# Effects of constant rate infusions (CRI) of S(+)-ketamine or medetomidine under isoflurane anaesthesia after induction with either S(+)-ketamine or racemic ketamine on minimal alveolar concentration (MAC), cardiopulmonary function and recovery quality in horses

Conrot, Aude

Abstract: The aim of this study was to compare the effects on MAC, cardiopulmonary function and recovery quality of S(+)-ketamine or medetomidine CRIs in isoflurane anaesthetised horses after induction of anaesthesia with either S(+)- or racemic ketamine. Fifty horses were randomly assigned to two groups, S-ket and Med, and the observer was blinded. Horses were sedated with medetomidine. Anaesthesia was induced with midazolam and 1.1 mg kg-1 S(+)-ketamine (S-ket) or 2.2 mg kg-1 racemic ketamine (Med) and maintained with isoflurane in oxygen and 0.5 mg kg-1 h-1 S(+)-ketamine or 3.5 µg kg-1 h-1 medetomidine and thiopental as rescue drug. Cardiopulmonary function was monitored and recovery timed and scored. No differences between the groups concerning age, weight and duration of anaesthesia were found. Both groups required the same concentrations of isoflurane to maintain anaesthesia, but the need for thiopental was higher in Sket group. No clinically relevant differences in cardiopulmonary function were found between groups. Only cardiac index was higher in S-ket group. Horses in S-ket group regained sternal and standing position faster. Recovery scores were better in Med group. In S-ket group, recovery quality decreased with CRI duration. It was concluded that S(+)-ketamine CRI and medetomidine CRI during isoflurane anaesthesia resulted in acceptable cardiopulmonary function in horses. Horses recovered faster with S(+)-ketamine, but quality of recovery was better with medetomidine. Ziel dieser Studie war, den Einfluss auf die MAC, die kardiopulmonäre Funktion und die Aufwachqualität einer CRI von S(+)-Ketamin oder Medetomidin während einer Isoflurananästhesie bei Pferden nach Einleitung mit S(+)-Ketamin oder Ketaminrazemat zu vergleichen. Für diese Doppelblindstudie wurden 50 Pferde zufällig in 2 Gruppen eingeteilt, S-ket und Med. Alle Pferde wurden mit Medetomidin sediert. Die Anästhesie wurde mit Midazolam und 1.1 mg kg-1 S(+)-Ketamin (S-ket) oder 2.2 mg kg-1 Ketaminrazemat (Med) eingeleitet und mit Isofluran in Sauerstoff und 0.5 mg kg-1 h-1 S(+)-Ketamin oder 3.5 µg kg-1 h-1 Medetomidin sowie bei Bedarf Thiopental aufrecht erhalten. Die kardiopulmonären Parameter wurden überwacht, die Aufwachphase bewertet. Alter, Gewicht und Anästhesiedauer waren in beiden Gruppen gleich. Beide Gruppen benötigten die gleiche Isoflurankonzentration um die Anästhesie aufrechtzuerhalten, jedoch war der Thiopentalbedarf in der S-ket Gruppe höher. Es gab keinen klinisch relevanten Unterschied in der kardiopulmonären Funktion. Einzig der Herzindex war in der S-ket Gruppe höher. Pferde der S-Ket Gruppe nahmen die sternale und stehende Position schneller ein. Die Aufwachscores waren in der Med Gruppe besser. In der S-ket Gruppe verschlechterte sich die Aufwachqualität mit der Infusionsdauer. Pferde der S-Ket und Med Gruppe zeigten eine akzeptable kardiopulmonäre Funktion. Mit S(+)-Ketamin standen die Pferde schneller auf, aber die Aufwachqualität war besser mit Medetomdin.

Other titles: Einfluss einer S(+)-Ketamin- oder Medetomidin-Dauertropfinfusion (CRI) unter Isoflurananästhesie nach Einleitung mit S(+)-Ketamin oder Ketaminrazemat auf die minimale alveoläre Konzentration (MAC), die kardiopulmonäre Funktion und die Aufwachqualität bei Pferden

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*À mes très chers parents*

*À mes sœurs*

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**SUMMARY**

The aim of this study was to compare the effects on MAC, cardiopulmonary function and recovery quality of S(+)-ketamine or medetomidine CRIs in isoflurane anaesthetised horses after induction of anaesthesia with either S(+)- or racemic ketamine.

Fifty horses were randomly assigned to two groups, S-ket and Med, and the observer was blinded. Horses were sedated with medetomidine. Anaesthesia was induced with midazolam and 1.1 mg $kg^{-1}$ S(+)-ketamine (S-ket) or 2.2 mg $kg^{-1}$ racemic ketamine (Med) and maintained with isoflurane in oxygen and 0.5 mg $kg^{-1}$ $h^{-1}$ S(+)-ketamine or 3.5 µg $kg^{-1}$ $h^{-1}$ medetomidine and thiopental as rescue drug. Cardiopulmonary function was monitored and recovery timed and scored.

No differences between the groups concerning age, weight and duration of anaesthesia were found. Both groups required the same concentrations of isoflurane to maintain anaesthesia, but the need for thiopental was higher in S-ket group. No clinically relevant differences in cardiopulmonary function were found between groups. Only cardiac index was higher in S-ket group.

Horses in S-ket group regained sternal and standing position faster. Recovery scores were better in Med group. In S-ket group, recovery quality decreased with CRI duration.

