at three different dose regimens are shown in Table 5.2. Median plasma propofol concentration following propofol bolus administration peaked after 1 minute to 3.1 (2.6-3.9) μg mL^{-1} during Treatment LPROP, 6.5 (5.0-9.9) μg mL^{-1} during Treatment MPROP and 21.1 (16.4-24.9) μg mL^{-1} during Treatment HPROP. Median plasma propofol concentration at the time of propofol-treatment isoflurane MAC determination was calculated to be 1.6 (1.2-1.8) μg mL^{-1} for Treatment LPROP, 2.5 (2.3-3.0) μg mL^{-1} for Treatment MPROP and 7.8 (7.3-8.4) μg mL^{-1} for Treatment HPROP. Peak plasma propofol concentration and plasma propofol concentration at the time of propofol-treatment isoflurane MAC determination during Treatment HPROP showed statistically significant differences from those of Treatment LPROP and Treatment MPROP.

Figure 5.3 illustrates that propofol concentrations at time of propofol-treatment isoflurane MAC determination (χ) and the corresponding isoflurane MACs (У) observed in this study indicates a strong correlation (rho = 0.91), characterized by a linear relationship whose best-fit equation was:

$$У = 1.273 - 0.096χ$$

The relationship between plasma propofol concentrations at time of propofol-treatment isoflurane MAC determination and corresponding isoflurane MACs observed in this study shows that propofol reduces isoflurane MAC in a dose-dependent manner.

The data obtained for cardiovascular system and respiratory system variables did not show any statistically significant differences between groups or between the baseline reading and any subsequent points within a group. Median mean arterial blood pressure (MAP) was above 60 mmHg at all recorded time points except at 2 minutes after administration of dose propofol when a median MAP of 56 mmHg was observed. The median SpO_2 stayed above 90% all the time. The median end-tidal carbon dioxide concentration was successfully maintained within the normal range of 35–45 mmHg by manipulating respiratory rate and intrathoracic pressure (tidal volume) settings on the mechanical ventilator. The median oesophageal temperature of the goats was maintained within 38.2–39.1ºC and there were no statistically significant differences between or within groups (Table 5.3).

The quality of recovery from anaesthesia was good following all propofol treatments. The variables used to assess quality of recovery from anaesthesia did not show any statistically significant differences between treatments (Table 5.4). The movements observed in the goats in response to claw-clamping ranged from subtle flexion of joints of the limbs, attempts to lift head up and subtle attempts at chewing the endotracheal tube. At no point did any goat spontaneously awake from general anaesthesia before the scheduled end of an experiment. The goats did not show any signs of lameness following recovery from anaesthesia, leading to the assumption that there was no residual pain from clamping the claws.

5.4.1 TABLES

Table 5.1 Median (inter-quartile range) of isoflurane MAC and related parameters after intravenous administration of propofol: 0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg $kg^{-1}min^{-1}$ (LPROP Treatment), 1.0 mg kg^{-1}g bolus followed by continuous infusion at 0.1 mg $kg^{-1}min^{-1}$ (MPROP Treatment), or 2.0 mg kg^{-1} bolus followed by continuous infusion at 0.2 mg $kg^{-1}min^{-1}$ (HPROP Treatment) in goats.

Treatment	**Isoflurane MAC (%vol)**	**Change post-treatment (%)**	**Time (minutes)[a]**
Control	1.37 (1.36-1.37)[*]	Not applicable	65.0 (65.0-65.0)
LPROP	1.15 (1.08-1.15)[*]	-16.4 (16.1-16.4)[*]	60.0 (60.0-71.3)
MPROP	0.90(0.87-0.93)[*]	-34.7 (32.3-36.3)[*]	75.0 (75.0-75.0)[#]
HPROP	0.55 (0.49-0.58)[*]	-59.7 (57.4-64.3)[*]	112.5 (105.0-120.0)[*]

[*] : statistically significantly different (P <0.05) from other three treatments
[#] : statistically significantly different (P <0.05) from LPROP treatment
[a] : indicates time taken to determine isoflurane MAC following beginning of respective treatment

Table 5.2 Plasma propofol concentrations (µg mL^{-1}) [expressed as median (inter-quartile range)] observed after intravenous administration of propofol: 0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg $kg^{-1}min^{-1}$ (LPROP Treatment), 1.0 mg $kg^{-1}g$ bolus followed by continuous infusion at 0.1 mg $kg^{-1}min^{-1}$ (MPROP Treatment), or 2.0 mg kg^{-1} bolus followed by continuous infusion at 0.2 mg $kg^{-1}min^{-1}$ (HPROP Treatment) in goats.

Treatment	Time (minutes)						
	Baseline	1	5	15	30	60	90	120
LPROP	0 (0-0)	3.1 (2.6-3.9) *	2.0 (1.8-2.1) *	1.5 (1.3-1.7) *	1.6 (1.3-1.8) *	1.7 (1.2-1.9) *	-	
MPROP	0 (0-0)	6.5 (5.0-9.9) *	3.1 (2.8-4.0) *	3.0 (2.8-3.3) *	2.5 (2.1-2.8) *	2.3 (1.6-2.8) *	3.0 (2.7-3.4) #	-
HPROP	0 (0-0)	21.1 (16.4-24.9) *	9.7 (8.6-10.9) *	8.6 (6.9-9.2) *	8.2 (7.8-8.5) *	8.4 (7.4-8.8) *	8.1 (7.8-8.5)	7.2 (6.4-8.0)

* : statistically significantly different (P <0.05) from other two treatments
# : statistically significantly different (P <0.05) from HPROP treatment

Table 5.3 Physiological parameters [median (inter-quartile range)] observed following intravenous administration of propofol: 0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg $kg^{-1}min^{-1}$ (LPROP Treatment), 1.0 mg $kg^{-1}g$ bolus followed by continuous infusion at 0.1 mg $kg^{-1}min^{-1}$ (MPROP Treatment), or 2.0 mg kg^{-1} bolus followed by continuous infusion at 0.2 mg $kg^{-1}min^{-1}$ (HPROP Treatment) in isoflurane-anaesthetised goats.