It was concluded that S(+)-ketamine CRI and medetomidine CRI during isoflurane anaesthesia resulted in acceptable cardiopulmonary function in horses. Horses recovered faster with S(+)-ketamine, but quality of recovery was better with medetomidine.

Key words: Horses, isoflurane anaesthesia, CRI, S(+)-ketamine, medetomidine

**ZUSAMMENFASSUNG**

Ziel dieser Studie war, den Einfluss auf die MAC, die kardiopulmonäre Funktion und die Aufwachqualität einer CRI von S(+)-Ketamin oder Medetomidin während einer Isoflurananästhesie bei Pferden nach Einleitung mit S(+)-Ketamin oder Ketaminrazemat zu vergleichen.

Für diese Doppelblindstudie wurden 50 Pferde zufällig in 2 Gruppen eingeteilt, S-ket und Med. Alle Pferde wurden mit Medetomidin sediert. Die Anästhesie wurde mit Midazolam und 1.1 mg $kg^{-1}$ S(+)-Ketamin (S-ket) oder 2.2 mg $kg^{-1}$ Ketaminrazemat (Med) eingeleitet und mit Isofluran in Sauerstoff und 0.5 mg $kg^{-1}$ $h^{-1}$ S(+)-Ketamin oder 3.5 µg $kg^{-1}$ $h^{-1}$ Medetomidin sowie bei Bedarf Thiopental aufrecht erhalten. Die kardiopulmonären Parameter wurden überwacht, die Aufwachphase bewertet.

Alter, Gewicht und Anästhesiedauer waren in beiden Gruppen gleich. Beide Gruppen benötigten die gleiche Isoflurankonzentration um die Anästhesie aufrechtzuerhalten, jedoch war der Thiopentalbedarf in der S-ket Gruppe höher. Es gab keinen klinisch relevanten Unterschied in der kardiopulmonären Funktion. Einzig der Herzindex war in der S-ket Gruppe höher.

Pferde der S-Ket Gruppe nahmen die sternale und stehende Position schneller ein. Die Aufwachscores waren in der Med Gruppe besser. In der S-ket Gruppe verschlechterte sich die Aufwachqualität mit der Infusionsdauer.

Pferde der S-Ket und Med Gruppe zeigten eine akzeptable kardiopulmonäre Funktion. Mit S(+)-Ketamin standen die Pferde schneller auf, aber die Aufwachqualität war besser mit Medetomdin.

Schlüsselwörter: Pferde, Isoflurananästhesie, CRI, S(+)-Ketamin, Medetomidin

## 1. INTRODUCTION

In horses, surgical procedures of longer duration are usually performed under inhalation anaesthesia. However, volatile anaesthetic agents cause a dose-dependent cardiovascular depression, a major cause of death in equine anaesthesia (Johnston et al. 2002). Respiratory depression is frequent in anaesthetised horses. Recovery is one of the most critical phases of equine anaesthesia and is often associated with bone fractures, joint dislocations, haemorrhages, head traumas and dehiscence of surgical wounds (Clark-Price et al. 2008).

The technique of balanced anaesthesia is based on the concept that the co-administration of a mixture of small amounts of several drugs shall produce an ideal anaesthetic state (Muir & Yamashita 2000). Thus, the advantages, but not the disadvantages, of the individual components of the mixture are summated.

Medetomidine is an alpha2-adrenoreceptor agonist that has been used in horses as constant rate infusion (CRI) together with isoflurane and its use is well established in equine anaesthesia. In clinical patients the use of a CRI of medetomidine in combination with isoflurane compared to isoflurane anaesthesia alone, resulted in significantly reduced isoflurane requirements (Neges et al. 2003). Further, adjustment of anaesthetic depth was easier with medetomidine and less additional drugs had to be administered in order to deepen anaesthesia. A comparison of medetomidine infusions during isoflurane anaesthesia with lidocaine infusions, a commonly used drug for balanced anaesthesia in horses, showed much better recoveries following medetomidine CRI (Ringer et al. 2007). At the Equine Department of the Vetsuisse Faculty of Zurich, anaesthesia is usually induced with racemic ketamine, and maintained with isoflurane and a constant rate infusion of medetomidine.

Commercially available ketamine for veterinary use is mostly a racemic mixture of two optical isomers: S(+)- and R(-)-ketamine. Racemic ketamine is considered to be safe and effective for most equine anaesthetic procedures, either during field surgery or hospital conditions. Addition of racemic ketamine to volatile anaesthetics (i.e. halothane, isoflurane) as part of balanced anaesthesia in horses reduced MAC and resulted in better cardiopulmonary function (Bettschart-Wolfensberger & Larenza 2007). However, racemic

ketamine can induce emergence reactions during the post-anaesthetic recovery period, characterized by muscular tremor, rigidity and ataxia that can turn into a fatal event in horses (Muir & Sams 1992, Larenza et al. 2009). Therefore the use of racemic ketamine infusion is limited to procedures lasting less than 2 hours (Larenza et al. 2009). In the last years, the single S(+)-isomer has become available for use in cats. A preliminary study in ponies demonstrated that S(+)-ketamine provided an identical depth of immobility after a single injection of half dose of racemic ketamine with a more rapid recovery (Larenza et al. 2007), probably caused by faster S(+)-ketamine elimination. In horses, a significantly better anaesthesia recovery was also observed after a constant rate infusion of S(+)-ketamine compared with racemic ketamine infusions (Larenza et al. 2009).