Variable	Unit	Treatment	Time	Period of Baseline Isoflurane MAC Determination (minutes)				Period of Propofol-treatment Isoflurane MAC Determination (minutes)				
			Baseline	2	15	30	45	2	15	30	45	60
Heart Rate	beats min^{-1}	LPROP	86 (73-102)	88 (86-108)	86 (79-101)	88 (81-94)	87 (81-92)	100 (82-118)	95 (80-112)	92 (81-110)	95 (82-116)	103 (87-121)
		MPROP	82 (74-84)	72 (70-80)	71 (70-80)	72 (70-85)	77 (70-89)	81 (70-92)	85 (77-88)	81 (74-83)	81 (77-89)	89 (76-101)
		HPROP	80 (80-83)	74 (73-80)	75 (74-78)	74 (72-81)	76 (69-80)	67 (64-72)	74 (71-86)	72 (68-74)	74 (72-77)	76 (72-76)
SAP	mmHg	LPROP	115 (107-121)	100 (87-114)	100 (87-110)	102 (88-108)	103 (91-106)	106 (93-118)	102 (92-110)	99 (94-108)	92 (90-105)	101 (99-110)
		MPROP	109 (103-124)	86 (81-92)	87 (82-97)	89 (86-97)	94 (89-97)	87 (81-93)	96 (87-103)	86 (85-89)	97 (91-110)	95 (92-105)
		HPROP	110 (100-120)	88 (78-91)	86 (83-88)	82 (78-91)	86 (82-91)	73 (69-79)	84 (76-89)	85 (77-97)	95 (81-104)	107 (88-114)
DAP	mmHg	LPROP	77 (71-87)	62 (56-82)	70 (57-83)	71 (59-81)	71 (64-77)	75 (62-95)	76 (66-87)	76 (72-87)	74 (68-84)	85 (76-88)
		MPROP	80 (74-92)	52 (48-63)	53 (52-63)	61 (57-64)	61 (54-68)	62 (65-67)	72 (62-79)	56 (53-68)	74 (66-83)	77 (75-79)
		HPROP	75 (69-80)	60 (47-63)	59 (47-59)	59 (49-68)	59 (51-66)	44 (42-49)	56 (53-65)	62 (48-74)	72 (53-80)	82 (60-90)
MAP	mmHg	LPROP	96 (85-104)	75 (71-96)	80 (71-94)	82 (69-93)	85 (74-90)	86 (74-105)	86 (75-96)	84 (81-94)	81 (76-92)	92 (84-97)
		MPROP	92 (86-109)	63 (59-65)	67 (65-76)	74 (67-77)	78 (69-84)	71 (64-79)	82 (71-89)	67 (65-76)	83 (75-95)	85 (84-88)
		HPROP	92 (83-95)	70 (58-72)	68 (58-72)	68 (59-77)	68 (62-76)	63 (59-65)	56 (53-65)	71 (55-83)	83 (61-91)	94 (68-101)
SpO_2	%	LPROP	-	99 (98-99)	98 (97-99)	98 (97-99)	98 (98-99)	98 (98-98)	98 (98-98)	98 (98-98)	98 (97-99)	98 (97-98)
		MPROP	-	99 (98-99)	98 (95-99)	97 (97-98)	97 (96-99)	97 (97-99)	98 (98-98)	98 (98-98)	98 (97-99)	98 (96-99)
		HPROP	-	99 (97-99)	97 (96-99)	97 (95-99)	97 (96-99)	97 (96-97)	97 (96-98)	97 (97-98)	97 (96-98)	97 (97-98)
$PE´CO_2$	mmHg	LPROP	-	38.3 (36.9-41.3)	40.5 (36.6-44.3)	39.8 (36.6-42.6)	41.7 (38.3-43.9)	44.3 (39.8-45.4)	43.9 (41.3-44.8)	44.6 (43.7-46.7)	42.8 (40.5-44.1)	42.3 (41.3-44.1)
		MPROP	-	41.8 (37.8-42.9)	41.3 (40.5-42.8)	42.6 (38.6-44.8)	43.7 (42.2-44.7)	42.6 (40.5-45.4)	42.9 (41.8-45.8)	42.2 (39.8-44.8)	42.6 (41.2-45.0)	40.5 (38.0-43.3)
		HPROP	-	40.5 (39.8-42.8)	41.8 (39.8-43.3)	40.5 (40.3-42.8)	40.5 (37.2-41.3)	38.0 (38.0-40.9)	37.2 (36.1-37.8)	37.2 (35.9-38.6)	36.5 (35.9-37.6)	39.8 (36.9-42.2)
Temp	(ºC)	LPROP	39.0 (38.9-39.1)	39.0 (38.9-39.1)	38.4 (38.3-38.8)	38.4 (38.2-38.8)	38.4 (38.2-38.8)	38.4 (38.2-38.8)	38.3 (38.2-38.7)	38.4 (38.1-38.7)	38.4 (38.2-38.6)	38.5 (38.2-38.7)
		MPROP	39.0 (38.8-39.2)	38.6 (38.5-38.8)	38.6 (38.4-38.8)	38.5 (38.3-38.7)	38.5 (38.3-38.7)	38.4 (38.3-38.4)	38.2 (38.0-38.4)	38.2 (38.0-38.3)	38.1 (38.0-38.2)	38.2 (38.1-38.3)
		HPROP	38.7 (38.6-38.9)	38.6 (38.4-38.7)	38.6 (38.4-38.7)	38.6 (38.3-38.7)	38.6 (38.4-38.7)	38.5 (38.4-38.7)	38.5 (38.2-38.6)	38.4 (38.2-38.5)	38.3 (38.1-38.6)	38.3 (38.1-38.6)

Note: No statistically significant differences ($P<0.05$) between or within groups.
SAP- systolic arterial pressure; DAP- diastolic arterial pressure; MAP- mean arterial pressure; SpO_2- saturation of haemoglobin with oxygen in peripheral blood; $PE´CO_2$- end-tidal carbon dioxide partial pressure; Temp- body temperature.

Table 5.4 Quality of recovery from anaesthesia [median (inter-quartile range)] observed in a study where the effects of intravenously administered propofol: 0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg $kg^{-1}min^{-1}$ (LPROP Treatment), 1.0 mg $kg^{-1}g$ bolus followed by continuous infusion at 0.1 mg $kg^{-1}min^{-1}$ (MPROP Treatment), or 2.0 mg kg^{-1} bolus followed by continuous infusion at 0.2 mg $kg^{-1}min^{-1}$ (HPROP Treatment) on the MAC of isoflurane in goats were investigated.

Treatment	Time to Extubation (minutes)	Time to Sternal Position (minutes)	Time to Standing (minutes)	Recovery Score
LPROP	2.5 (1.3-3.0)	4.0 (2.3-5.0)	6.0 (5.0-7.0)	2 (2-2)
MPROP	2.0 (2.0-2.8)	2.0 (2.0-2.8)	5.0 (3.5-8.0)	2 (2-2)
HPROP	2.5 (2.0-3.0)	2.5 (2.0-3.0)	5.0 (5.0-7.3)	2 (2-2)

Note: No statistically significant differences (P<0.05) between any treatments

5.4.2 FIGURES

Figure 5.1 Box-and-Whiskers plot of median isoflurane MAC (% volume) observed in isoflurane anaesthetised goats (Control) and after intravenous administration of propofol (LPROP Treatment, MPROP Treatment, or HPROP Treatment) to isoflurane-anaesthetised goats. Each box represents data from the 25th to the 75th percentiles, the bold line represents the median value, and the whiskers represent the range of scores, while the small dots outside the box represent outliers. Also highlighted are the *P*-values obtained when the isoflurane MAC were compared statistically between treatments.

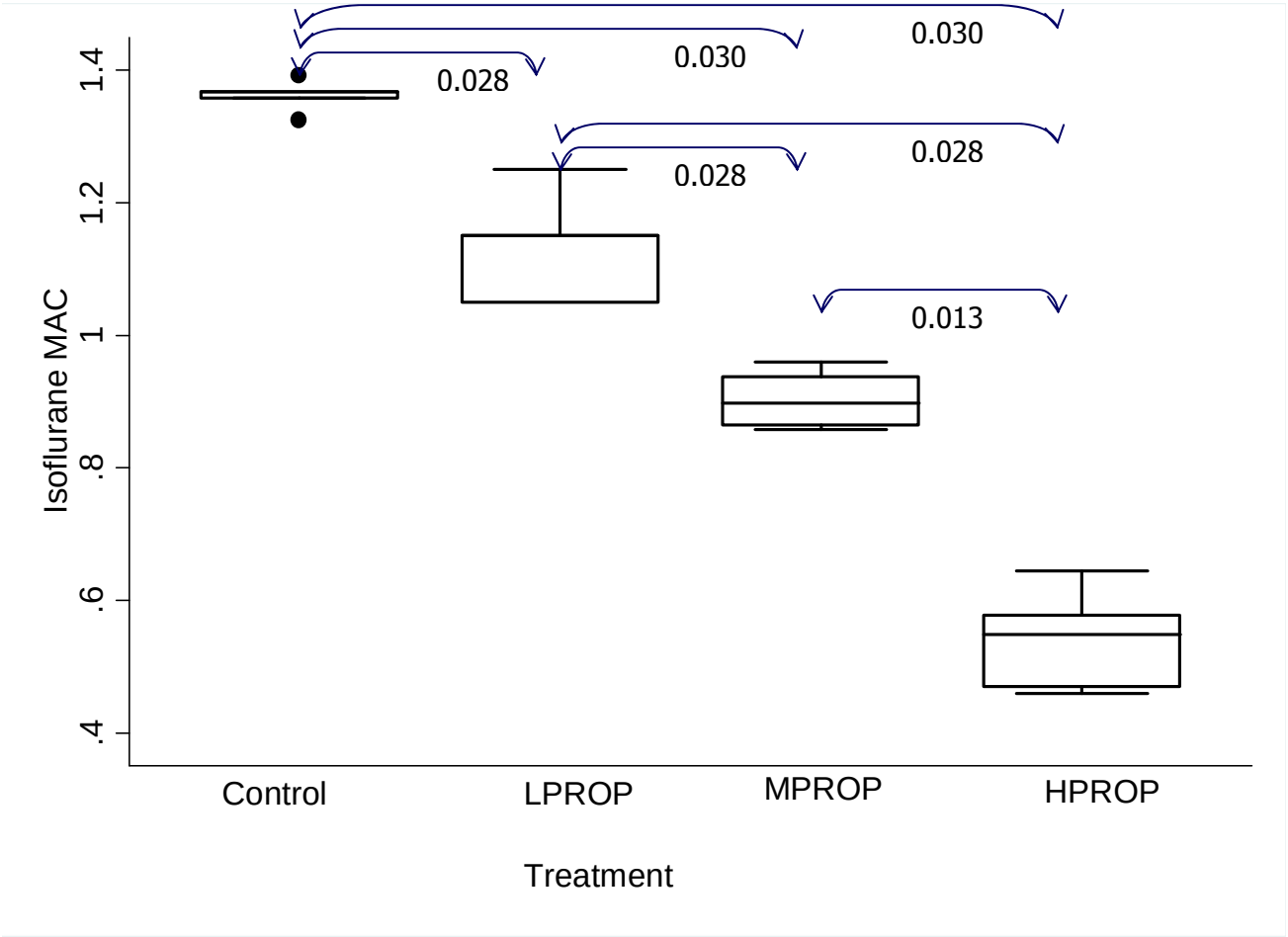


Figure 5.2 Percentage change in isoflurane MAC observed in isoflurane anaesthetised goats (Control) and after intravenous administration of midazolam (LFENT Treatment, MFENT Treatment, or HFENT Treatment) to isoflurane-anaesthetised goats. Also highlighted are the *P*-values obtained when the change in isoflurane MAC following propofol treatement were compared statistically between treatments.

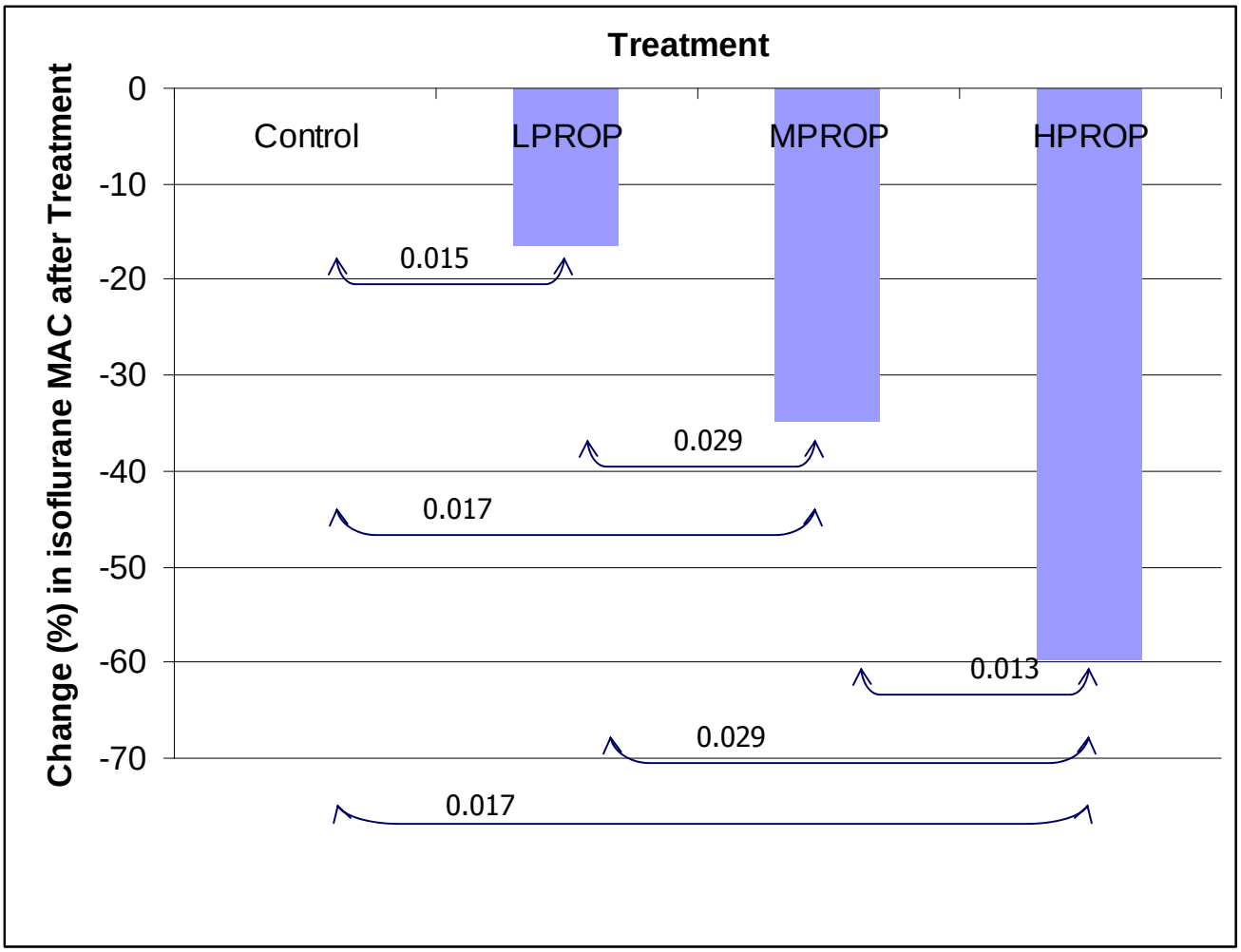
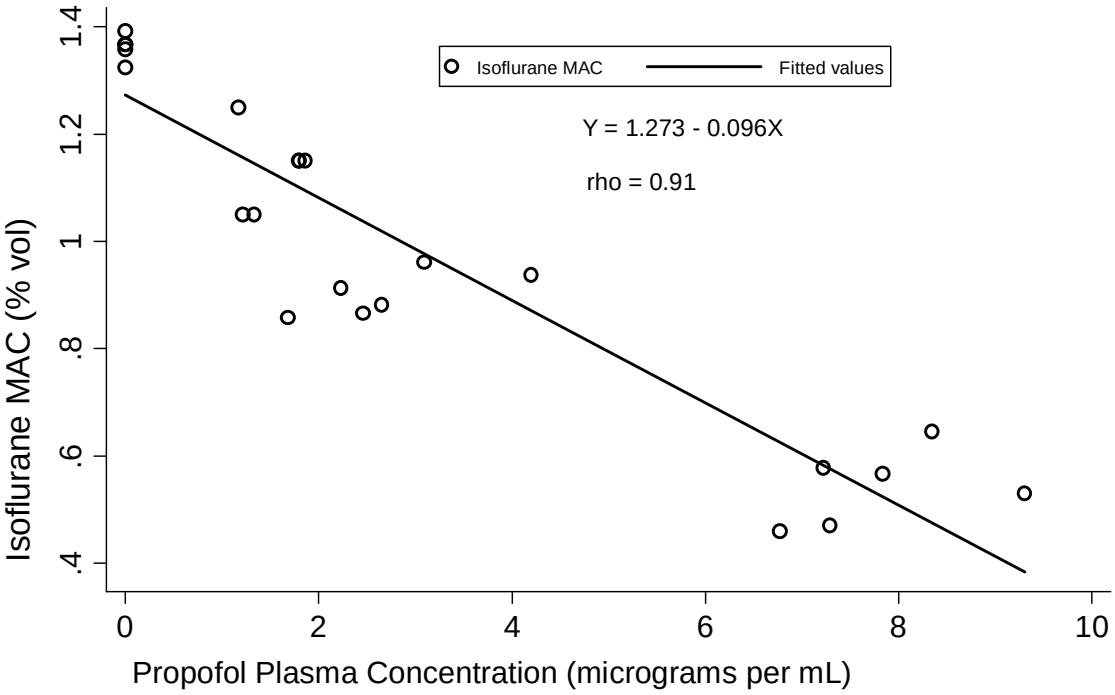


Figure 5.3 Plot of median isoflurane MAC against median plasma propofol concentration at time of propofol-treatment isoflurane MAC determination for individual goat treatments following intravenous administration of propofol: 0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg $kg^{-1}min^{-1}$, 1.0 mg $kg^{-1}g$ bolus followed by continuous infusion at 0.1 mg $kg^{-1}min^{-1}$, or 2.0 mg kg^{-1} bolus followed by continuous infusion at 0.2 mg $kg^{-1}min^{-1}$ in goats.