The aim of the present study was to compare the effects of a S(+)-ketamine CRI with a medetomidine CRI during isoflurane anaesthesia in horses induced with either S(+)-ketamine or racemic ketamine on MAC and cardiopulmonary function and the resulting recovery .

# 2. BACKGROUND

## 2.1 Isomerism

### 2.1.1 Introduction

In chemistry, isomers are compounds with the same molecular formula, but different structures (Kemnitz & Simon 2005). Isomerism is the occurrence of such compounds. The term "isomer" is derived from greek words "isos" which means "equal", and "méros" which means "part" (Haubrich 2003). Even if isomers have the same number of atoms and the same molecular weight, they differ in their properties.

Friedrich Wöhler and Justus von Liebig were both involved in the discovery of isomerism. They found that cyanic acid had the same composition as fulmaric acid, but that its properties were different. During the years 1849 - 1853, Louis Pasteur investigated on tartaric and racemic acids. He separated racemic tartaric acid into two optically active components by crystallization. These acids

had an identical chemical composition and structure, but different properties (Wheland & Mills 1964).

In 1992, the Food and Drug Administration claimed that separation of isomers had not received adequate attention in drug development and that, despite technical difficulties and high costs, focussing on this new issue could open new therapeutic horizons (Kohrs & Durieux 1998).

### 2.1.2 Classification

There are two main forms of isomerism: structural (or constitutional) isomerism and stereoisomerism.

#### 2.1.2.1 Structural isomerism

Two or more substances are said to be structural isomers of each other if they have the same chemical composition, but different structures (Wheland 1964). That means that their atoms are bonded together in different orders. These differences give the molecules different chemical and physical properties. There are different categories of structural isomerism: chain isomerism (or skeletal isomerism), position isomerism, functional group isomerism (Wheland 1964) and tautomerism.

**A. Chain isomerism**

Chain or skeletal isomers occur because of different possibilities of a branching component of the skeleton.

In the example below, butane is constituted of a straight chain, whereas its isomer isobutane has a branched chain (Figure 1). Both molecules have the same molecular formula $C_4H_{10}$, but different constitutions.

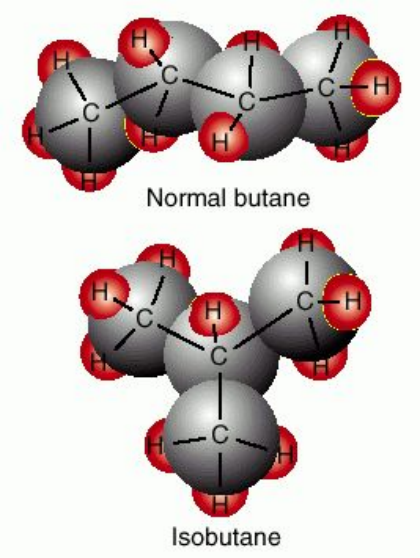



**Figure 1-** Chain isomerism (http://medical-dictionary.thefreedictionary.com)

### B. Position isomerism

In position isomerism, the basic carbon skeleton remains unchanged, but important functional groups change their position in the chain. For example, 1-propanol, $CH_3CH_2CH_2OH$, and 2-propanol, $CH_3CH(OH)CH_3$, are position isomers, because their functional group (the OH-group) has a different location in the carbon chain.

The functional group can also be located on different parts of a benzene ring (Figure 2). There are three isomers of toluidine which all have the same molecular formula $C_7H_9N$. The difference between these three isomers is the position where the methyl group ($-CH_3$) is bonded to the ring relative to the amino functional group ($-NH_2$). In *o*-toluidine, the methyl group is located next to the amino group, in *m*-toluidine it is located next-but one to the amino group and in *p*-toluidine it is located opposite to the amino group.

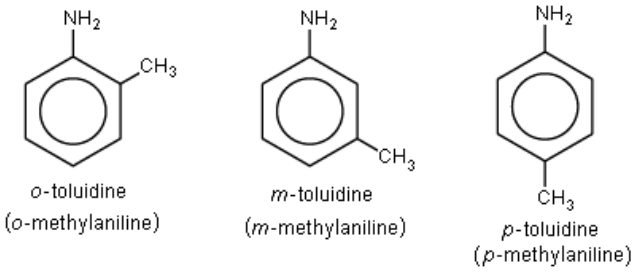
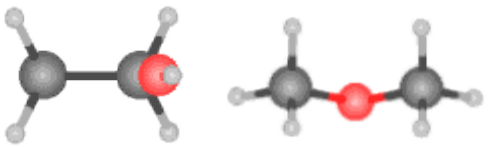



**Figure 2-** Position isomers of toluidine (Wikipedia)

### C. Functional group isomerism

Functional isomerism occurs when substances have the same molecular formula, but different functional groups.



Ethanol Methoxymethane

**Figure 3-** Functional group isomerism (www.creative-chemistry.org.uk)

Ethanol ($CH_3CH_2$-OH), an alcohol, has the functional hydroxyl group (-OH), methoxymethane ($CH_3$-O-$CH_3$) the functional R-O-R' group. Both substances have the same molecular formula $C_2H_6O$ (Figure 3).

### D. Tautomerism

Tautomers are structural isomers that easily convert between two isomeric forms by a chemical reaction called tautomerization. They therefore exist in equilibrium. This reaction commonly results in the migration of a hydrogen atom and a switch of a single bond to an adjacent double bond. A common example is the keto-enol tautomerism (Figure 4). The hydrogen atom bonded to the carbon atom in the carbonyl (keto) group (-CH-C=O) moves to the oxygen atom, making it an enol group (-C=C-OH).