5.5 DISCUSSION

The median isoflurane MAC in goats of 1.37 (1.36-1.37)% volume obtained in the present study closely resembles those in literature. Studies by different research teams have reported isoflurane MAC values in goats ranging from 1.23% volume to 1.50% volume (Antognini & Eisele 1993, Hikasa et al. 1998; Doherty et al. 2002a; Doherty et al. 2002b). There are several reasons for variations in minimum alveolar concentrations among different studies, including physiological factors like breed, sex, body temperature, blood pressure and tissue oxygenation status as well as other factors like method of testing (type of noxious stimuli), subjectivity in interpretation of response to method of testing, differences in anatomical area of stimulus application (Stanski, 2000; Wilson et al. 2008). Various other types of noxious stimuli such as tail clamping, paw pressure or nerve stimulation have been applied to determine MAC (Levionnois et al. 2009). Of these types of noxious stimuli, electrical stimulation is the best as it can be applied at a consistent intensity, is totally reversible and maintains an intact neurophysiology and tissue integrity (Le Bars et al. 2001). Variation in this study was minimized by use of a single observer to test response to stimulus, use of one anatomical structure (claw) as test site and provision of cardiopulmonary support (intravenous fluids for maintaining blood pressure, artificial ventilation with oxygen supplementation for maintaining adequate tissue oxygenation and temperature support). In addition the baseline (control) isoflurane MAC and the propofol-treatment isoflurane MAC for each goat were determined during the same experimental setting with a short time interval, minimizing the impact of variation due to ambient conditions.

The baseline isoflurane MAC value was statistically significantly different from the propofol-treatment isoflurane MAC values at the three propofol infusion rates used in the present study indicating that propofol reduces isoflurane MAC in goats. The degree of reduction in isoflurane MAC by propofol was dose-dependent as shown by the reduction in isoflurane MAC by 16.4%, 34.7% and

59.7% following LPROP, MPROP and HPROP treatment respectively. Propofol, like isoflurane, has been reported to directly depress dorsal horn neuronal responses to noxious mechanical stimulation (Antognini et al. 2000c).

In the present study median plasma propofol concentration following administration of the highest dose of propofol (bolus of 2.0 mg kg^{-1} followed by a maintenance dose of 0.2 mg kg^{-1} $minute^{-1}$) was 7.8 µg mL^{-}. This plasma propofol concentration caused a reduction in isoflurane MAC of about 60%. There are pharmacokinetic studies on administration of a single intravenous dose of propofol in goats in literature (Bettschart-Wolfensberger et al. 2000; Pablo et al. 1997), but none following administration of propofol by CRI in goats. In sheep, plasma propofol concentrations were reported to be 2.0 – 4.7 µg mL^{-} following administration of propofol by CRI at 0.2 mg kg^{-1} $minute^{-1}$ together with ketamine at 0.1 mg kg^{-1} $minute^{-1}$ (Correia et al. 1996), which is lower than propofol plasma concentrations obtained in this study. Species differences in metabolism of propofol or differences in pharmacological interactions of the drugs involved may have caused the differences in propofol plasma concentrations. In ponies, plasma propofol concentrations were reported to be within a range (2.3 – 6.5 µg mL^{-}), which is close to the concentrations reported in the present study, following administration of propofol by CRI at 0.2 mg kg^{-1} $minute^{-1}$ (Nolan et al. 1996). In dogs, the plasma propofol concentration achieved when propofol was administered on its own at 0.4 mg kg^{-1} $minute^{-1}$ were reported to be within a range 3.77 to 5.84 µg mL^{-} (Nolan & Reid 1993). In a target controlled infusion (TCI) study in dogs, plasma concentrations required for surgical anaesthesia ranged from 3.5 to 7.0 µg mL^{-} (Beths et al. 2001). It is apparent that the present study observed relatively higher plasma propofol concentrations, but not correspondingly higher anaesthetic effects when compared to other species. The plasma concentration of propofol that caused only 60% reduction in isoflurane MAC in this study is higher than the range reported for full anaesthesia in dogs. The reasons why apparently higher plasma propofol concentrations observed in this study produced relatively less

isoflurane MAC reduction when compared to sheep and dogs cannot be determined from this study and requires further investigation.

The strong correlation between the decrease in isoflurane MAC and the rise in plasma propofol concentration in this study further supports the fact that propofol reduces isoflurane MAC in a dose-dependent manner. From the linear relation illustrated in Figure 2, propofol and isoflurane interact additively with respect to suppression of movement in response to a supramaximal stimulus in goats. In 2008, Hendrickx and colleagues demonstrated that interaction plots that form straight lines indicate an additivity type of drug interaction. Both propofol and isoflurane have been reported to cause anaesthetic effects due to interaction with γ-aminobutyric acid (GABA) receptor sites (Hui et al. 1995; Hendrickx et al. 2008). Thus perhaps, the finding of additivity is not surprising since both drugs have effects on similar receptor sites. No previous study could be found in available literature on the interaction of propofol and isoflurane in goats, but additivity was suggested when propofol was co-administered with sevoflurane, a newer inhalation anaesthetic agent (Harris et al. 2006).

Propofol, administered by CRI at low dosages together with isoflurane to mechanically ventilated goats, had minimal impact on cardiovascular function as demonstrated by the results of the present study. The baseline (reference) values for cardiovascular parameters (heart rate, arterial blood pressure and SpO_2) are similar to those observed in anaesthetized goats in other studies (Reid et al. 1993; Bettschart-Wolfensberger et al. 2000; Prassinos et al. 2005; Dzikiti et al. 2009).

The oesophageal temperature of the goats did not decrease by more that 1 ºCelsius in any of the three groups indicating that heat conservation methods employed (covering with ordinary blankets and warming with a warm-air heating blanket) were successful in preventing heat loss. It was important to prevent excessive heat loss from the anaesthetized goats as hypothermia is known to cause a reduction in MAC (Stanski, 2000).

In a number of studies in which propofol was administered alone for general anaesthesia in goats, myoclonic activity was reported as an adverse effect (Pablo et al. 1997; Bettschart-Wolfensberger et al. 2000; Prassinos et al. 2005). The tendency towards myoclonic activity could probably have been absent in the present study due to the fact that propofol was co-administered with isoflurane.

Recovery from isoflurane-propofol anaesthesia was fast and excitement-free all the time as reported in previous publications on propofol anaesthesia in goats (Reid et al. 1993; Prassinos et al. 2005). The short recovery times associated with propofol are mostly due to its pharmacokinetic profile that is characterized by a high volume of distribution, rapid metabolism and a very high clearance rate even when administered as repeated doses or continuous intravenous infusion (Reid et al. 1993; Bettschart-Wolfensberger et al. 2000; Prassinos et al. 2005). Isoflurane is also known to be associated with rapid recovery from anaesthesia in goats (Antognini & Eisele 1993), moreso when used in low dosages as in this study. Rapid recovery from anaesthesia is important in ruminants as they are prone to tympany and regurgitation of ruminal contents which increases the risk of hypoxaemia and aspiration of regurgitated ruminal contents (Correia et al. 1996; Prassinos et al. 2005).

It was concluded that propofol reduced isoflurane MAC in response to claw-clamping in a dose-dependent manner with minimal adverse effects on cardiovacular function in goats. It seems that higher infusion rates of propofol are required to prevent responses to a noxious stimulus in goats when compared to other studied species.

Total intravenous anaesthesia with propofol-fentanyl or propofol-midazolam in spontaneously-breathing goats

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6.1 ABSTRACT

Objective To evaluate and compare the efficacy of propofol and fentanyl, and propofol and midazolam for total intravenous anesthesia in goats.

Study Design Prospective, randomized, crossover experimental study.

Animals Six healthy goats; 3 does and 3 wethers.

Methods Anaesthesia was induced with propofol, one minute after the goats were given either fentanyl 0.02 mg kg^{-1} (Treatment FP) or midazolam 0.3 mg kg^{-1} (Treatment MP) intravenously. Anaesthesia was maintained by constant rate infusion of propofol 12.0 mg kg^{-1} $hour^{-1}$ and fentanyl 0.02 mg kg^{-1} $hour^{-1}$ (Treatment FP) or propofol 12.0 mg kg^{-1} $hour^{-1}$ and midazolam 0.3 mg kg^{-1} $hour^{-1}$ (Treatment MP) for 90 minutes. Depth of anaesthesia was assessed every 10 minutes and the propofol dosage was adjusted to maintain a surgical plane of anaesthesia. Cardiopulmonary parameters were monitored continuously. Arterial blood-gas analysis was performed intermittently. Quality of recovery was scored.

Results There was no significant difference in the propofol induction dose (4.00 and 3.97 mg kg^{-1} following Treatment FP and Treatment MP, respectively). Quality of induction for both treatments was smooth. The propofol dose required for maintenance of general anaesthesia was statistically less ($p = 0.004$) in Treatment FP (12.0 mg kg^{-1} $hour^{-1}$) than in Treatment MP (18.0 mg kg^{-1} $hour^{-1}$). No adverse effects on cardiopulmonary function were observed. Quality of recovery was acceptable in both treatments, but abnormal behavioural signs were observed in Treatment FP.

Conclusions and clinical relevance Total intravenous anaesthesia with propofol and fentanyl or propofol and midazolam, at the doses used in the present study, in spontaneously-breathing, oxygen-supplemented goats is feasible. Recovery from fentanyl-propofol combination might be rough in individual cases.

Keywords goat, propofol, fentany, midazolam, TIVA, surgical anaesthesia

6.2 INTRODUCTION

Use of propofol as the sole agent for TIVA is unsatisfactory, since the dosages required to eliminate responses to surgery might cause significant cardiopulmonary depression (Smith 1994). There is a paucity of information on TIVA in ruminants; however, in goats, propofol in combination with ketamine caused a degree of immobility and cardiopulmonary effects comparable to those associated with sevoflurane anaesthesia (Larenza et al. 2005).

Propofol is the most suitable of the induction drugs for administration as a constant rate infusion (CRI) due to its short context-sensitive half-time (Bettschart-Wolfensberger et al. 2000). Fentanyl, a short-acting μ opioid agonist, is used for treatment of moderate to severe pain in dogs and humans (Carroll et al. 1999). There is very little information in the literature on the use of fentanyl in goats; however, fentanyl has been reported to have a short half-life following intravenous administration to goats, thus necessitating its use by means of CRI (Carroll et al. 1999). Midazolam, a water-soluble benzodiazepine, is used as a sedative, muscle relaxant and an anticonvulsant in human patients (Cao et al. 2002). Midazolam's sedative effects are due to its agonist actions at gamma-aminobutyric acid (GABA) receptors (Cao et al. 2002). In goats, midazolam administered at 0.3 mg kg^{-1} caused clinically significant sedation and a 40% reduction in the dose of propofol required for induction of anaesthesia (Dzikiti et al. 2009).

In the present study, we assessed the anaesthetic efficacy and cardiopulmonary effects of TIVA from propofol co-administered with either fentanyl or midazolam, in goats. We tested the hypothesis that TIVA with either propofol-fentanyl or propofol-midazolam would produce similar anaesthestic and cardiopulmonary effects.

6.3 MATERIALS AND METHODS

6.3.1 EXPERIMENTAL DESIGN AND INSTRUMENTATION

Six, adult, mixed-breed goats (three does and three wethers) were used. The goats were determined to be healthy based on physical examination, a complete blood count and serum biochemical analysis. The age of the goats ranged between 20.0 and 21.0 months while the weight ranged between 39.6 and 46.5 kg. Each goat was studied on two occasions, receiving each treatment in a randomized manner with a four-week washout between treatments. Food and water were withheld for 18–24 hours before anaesthesia.

Baseline rectal temperature was measured by a digital thermometer, and heart rate and respiratory rate were measured by thoracic auscultation for one minute before the goats were placed on a custom-made sling-cum-table for ease of restraint. A 24-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) was inserted into an auricular artery and connected to a calibrated strain gauge transducer (DTX Plus transducer, BD Medical, Johannesburg, South Africa) for measurement of systolic, diastolic and mean arterial pressures. The arterial pressure readings were obtained from a multi-parameter monitor (Cardiocap/5, Datex-Ohmeda Corporation, Helsinki, Finland), which had been calibrated against a mercury column within a month of commencement of the study. For transducer calibration to atmospheric pressure, the scapulo-humeral joint or the point of the sternum were used as zero reference points in sternally-recumbent or laterally-recumbent goats, respectively. An 18-SWG catheter (Jelco, Medex Medical Ltd, Rossendale, Great Britain) was inserted into each cephalic vein for administration of drugs and intravenous fluids, respectively.

Fentanyl, 0.02 mg kg^{-1} or midazolam, 0.3 mg kg^{-1} were administered intravenously over a one minute period. The degree of sedation was assessed one minute later, immediately before the administration of propofol, using a 0 – 2 scale, where 0 = no sedation, 1 = moderate sedation: the

goat assumed sternal recumbency, 2 = heavy sedation: the goat failed to maintain sternal recumbency and unable to hold its head up. Propofol was administered initially as a bolus at 2.0 mg kg^{-1} over 15 seconds and this was followed by incremental dosages at 0.5 mg kg^{-1} every 15 seconds until the goats were judged to be anaesthetized sufficently to allow placement of an endotracheal tube, as determined by presence of a weak palpebral reflex and relaxation of the jaws. Immediately after tracheal intubation, the goats were placed in left lateral recumbency and the dose of propofol required for induction recorded. The goats were then connected to a circle breathing system (Anaesthesia System, Clinicare, Crest Health Technology, Chatham, UK) with an oxygen flow rate of 2 L min^{-1}. The goats breathed spontaneously, but were to be mechanically ventilated if the end-tidal carbon dioxide partial pressure ($PE´CO_2$) increased to 55 mmHg or if the peripheral oxygen saturation (SpO_2), read from an infrared probe (describe) on the tongue, decreased below 90%. Quality of induction was scored using a 0-2 scale where: 0 = excitement, jumps or attempts to stand after becoming recumbent, unable to place orotracheal tube; 1 = slightly prolonged (>2 minutes) induction or mild excitement; 2 = smooth induction, no excitement, orotracheal intubation easy.

A CRI, of propofol (12.0 mg kg^{-1} $hour^{-1}$) with either with fentanyl (0.02 mg kg^{-1} $hour^{-1}$) (Treatment FP) or midazolam (0.3 mg kg^{-1} $hour^{-1}$) (Treatment MP) was started immediately after induction. Propofol for CRI was drawn up to fill a 60 mL syringe; while fentanyl or midazolam were mixed with normal saline to 60 mL in a separate syringe. Syringe-driving pumps (Perfusor Compact, BBraun, Melsungen, Germany) were used to deliver propofol and fentanyl CRIs (Figure 6.1). The accuracy of the pumps was checked before the study by measuring the volume of solution delivered over time into a graduated cylinder. Ringer's lactate was infused at 4 mL kg^{-1} $hour^{-1}$ by a volumetric pump (Infusomat, BBraun, Melsungen, Germany).

Figure 6.1 Syringe-driving pumps (Perfusor Compact, BBraun, Melsungen, Germany) that were used to deliver propofol and fentanyl or midazolam by CRI.

Instrumentation for recording of clinical parameters was set up using a multi-parameter monitor (Cardiocarp/5, Datex-Ohmeda Corporation, Helsinki, Finland). The ECG was monitored continuously using a lead II tracing. A pulse oximeter probe was attached to the tongue to estimate haemoglobin saturation and calculate heart rate. Inspired and expired partial pressures of CO_2 and oxygen were obtained from a side-stream gas sampler connected between the endotracheal tube and the Y-piece of the breathing system. The flow rate through the gas sampling line was 200 mL minute^{-1}. Respiratory rate was calculated from the capnogram. The gas analyzer had been calibrated with calibration gas composed of 3% isoflurane in 5.0% CO_2, 55.0% O_2, 33.0% N_2O and N_2 as balance (Datex-Ohmeda Corporation, Helsinki, Finland) within a month of commencement of the studies and automatically self-calibrated to atmospheric air at the beginning of the experiment. Temperature was

measured by an electronic oesophageal probe placed as close to the base of the heart as possible. This was done by marking how far the temperature probe had to be placed to reach the point of the elbow. Oesophageal temperature was maintained between 37.5 and 39.5ºC using a forced warmed air blanket (Bair Hugger, Augustine Medical, Eden Prairie, USA). Clinical parameters were recorded at 3 and 10 minutes after induction and every 10 minutes thereafter.