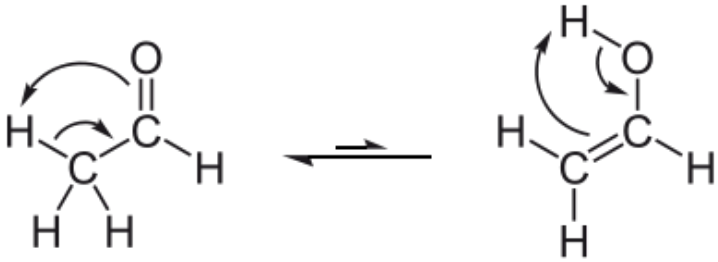


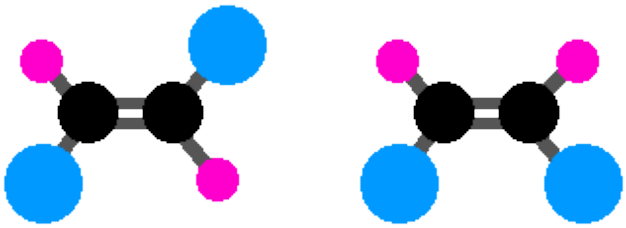

**Figure 4-** Keto-enol tautomerism (Wikipedia)

### 2.1.2.2 Stereoisomerism

Stereoisomers are molecules with the same basic arrangement of their atoms, but with different geometrical positioning of atoms and functional groups in space. This category includes enantiomers, where isomers are mirror images of each other and *cis-trans* isomers.

#### A. *Cis-trans* isomerism

*Cis-trans* isomerism, often named geometrical isomers, even if this synonym is considered obsolete, describes the orientation of functional groups within the molecule (IUPAC Compendium of Chemical terminology). In general, these isomers contain double bonds which cannot rotate, or some other feature that gives the molecule a certain structural rigidity. In the *cis*-isomer, the atoms are orientated on the same side, in the *trans*-isomer they are on the opposite side (IUPAC Compendium of Chemical terminology) (Figure 5). *Cis* and *trans* isomers often differ in physical properties, such as melting and boiling points.



*trans*-isomer *cis*-isomer

**Figure 5-** *Cis-trans* isomers (http://www.chemguide.co.uk)

#### B. Enantiomers

Enantiomers are molecules which contain the same number and kinds of atoms and bonds, but different spatial arrangements of the atoms. The simplest optical isomer has a single carbon atom in its molecule bonded to four different functional groups. This carbon atom is called asymmetric carbon or chiral centre (Figure 6).

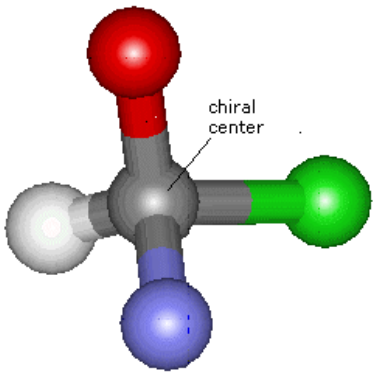



**Figure 6-** Chiral centre

Enantiomers are non-superimposable mirror images of each other, like the left and right hand (Figure 7).

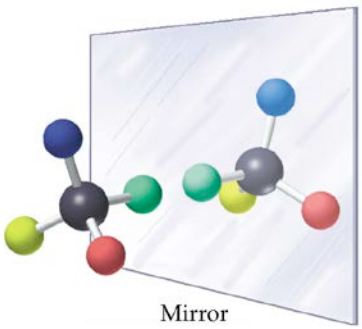



**Figure 7-** Enantiomers
(http://astrobiology.berkeley.edu)

Enantiomers are optical isomers that can rotate the plane of polarised light. If the optical isomer rotates the plane of polarised light to the right, it is known as (-) or dextrorotatory (D) enantiomer. In contrary, if the optical isomer rotates the plane of polarisation to the left, it is called (+) or laevo-rotatory (L) enantiomer.
The R (R is for rectus, the Latin for right) and S (S is for sinister, the Latin for left) notation describes the arrangement of the molecules around the chiral centre. The atom of the lowest atomic number is imagined to lie behind the plane of the page. The other three atoms lie in the plane of the page and if their atomic numbers descend in clockwise direction, it is the R-enantiomer, in anticlockwise direction it is the S-enantiomer. They may be laevo- or dextrorotatory to polarised light, demonstrating that there is no relationship between these classifications.

A racemic mixture, or a racemate, is a 1:1 mixture of two enantiomers. A racemic mixture has no effect on plane-polarised light, as the amount of polarised light rotation caused by the two enantiomers is the same, but in opposite direction.

## 2.2 General anaesthesia in horses

General anaesthesia in horses is associated with more problems than in other species and anaesthetic mortality has always been considered to be relatively high, when compared to companion animals or humans (Hall & Clarke 1991a, Jones 2001). Most fatalities are related to poor cardiovascular or respiratory performances during anaesthesia or to fatal injuries during poor quality anaesthetic recovery.

In 1993, Young and Taylor reported an equine anaesthesia mortality rate of 0.68%. Ischaemic myopathy, cardiac arrest and bone fractures were found to be the main causes of death. Similarly, Mee et al. (1998a) reported a surgical/anaesthetic death rate in elective procedures of 0.63%. In horses undergoing emergence procedures, mortality was 31.4% (Mee et al. 1998b). Horses anaesthetised for surgical colic had a higher mortality rate than animals anaesthetised for non-colic emergence procedures.

Johnston et al. (1995) surveyed a total of 6'225 general anaesthesias in horses and reported an overall mortality of 1.6%. When abdominal surgeries and deliveries of foals under general anaesthesia were excluded, the mortality rate fell to 0.9%. Factors influencing a high mortality were: anaesthesia performed in the third trimester of pregnancy, emergence abdominal surgeries, bone fractures, administration of xylazine, foals under 1 year old and surgeries performed outside normal working hours. The risk of death also increased with the duration of anaesthesia. In another survey, the same author studied more than 41'000 anaesthesia procedures (Johnston et al. 2002). The overall death rate was 1.9%. When emergence abdominal surgery was excluded, the rate fell to 0.9%. For abdominal surgery, the rate of mortality was high (11%). Cardiac arrest (33%), fractures (25%) and ischaemic myopathy (7%) were defined as the most common causes of death.

In a more recent retrospective study, the prevalence of fatalities in horses directly related to anaesthesia was 0.12% (Bidwell et al. 2007). This incidence is lower than that reported in previous studies. Causes of death directly related to anaesthesia were cardiac arrest, bone fractures in recovery stall, neuropathy and myopathy necessitating euthanasia. Possible explanations for the discrepancy from previous studies were the familiarity with the anaesthetic protocol, a reduced duration of anaesthesia and an adequate pre-operative examination. Horses in which anaesthesia was maintained with volatile agents showed higher risk of death than horses in which anaesthesia was maintained with total intravenous agents (Johnston et al. 2002).

In horses more than in other species, maintenance of good intra-operative cardiopulmonary function and a calm and coordinated anaesthetic recovery is very important.

### 2.2.1 Cardiovascular effects

Inhalational anaesthetic agents cause a dose-dependent depression of the cardiovascular system. The mechanism of cardiovascular effects includes direct myocardial depression and a decrease in sympathoadrenal activity (Tranquilli et al. 2007).

All inhalation anaesthetics decrease cardiac output in a dose-related manner due to a decrease in stroke volume as a cause of myocardial depression (Tranquilli et al. 2007). Blood pressure is also decreased in a dose-dependent manner. Inhalation agents sensitise the myocardium to the arrhythmogenic effects of catecholamines. Injectable drugs, such as acepromazine, ketamine and alpha2-adrenergic agonists are often administered to horses as part of the anaesthetic regimen. These drugs possibly accentuate cardiovascular depression (Tranquilli et al. 2007). During equine anaesthesia, sympathomimetic drugs such as dopamine and dobutamine are often given at low-doses to improve cardiac output and blood pressure (Swanson et al. 1985). In horses anaesthetised with halothane, hypercapnia was associated with improved cardiovascular function associated with an increase in circulating catecholamines (Wagner et al. 1990).

The American College of Veterinary Anesthesiologists suggests that for horses anaesthetised with inhalant anaesthetics or undergoing anaesthesia lasting more than 45 minutes, a continuous electrocardiogram and a blood pressure monitor should be employed. Hypotension has to be treated with fluid therapy and in severe cases with vasoactive agents such as dobutamine.

### 2.2.2 Respiratory effects

Respiratory depression is frequent in anaesthetised horses and can result in hypercapnia, hypoxaemia and acid-base abnormalities due to hypoventilation, ventilation-perfusion impairment and right-to-left vascular shunts (Kalchofner et al. 2009).

During equine anaesthesia, arterial oxygen tension is lower than might be expected from inspired oxygen tension, and thus the alveolar-arterial oxygen tension gradient ((A-a)$PO_2$) increases very soon after anaesthesia induction (Hall & Clarke 1991a). The arterial oxygen tension ($PaO_2$) partially depends on the size of the horse and its position during recumbency (Hall et al. 1968). Other factors inducing a large (A-a)$PO_2$ are a reduced cardiac output, hypoventilation, intrapulmonary vascular shunts, ventilation/perfusion mismatches and atelectasis (Hall & Clarke 1991a). The lung is partially collapsed creating atelectasis, probably due to the compression by abdominal and thoracic viscera, and may act as a venous-arterial shunt and thus cause hypoxaemia (Nyman et al. 1990, Hall & Clarke 1991a). During anaesthesia of horses in dorsal or lateral recumbency a shunt developed, independently whether the horses were breathing spontaneously or were mechanically ventilated (Nyman & Hedenstierna 1989). The shunt was greater in dorsal than in lateral recumbency.

The relationship between ventilation and perfusion is often disturbed during equine anaesthesia. Given that ventilation of the lower lung regions of the recumbent horse is restricted by limited movements of the diaphragm and rib cage, lower lung regions receive more blood than can be oxygenated by the prevailing ventilation (Hall & Clarke 1991a).

In horses anaesthetised for more than 45 minutes, assisted ventilation may be beneficial to reduce the degree of hypercarbia, but the occurrence of hypoxaemia cannot be completely avoided.