Anaesthetic depth was assessed, by the same person, every 10 minutes, immediately after recording the cardiopulmonary parameters, by application of a noxious stimulus. The noxious stimulus consisted of clamping a claw with Vulsellum forceps, closed tightly to the second ratchet, for 60 seconds or until purposeful movement occurred. The four claws on the two uppermost limbs were clamped consecutively in a clockwise fashion. Purposeful movement was defined as gross movement of the head or limbs. If purposeful movement occurred, the propofol infusion rate was increased by 10% and held constant for at least 10 minutes, otherwise, it was decreased by 10% and the stimulus was re-applied. In the event that the goat swallowed or moved spontaneously, a bolus of propofol (1.0 mg kg^{-1}) was to be administered.

Arterial blood samples were collected anaerobically into heparinised 1 mL syringes immediately prior to sedation (baseline), and at 3, 30 and 60 minutes after induction. Syringes were sealed and placed in ice, and analysis occurred within 30 minutes of collection. From these samples, PaO_2, $PaCO_2$, pH and bicarbonate concentration were determined by use of a pre-calibrated machine (Rapidlab ^{TM} 348 Analyser, Siemens Diagnostics, Midrand, South Africa).

Administration of anaesthestic agents was discontinued after 90 minutes and the goats were allowed to recover. The endotracheal tube was removed once the swallowing reflex was regained. Times (minutes) to extubation, sternal recumbency, and standing were recorded. All times were determined as the interval between the termination of anaesthesia and the occurrence of a particular

event. Quality of recovery was scored using a 0 – 2 scale where: 0 = restlessness, 1 = relatively smooth, with some restlessness, 2 = smooth.

6.3.2 STATISTICAL ANALYSIS

Data were analysed using the R statistical software (The R Foundation for Statistical Computing, Vienna, Austria). All data were assumed to be non-parametric because of the small sample size and are expressed as median and inter-quartile ranges.

Data on sedation scores, propofol induction doses, induction scores, propofol doses required for maintenance of anaesthesia, time to extubation, time to sternal position, time to standing and recovery scores were tested for statistical differences between the 2 treatments using the Wilcoxon matched-pairs signed rank test.

Repeatedly measured data (cardiopulmonary parameters and arterial blood gas data) were tested for statistically significant differences between and within treatment groups using repeated measures analysis of variance (ANOVA) by ranks. If statistically significant differences were found, a post-hoc analysis (pair-wise Wilcoxon test with a Bonferroni adjustment for multiple testing) was conducted to identify any two different time points. Two arterial blood sample results (one at baseline and one at 30 minutes after induction) from Treatment MP were considered to be outliers, suggesting contamination of the blood sample with atmospheric air and were therefore excluded from statistical analysis. Exclusion of a data point has the advantage of narrowing the confidence interval, but it negatively impacts on statistical power as it brings in some bias which might lead to a wrong conclusion. In this instance only value is excluded on each of the two time points and hence complete case analysis is not expected to bias the results by a large extend. A value of $P < 0.05$ was considered significant.

6.4 RESULTS

The goats consistently exhibited heavy sedation following intravenous administration of either fentanyl or midazolam. The sedation scores were 2.0 (1.5–2.0) for both groups. There was no significant difference in median induction doses of propofol which were 4.00 (3.96-4.01) and 3.97 (3.91-4.00) mg kg^{-1} for Treatment FP and Treatment MP, respectively. Induction was excitement-free on all occasions (i.e. induction score of 2 for each goat). A median propofol dose of 12.0 mg kg^{-1} $hour^{-1}$ was required for maintenance of anaesthesia for Treatment FP and this was significantly less than ($P = 0.004$) the dose of 18.0 mg kg^{-1} $hour^{-1}$ required for Treatment MP. No additional boluses of propofol were required.

The cardio-respiratory data did not differ significantly between treatments. Respiratory rates were statistically significantly less at all time points when compared to baseline readings in both treatment groups. No goat required artificial ventilation as intervention for hypoventilation ($PE'CO_2$ > 55 mmHg or SpO_2 < 90%). There were no significant differences between or within treatment groups for blood-gas parameters. The oesophageal temperatures of the goats were maintained within the pre-determined range (37.5-39.5ºC) and there were no significant differences between groups at any time point. The temperature readings observed following Treatment FP were significantly less ($P <$ 0.05) than baseline readings from 30 minutes of anaesthesia onwards, and with Treatment MP they were significantly less than baseline readings from 70 minutes of anaesthesia onwards (Table 6.1).

Copius salivation was observed in all goats, and regurgitation was observed in one goat during Treatment FP. Median time to extubation was 3.0 (3.0-3.0) and 4.5 (3.3-5.0) minutes following Treatment FP and Treatment MP, respectively and there were no statistically significant differences between the treatments. Median time to sternal position was 4.5 (3.3-5.0) and 5.0 (5.0-6.5) minutes for Treatment FP and Treatment MP, respectively and there were no statistically significant

differences between the treatments. Median time to standing was 13.0 (10.3-15.0) and 15.0 (11.3-17.3) minutes for Treatment FP and Treatment MP, respectively and there were no statistically significant differences between the treatments. The scores for quality of recovery from anaesthesia were 0.5 (0-2) and 2 (2-2) for Treatment FP and Treatment MP, respectively; however, there was no difference ($P > 0.05$) between groups. Nevertheless, 4 out of 6 times, goats recovering from fentanyl-propofol anaesthesia showed abnormal behavioural signs such as exaggerated tail-wagging, nibbling at surrounding objects and restlessness. There were no significant differences between groups in terms of time to extubation, sternal position and standing.

6.4.1 TABLES

Table 6.1 Physiological parameters [median (inter-quartile range)] during total intravenous anaesthesia with fentanyl and propofol (Treatment FP) or midazolam and propofol (Treatment MP) in goats breathing 100% oxygen.