### 2.2.3 Recovery

Recovery is one of the most critical phases of equine anaesthesia and is often associated with bone fractures, joint dislocations, haemorrhages, head traumas and dehiscences of surgical wounds (Clark-Price et al. 2008). Unfortunately anaesthesia recovery is little controllable (Hubbell 2007). Horses may try to stand up prematurely and subsequently fall and panic, with the result of further attempts to stand with poor results. Ideally, horses regain consciousness within 20 to 30 minutes after the end of anaesthesia (Bettschart-Wolfensberger, personal communication), take briefly sternal position and then stand up (Hubbell 2007). As prolonged anaesthesia increases the risk of poor recovery, duration of anaesthesia has to be minimised (Auckburally & Flaherty 2009). The administration of alpha2-adrenoceptor agonists during recovery from anaesthesia prolonged the recovery period but improved its quality without producing significant cardiorespiratory effects (Santos et al. 2003). This allows volatile agents to be eliminated and permits horses to regain their cognitive function before trying to stand (Auckburally & Flaherty 2009). In addition, adequate pain relief in form of systemic or local analgesia is very important during recovery. Hubbell (2007) presumed that placing horses in a quiet and darkened environment improves recovery quality. This could not be demonstrated in a prospective randomized clinical trial performed in 29 horses (Clark-Price et al. 2008). In that study, no significant differences between horses recovering in a darkened stall or in an illuminated environment could be detected.

Horses that fail to rise within reasonable time possibly suffer from post anaesthetic myositis (Hubbell 2007). Usually, muscle groups which were on the underside of the horse during anaesthesia are affected. The muscles are swollen, firm, painful and non-functional (Young 2005). Creatine kinase values are elevated (Trim & Mason 1973). Myoglobin may be released in large quantities and may result in myoglobinuria with red or red-brown urine. In

severe cases, renal failure can occur (Young 2005). The underlying cause of post-anaesthetic myopathy is probably ischaemic muscle damage (Young 2005). The weight of the horses, the non-physiological recumbency during anaesthesia, and hypotension during anaesthesia can induce post anaesthetic myositis (Schatzmann & Girard 1984). Horses with prolonged halothane-induced hypotension were more likely to develop post-anaesthetic myopathy than horses in which normotension was maintained (Grandy et al. 1987). The occurrence of post-anaesthetic myositis may be reduced by using soft surfaces to support the recumbent horse and by maintaining mean arterial blood pressure above 70 mmHg.

### 2.2.4 Balanced anaesthesia

The technique of balanced anaesthesia is based on the concept that the co-administration of a mixture of small amounts of several drugs shall produce an ideal anaesthetic state (Muir & Yamashita 2000). Thus, the advantages, but not the disadvantages, of the individual components of the mixture are summated. In horses, procedures of longer duration are usually performed under inhalation anaesthesia with agents causing a dose-dependent cardiovascular depression (Johnston et al. 2002). Ideally, their use should be reduced to a minimal dose rate for induction of unconsciousness while the addition of other drugs provide analgesia and muscle relaxation. With balanced anaesthesia the quality of anaesthesia induction, maintenance and recovery can be improved and the need for additional drugs to support cardiac output or arterial blood pressures as well as to support recovery can be reduced (Muir & Yamashita 2000).
Usually used drugs for balanced anaesthesia are lidocaine, ketamine, alpha2-adrenergic agonists, opioids and central muscle relaxants.

<u>Lidocaine</u>

Lidocaine is rapidly metabolised in the liver and has a very short half-life (Engelking et al. 1987). In order to achieve constant plasma levels within acceptable time, an induction bolus has to be administered followed by a constant rate infusion (CRI) (Ringer et al. 2007). Lidocaine administered to ponies decreased MAC of halothane in a dose-dependent manner (Doherty &

Frazier 1998). In horses undergoing surgery, the requirements for isoflurane were reduced by 25% when lidocaine was administered during anaesthesia (Dzikiti et al. 2003). The effect of a CRI of lidocaine on the quality of recovery from sevoflurane or isoflurane anaesthesia was studied in horses (Valverde et al. 2005). Horses that received a CRI of lidocaine had a significantly higher degree of ataxia and a lower quality of recovery than horses receiving a saline solution. Therefore the authors recommended discontinuing the lidocaine infusion 30 minutes before the end of surgery. Another study compared the effects of balanced anaesthesia with lidocaine with those of balanced anaesthesia with medetomidine (Ringer et al. 2007). Both protocols were successfully used to perform surgery. Cardiovascular function was well maintained in both groups. However, a more stable depth of anaesthesia and a better quality of recovery was observed in horses receiving medetomidine.

Alpha2- adrenergic agonists

Alpha2-adrenergic agonists are potent analgesics and reduce the MAC of inhalant anaesthetic agents. In horses, xylazine administration in isoflurane anaesthetised horses reduced the MAC in a dose- and time-dependent manner (Steffey et al. 2000). In ponies, mean MAC of desflurane was reduced by 28% when inhalation anaesthesia was combined with a CRI of medetomidine (Bettschart-Wolfensberger et al. 2001). Cardiopulmonary parameters remained stable during the whole anaesthetic episode and the quality of recovery was good to excellent. In a retrospective study reporting the use of a CRI of medetomidine during isoflurane anaesthesia, 300 equine cases were studied (Kalchofner et al. 2006). Only 4 out of 300 horses needed artificial ventilation and only one horse had a poor recovery quality. The effects of a CRI of dexmedetomidine, the dextrorotatory active enantiomer of medetomidine, were studied in isoflurane anaesthetised ponies (Marcilla et al. 2010). No severe side effects were observed. Cardiovascular function was well maintained: the typical decrease in heart rate was minimal and arterial blood pressure values were considered acceptable. However, further studies are necessary to investigate the dose-sparing effect of dexmedetomidine CRI.