Variable	Unit	Treatment	Time (minutes)					
			Baseline	3	10	30	60	90
Heart Rate	beats min^{-1}	FP	80 (80-80)	84 (81-92)	80 (67-84)	70 (61-72)	65 (56-67)	58 (56-64)
		MP	74 (72-79)	102 (97-110)	101 (94-105)	97 (87-99)	91 (85-100)	92 (83-100)
SAP	mmHg	FP	124 (116-128)	109 (101-115)	111 (101-117)	104 (100-112)	101 (99-107)	104 (95-105)
		MP	120 (118-121)	123 (108-131)	113 (107-118)	109 (103-110)	109 (96-118)	114 (101-127)
DAP	mmHg	FP	85 (70-91)	77(67-82)	81 (75-84)	77 (69-81)	72 (66-75))	74 (64-80)
		MP	84 (82-92)	98 (73-104)	84 (78-90)	79 (69-84)	80 (68-90)	85 (76-98)
MAP	mmHg	FP	105 (95-110)	92 (84-94)	96 (87-100)	90 (85-95)	84 (80-91)	85 (80-93)
		MP	102 (97-104)	109 (88-116)	98 (92-102)	94 (85-95)	94 (81-102)	99 (88-111)
SpO_2	%	FP	-	99 (98-100)	100 (96-100)	99 (98-100)	100 (100-100)	100 (98-100)
		MP	-	100 (100-100)	100 (99-100)	99 (98-100)	99 (98-99)	99 (98-100)
f_R	b min^{-1}	FP	28 (28-34)	6 (5-7)*	5 (3-6)*	6 (5-10)*	9 (6-12)*	8 (6-9)*
		MP	30 (28-30)	10 (5-17)*	13 (9-14)*	15 (13-16)*	15 (15-17)*	15 (14-18)*
$PE´CO_2$	mmHg	FP	-	33.5 (30.5-35.8)	32.0 (31.0-35.3)	29.5 (29.0-33.8)	31.0 (29.5-33.3)	32.0 (29.8-32.8)
		MP	-	33.5 (30.8-34.8)	33.5 (31.5-35.5)	32.5 (32.0-33.0)	32.5 (32.0-34.5)	31.5 (30.0-35.0)
P_aO_2	mmHg	FP	80 (75-105)	277 (271-288)	-	315 (307-335)	338 (311-356)	-
		MP	83 (82-85)	286 (271-293)	-	300 (277-305)	307 (299-325)	-
P_aCO_2	mmHg	FP	28.8 (24.7-32.3)	42.7 (39.8-44.3)	-	38.7 (36.8-40.3)	38.8 (36.4-42.5)	-
		MP	32.0 (30.3-34.0)	33.9 (33.8-37.6)	-	38.7 (35.1-39.2)	39.8 (38.8-42.3)	-
pH_a		FP	7.47 (7.43-7.48)	7.34 (7.32-7.35)	-	7.38 (7.36-7.39)	7.39 (7.39-7.39)	-
		MP	7.43 (7.43-7.44)	7.36 (7.34-7.38)	-	7.35 (7.34-7.37)	7.36 (7.33-7.38)	-
$[HCO_3^-]$	mmol L^{-1}	FP	20.8 (17.5-21.2)	21.6 (21.0-22.5)	-	22.5 (22.3-23.0)	23.3 (22.8-24.0)	-
		MP	22.6 (22.0-23.8)	18.9 (17.9-21.1)	-	22.2 (21.4-22.9)	22.2 (21.2-22.5)	-
Temp	^oC	FP	39.1 (38.9-39.4)	38.8 (38.7-38.8)	38.6 (38.5-38.6)	38.4 (38.3-38.5)	38.2 (38.1-38.4)	38.1 (38.0-38.3)
		MP	39.2 (39.2-39.6)	38.8 (38.6-39.1)	38.8 (38.6-39.1)	38.8 (38.5-39.0)	38.6 (38.3-39.1)	38.5 (38.1-39.0)

*: significantly different ($P<0.05$) from baseline reading within group
SAP- systolic arterial pressure; DAP- diastolic arterial pressure; MAP- mean arterial pressure; SpO_2- saturation of haemoglobin with oxygen in peripheral blood; f_R - respiratory rate; $PE´CO_2$- end-tidal carbon dioxide partial pressure; P_aO_2 - arterial oxygen partial pressure; P_aCO_2 - arterial carbon dioxide partial pressure; pH_a - arterial hydrogen ion concentration negative logarithm; $[HCO_3^-]$ - arterial bicarbonate ion concentration; Temp- body temperature.

6.5 DISCUSSION

Total intravenous anaesthesia using propofol combined with either fentanyl or midazolam for induction and maintenance of general anaesthesia produced adequate immobilization of goats, although recovery from anaesthesia with propofol-fentanyl TIVA was characterized by abnormal behavioural signs.

The dosages of fentanyl and midazolam were chosen because they had been shown to reduce isoflurane minimum alveolar concentration by about 30% in unpublished studies from the authors' laboratory (see Chapters 3 and 4). There is no study documenting the sedative effects of intravenously administered fentanyl in goats. Fentanyl has been administered to goats intravenously for its anti-nociceptive effects (Carroll et al. 1999), but its sedative effects were not described. Likewise, there is no information on the effect of fentanyl on the propofol induction and maintenance dose in goats. Morphine, a μ opioid agonist like fentanyl, was reported to decrease isoflurane minimum alveolar concentration by 30% after administration at 2 mg kg^{-1} as single intravenous dose (Doherty et al. 2004. Midazolam has been reported to produce sedation in goats (Stegmann & Bester 2001; Dzikiti et al. 2009) and decrease the induction dose of propofol in goats (Dzikiti et al. 2009). In a previous study, the maximal sedative effects of midazolam were observed at 5 minutes following intravenous administration (Stegmann & Bester 2001). Although midazolam induced heavy sedation in the present study, it was likely that the maximal effect of midazolam was not achieved by the time propofol was administered. Also, the median induction dose of propofol (3.97 mg kg^{-1}) with Treatment MP was greater than the mean propofol induction dose (3.2 mg kg^{-1}) previously reported after intramuscular administration of midazolam (Dzikiti et al. 2009), which is further evidence that the maximal sedative effect of midazolam was not achieved by one minute. In an earlier study by Dzikiti et al. 2009 using the same goats as in the present study, a mean propofol dose of 5.3 mg kg^{-1} was needed for induction in non-premedicated goats and a median propofol dose of 5.1 mg kg^{-1} was

reported in the study by Pablo et al., 1997. In the study reported here, propofol provided a smooth induction of general anesthesia. Similar results were also achieved in sedated goats (Dzikiti et al. 2009) and even unsedated goats (Prassinos et al. 2005; Dzikiti et al 2009). Previous studies have reported adverse effects such as apnoea and myoclonus in some goats following induction of general anaesthesia with propofol (Pablo et al. 1997; Bettschart-Wolfensberger et al. 2000; Dzikiti et al. 2009). No such adverse effects were observed immediately after induction of anaesthesia in the present study.

The proposed dose of propofol for maintenance of anaesthesia (12.0 mg kg^{-1} $hour^{-1}$) proved to be sufficient to prevent purposeful movement to noxious stimulation when combined with fentanyl, but a propofol dose of 18.0 mg kg^{-1} $hour^{-1}$ was required when combined with midazolam. In retrospect, use of a higher dose of midazolam might have allowed a smaller dose of propofol to be used for maintenance of anaesthesia, since the sedative effects of midazolam have been reported to be dose-dependent (Stegmann & Bester 2001). The propofol infusion rates were chosen based on the results of a pilot study by our laboratory which showed that propofol administered at 12.0 mg kg^{-1} $hour^{-1}$ reduced isoflurane minimum alveolar requirements by about 60%. A weakness of the present study is that the plasma concentration of propofol was not measured and thus the possibility exists that there was a difference in propofol plasma concentrations between the groups. However, this is not considered likely as a randomized crossover design was used and the goats' body mass was calculated on each occasion. Increasing the propofol rate by 10% for 10 minutes appeared to increase the plasma concentration as this manoeuvre was effective in preventing purposeful movement on the subsequent stimulation, although the data from this study cannot substantiate that the propofol plasma concentration had changed within the 10-minute period.

Total intravenous anaesthesia with fentanyl-propofol or midazolam-propofol has minimal negative impact on cardiopulmonary function based on cardiovascular and respiratory findings of this

study. Although respiratory rate decreased in comparison to baseline values in both groups during maintenance of anaesthesia, SpO_2, $PE´CO_2$, and PaO_2 remained within clinically acceptable limits throughout the anaesthetic procedure.

In the present study, recovery times were short for both groups, and consistent with data available for premedicated, goats (Dzikiti et al. 2009). Recovery from anaesthesia was smooth and uneventful in all goats recovering from midazolam-propofol anesthesia, but abnormal behavioural signs and restlessness were observed in 4 of the 6 goats recovering from fentanyl-propofol anaesthesia. The latter finding is consistent with a previous report on the effects of fentanyl in goats (Carroll et al. 1999). It might be prudent to sedate the goats with effective sedatives like midazolam during the recovery period to ameliorate fentanyl's excitatory effects. This may improve quality of recovery by calming the goat while providing more time for metabolism and excretion of fentanyl. Adverse effects such as myoclonus, that have have been reported previously in goats recovering from a single dose propofol (Dzikiti et al. 2009), were not observed in the present study probably because the goats were recovering from a lower plasma concentration of propofol, following CRI. Had the present study included a control group, in which only propofol CRI was administered and another group, in which propofol was combined with both fentanyl and midazolam CRI, answers to whether the excitatory effects of fentanyl during recovery from anaesthesia can be ameliorated by midazolam or whether myoclonus is only observed with high doses of propofol in goats could have been obtained.