Ketamine and S(+)-ketamine

In 1992, Muir and Sams investigated the effects of ketamine on the MAC of halothane in horses. The degree of MAC reduction was correlated with plasma ketamine concentrations and reached up to 37%. Cardiac output significantly increased during ketamine infusions. In comparison to the single use of halothane, the concurrent administration of ketamine and guaifenesin allowed for a reduced halothane MAC, a more stable anaesthesia and a lower need for dobutamine to maintain blood pressure (Spadavecchia et al. 2002). In horses receiving an infusion of ketamine and lidocaine in combination with isoflurane, the requirements for isoflurane, dobutamine and thiopental were lower than in horses receiving isoflurane alone (Enderle et al. 2008). The quality of recovery was comparable in both groups.

Unfortunately, ketamine can induce emergence reactions during the recovery period like muscular tremor and rigidity, excitation and ataxia (Muir & Sams 1992, Larenza et al. 2009). These effects are related to the plasma concentrations of ketamine and norketamine and to the length of infusion (Muir & Sams 1992). Therefore, the use of racemic ketamine infusion is limited to procedures lasting less than 2 hours (Larenza et al. 2009).

A recent study compared the recovery quality after low doses of racemic and S(+)-ketamine in 10 horses (Larenza et al. 2009). Heart rate values before surgical stimulation were comparable between both groups, but horses receiving racemic ketamine had significantly higher heart rates during surgical stimulation, suggesting a stronger analgesic effect of S(+)-ketamine. Horses in S(+)-ketamine group had significantly better recovery scores than horses in racemic ketamine group. Visual Analogue Scale (VAS) values were positively correlated to the length of the infusion in horses receiving racemic ketamine: the longer the duration of infusion, the worse was the quality of recovery. In horses receiving S(+)-ketamine, no such correlation was evident.

Opioids

The adjunction of opioids as part of balanced anaesthesia is widespread in human and small animal anaesthesia. However, their effect in horses is disputed. Opiods are often used in horses to provide analgesia during and after surgery and in combination with sedatives for restraint. Most opioids cause a

dose-dependent respiratory depression, and excitement and ataxia are frequent side-effects (Hall & Clarke 1991a). Morphine, butorphanol and alfentanil did not reduce the MAC of inhalant agents in horses (Matthews & Lindsay 1990, Pascoe et al. 1993, Steffey et al. 2003). Morphine had an inconsistent influence on the MAC of isoflurane, as some horses showed either an increase or a small decrease in isoflurane requirements (Steffey et al. 2003). A retrospective clinical study evaluated the effects of butorphanol on cardiovascular parameters in isoflurane anaesthetised horses (Hofmeister et al. 2008). Butorphanol deepened the anaesthetic plane and obtund sympathetic effects following surgical stimulation. No adverse effects on heart rate and arterial blood pressure were observed. Fentanyl, a synthetic μ-receptor agonist, was administered to horses anaesthetised with isoflurane (Thomasy et al. 2006). MAC of isoflurane was reduced by 18% at a fentanyl concentration of 13.31 ng $mL^{-1}$. A dose-dependent increase of arterial blood pressures was observed. Two horses needed active cooling of the skin to maintain their body temperature below 38.6˚C. One horse had a recovery of bad quality, falling down several times.

Central muscle relaxants

Guaifenesin and benzodiazepines can be added to balanced anaesthesia to increase muscle relaxation. However, both drug classes will impair recovery by causing ataxia during this crucial phase.

## 2.3 Ketamine and its isomers

### 2.3.1 History

Ketamine is a derivate of phencyclidine and belongs to the group of dissociative anaesthetics. It was developed by Parke-Davis in 1962 as part of an effort to find a safer anaesthetic alternative to phencyclidine. In 1965, it was tested for the first time in twenty volunteers (Domino et al. 1965). It was officially released for clinical use in humans in the US in 1970 and became popular as a battlefield anaesthetic (Jansen 2000).

Ketamine is still widely used in humans. The incidence of its recreational use increased through the end of the 20th century. That's why it is considered as a controlled narcotic substance in many countries worldwide.

In the seventies, racemic ketamine was also registered and marketed for cats. Since then, it was widely used as an anaesthetic in veterinary medicine because of its large therapeutic window in vertebrates.

In 1992 the Food and Drug Administration stated that separation of stereoisomers had not received appropriate attention and that focussing on this issue could open new horizons in therapeutics (Kohrs & Durieux 1998). In the late nineties, Pfizer launched Ketanest S®, a product containing the single S(+)-isomer for use in humans. In 2006, a formulation containing S(+)-ketamine was approved for use in cats in Switzerland.

### 2.3.2 Physicochemical characteristics

Ketamine is chemically related to phencyclidine (PCP) and cyclohexamine (Reich & Silvay 1989) (Figure 8). Its chemical formula is $C_{13}H_{16}ClNO$ and its systemic IUPAC name (International Union of Pure and Applied Chemistry) is 2-(2-chlorophenyl)-2-methylamino-cyclohexanone.