The advantages of TIVA in comparison to inhalation anaesthesia include absence of pollution of working environment and requirement of minimal equipment which allows its use in remote settings (Larenza et al. 2005). Fentanyl has benefits of analgesic effects in addition to mild sedation. Midazolam causes mild cardiopulmonary depression, but lacks analgesic effects (Cao et al. 2002).

The results of this study indicate that TIVA achieved by co-administration of propofol and either fentanyl or midazolam for induction and maintenance of anaesthesia in spontaneously-breathing, oxygen-supplemented goats is satisfactory, but caution must be exercised with the fentanyl-propofol combination as recovery from anaesthesia might be rough.

General Conclusions

The objectives of the present series of studies which were geared towards providing information that would improve the literature resource on goat anaesthesia with specific information on total intravenous anaesthesia were successfully achieved.

The specific objectives of the present series of studies were:

a) Investigation of the sedative, propofol-sparing and cardiopulmonary effects of acepromazine, midazolam, butorphanol and combinations of butorphanol with acepromazine or midazolam in goats.
b) Evaluation of the effects of different dosages of midazolam on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
c) Evaluation of the effects of different dosages of fentanyl on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
d) Evaluation of the effects of different dosages of propofol on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats.
e) Evaluation and comparison of the efficacy of propofol and fentanyl, and propofol and midazolam for total intravenous anesthesia in goats.

From the outcomes of the present series of studies, the following conclusions were drawn:

Acepromazine and midazolam, in agreement with currently available literature, produced a significant ($p < 0.05$) degree of sedation when administered alone (Bertens 1993; Stegmann & Bester 2001), but when combined with butorphanol the sedation score did not show any significant

difference when compared to the unpremedicated goats. Butorphanol administered alone did not show a consistent significant degree of sedation. Previous studies have also reported the sedative effects of butorphanol in goats to be variable and unpredictable (Doherty et al. 2002a). The observed dose of propofol for induction of general anaesthesia in unpremedicated goats of 5.05 mg kg^{-1} is similar to doses of 5.1 mg kg^{-1} (Pablo et al. 1997) and 5.6 mg kg^{-1} (Amarpal et al. 2002) previously reported in the literature. Significant reductions in propofol induction dose requirements were observed in goats that received midazolam alone (39.7%), midazolam combined with butorphanol (38.1%) and acepromazine combined with butorphanol (27.8%). Sedation of goats with acepromazine, midazolam, acepromazine-butorphanol and midazolam-butorphanol followed by propofol general anaesthesia was associated with minimal adverse cardiopulmonary effects.

Intravenous administration of low dose midazolam (bolus at 0.1 mg kg^{-1} followed by 0.1 mg kg^{-1} $hour^{-1}$ maintenance dose), moderate dose midazolam (bolus at 0.2 mg kg^{-1} followed by 0.2 mg kg^{-1} $hour^{-1}$ maintenance dose), high dose midazolam (bolus at 0.9 mg kg^{-1} followed by 0.9 mg kg^{-1} $hour^{-1}$ maintenance dose) reduced isoflurane MAC in goats by 16.8%, 35.1% and 54.7%, respectively. Midazolam produced a substantial and dose-dependent reduction of isoflurane MAC without significantly affecting cardiovascular function in goats.

The reductions in isoflurane MAC by about 27.6%, 40.7% and 56.6% observed in the present study after administration of a low dose fentanyl (0.005 mg kg^{-1} bolus followed by continuous infusion at 0.005 mg kg^{-1} $hour^{-1}$), moderate dose fentanyl (0.015 mg kg^{-1} bolus followed by continuous infusion at 0.015 mg kg^{-1} $hour^{-1}$) and high dose fentanyl (0.03 mg kg^{-1} bolus followed by continuous infusion at 0.03 mg kg^{-1} $hour^{-1}$) show that fentanyl administered intravenously reduces isoflurane MAC in a dose-dependent manner and could have a role as an analgesic adjunct in balanced anaesthesia protocols in goats. Fentanyl seems to be less potent in reducing isflurane MAC in goats than in dogs, but is more potent in reducing isoflurane MAC in goats when compared to the

horse and swine (Moon et al. 1995; Thomas et al. 2006; Wilson et al. 2006). Exaggerated tail-wagging was observed in the majority of goats that received higher doses of fentanyl, but this was not considered to be deleterious to the quality of recovery from anaesthesia since the general behaviour of the goats was acceptable at all times during recovery.

The baseline isoflurane MAC was statistically significantly different ($p < 0.05$) from the propofol-treatment isoflurane MACs while cardiovascular parameters remained largely unchanged at the three propofol infusion rates used in the present study, indicating that propofol reduces isoflurane MAC in goats without causing significant adverse cardiovascular effects. The degree of reduction in isoflurane MAC by propofol was dose-dependent. Median plasma propofol concentration at the time of propofol-treatment isoflurane MAC determination was 1.6 (1.2-1.8) μg mL^{-1}, 2.5 (2.3-3.0) μg mL^{-1} and 7.8 (7.3-8.4) μg mL^{-1} following administration of low dose propofol (0.5 mg kg^{-1} bolus followed by continuous infusion at 0.05 mg kg^{-1} $minute^{-1}$), moderate dose propofol (1.0 mg kg^{-1} followed by continuos infusion at 0.1 mg kg^{-1} $minute^{-1}$) and high dose propofol (2.0 mg kg^{-1} followed by continuous infusion at 0.2 mg kg^{-1} $minute^{-1}$) respectively. Relatively higher plasma propofol concentrations, but not correspondingly higher anaesthetic effects were observed in the present study when compared to results from other studies in sheep, dogs, ponies and dogs (Nolan & Reid 1993; Correia et al. 1990; Nolan et al. 1996; Beths et al. 2001).

Total intravenous anaesthesia using propofol combined with either fentanyl or midazolam for induction and maintenance of general anaesthesia produced adequate immobilization of goats, although recovery from anaesthesia following propofol-fentanyl TIVA was characterized by abnormal behavioural signs.

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APPENDIX

9.1 Scientific Publications Associated with this Thesis

1 Sedative and cardiopulmonary effects of acepromazine, midazolam, butorphanol, acepromazine-butorphanol and midazolam-butorphanol on propofol anaesthesia in goats – TB Dzikiti, GF Stegmann, LJ Hellebrekers, LN Dzikiti (2009) Journal of the South African Veterinary Association 80 (1) pp 10-16 http://www.ncbi.nlm.nih.gov/pubmed/19653513

2 Effects of propofol on isoflurane minimum alveolar concentration and cardiovascular function in artificially-ventilated goats TB Dzikiti, GF Stegmann, D Cromarty, LN Dzikiti, LJ Hellebrekers. Vet Anaesth Analg (Accepted)

3 Total intravenous anaesthesia with propofol-fentanyl or propofol-midazolam in spontaneously-breathing goats TB Dzikiti, GF Stegmann, LN Dzikiti, LJ Hellebrekers. Vet Anaesth Analg (Accepted)

9.2 Congress / Seminar Presentations Associated with this Thesis

1. Dzikiti TB, Stegmann GF, Dzikiti LN, Hellebrekers LJ (2009): Total intravenous anaesthesia (TIVA) with propofol-fentanyl and propofol-midazolam in spontaneously-breathing goats. Abstract presented at the 10th World Congress of Veterinary Anaesthesia, Glasgow, Scotland **http://www3.interscience.wiley.com/cgi-bin/fulltext/123341135/PDFSTART**

2. Dzikiti TB, Stegmann GF, Auer AEJ, Dzikiti LN, Hellebrekers LJ (2009): Sedative and cardiopulmonary effects of acepromazine, midazolam, butorphanol, acepromazine-butorphanol and midazolam-butorphanol on propofol anaesthesia in goats. Presented at the Faculty Day Faculty of Veterinary Science, University of Pretoria, 2009. Onderstepoort, RSA

3. Dzikiti TB, Stegmann GF, Dzikiti LN, Hellebrekers LJ (2009): Total intravenous anaesthesia with propofol-fentanyl or propofol-midazolam in spontaneously-breathing goats TB Dzikiti, GF Stegmann, LN Dzikiti, LJ Hellebrekers. Presented at the Faculty Day Faculty of Veterinary Science, University of Pretoria, 2009. Onderstepoort, RSA