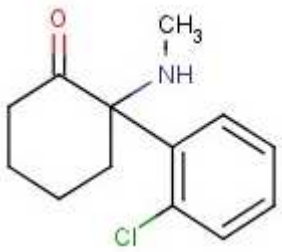
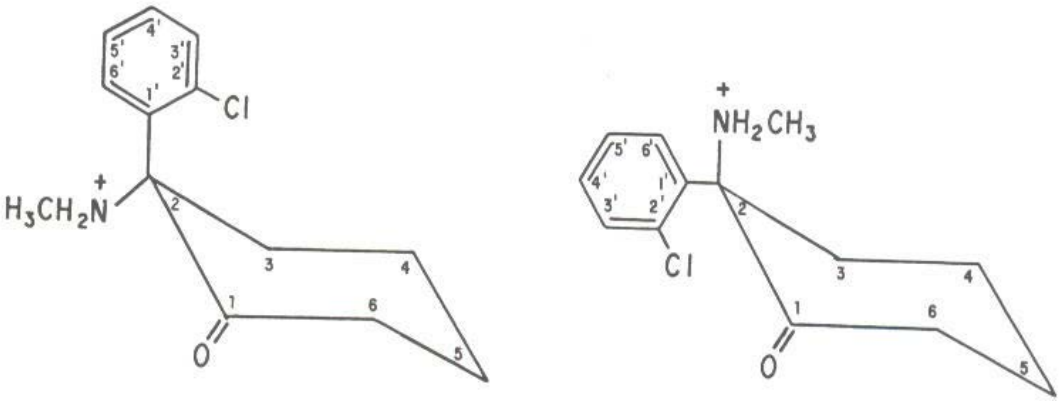



**Figure 8-** Molecular structure of ketamine (www.lookchem.com)

Ketamine has a molecular weight of 238 and a $pK_a$ of 7.5 (White et al. 1982). Although ketamine hydrochloride is water-soluble, ketamine has a high lipid solubility, ten times that of thiopentone (Reich & Silvay 1989). pH value of watery ketamine is between 3.5 and 5.5. Therefore tissue irritation may occur after intramuscular administration (Booth 1988).

Ketamine contains a chiral centre at the $C_2$-carbon of the cyclohexanone ring and therefore, two enantiomers exist: S(+)-ketamine and R(-)-ketamine (Reich & Silvay 1989) (Figure 9). The most widely commercially available ketamine

preparations contain a racemic mixture of the two enanatiomers, but the pure S(+)-enantiomer is also registered for clinical use in humans and cats.




S(+)-ketamine hydrochloride R(-)-ketamine hydrochloride

**Figure 9-** Ketamine enantiomers

### 2.3.3 Pharmacokinetics

Ketamine can be applied intramuscularly, subcutaneously and intravenously. Rectal, oral and nasal applications have also been described in humans (Ĉetina 1982, Paddleford & Erhardt 1992, Adams & Werner 1997), which made ketamine a popular drug in emergence medicine. Due to extensive first pass metabolism, plasma levels of ketamine are low after oral administration.

After parenteral administration, ketamine is rapidly absorbed. After a single intravenous administration, the highest ketamine plasma concentration is achieved after one minute in most species and within five to ten minutes following an intramuscular administration (Kaka et al. 1979, White et al. 1982, Waterman 1984, Adams & Werner 1997, Pypendop & Ilkiw 2005, Larenza et al. 2007).

Ketamine is first distributed in high-perfused tissues, including the brain. Because of its high lipid solubility, ketamine penetrates the blood brain barrier fast and develops its effects on the central nervous system (CNS) after a very short period of time (Cohen & Trevor 1974). The highest concentration in the brain is 4 to 6.5 times higher than that of the plasma (White et al. 1982, Adams & Werner 1997). This initial distribution from the central compartment (plasma) to peripheral tissues occurs with a half-life of 7 to 11 minutes (White et al. 1982). Subsequently, ketamine is redistributed from well-perfused tissues to

less perfused tissues. This redistribution has been found responsible for termination of the hypnotic and anaesthetic effects (Cohen & Trevor 1974, White et al. 1982). Ketamine is not strongly bound to plasma proteins (Kaka et al. 1979, Hanna et al. 1988a).

Ketamine is extensively metabolised in the liver. Cytochrome $P_{450}$ enzymes are involved in the metabolism of racemic ketamine. Ketamine is n-demethylated to norketamine. Norketamine possesses approximately 1/3 of the effect of the original compound (Craven 2007, Gehring et al. 2009). Norketamine is further hydroxylated to hydroxy-norketamine and then conjugated to a more water-soluble glucuronide derivate (5-6- dehydronorketamine) that is finally eliminated with the urine (Chang & Glazko 1974, White et al. 1982). Approximately 5% is eliminated with the faeces. The elimination half-life ranges between 1 to 3 hours (White et al. 1982, Hanna et al. 1988b, Adams & Werner 1997, Kohrs & Durieux 1998).

**Stereoselective pharmacokinetics**

Equianaesthetic doses of 1 mg $kg^{-1}$ S(+)-ketamine, 2 mg $kg^{-1}$ racemic ketamine and 3 mg $kg^{-1}$ R(-)-ketamine were administered to human patients (White et al. 1980). The dose-response curves of norketamine and the metabolite of norketamine were qualitatively identical among the three treatment regimens, indicating that there were no major differences in the pharmacokinetic properties of the enantiomers which could be attributed to stereospecificy.

However, a higher clearance was found for S(+)-ketamine, when compared to the racemate and R(-)-ketamine. In vitro data suggest that human microsomes n-demethylate S(+)-ketamine at a higher rate than R(-)-ketamine (Henthorn et al. 1999, Ihmsen et al. 2001). Ihmsen et al. (2001) also demonstrated that if racemic ketamine is used, the elimination of S(+)-ketamine is more rapid than that of R(-)-ketamine, but is inhibited by R(-)-ketamine. In the same way, in vitro biotransformation of ketamine in equine liver and lung occurs with a slower elimination of S(+)-ketamine in the presence of R(-)-ketamine (Schmitz et al. 2008).

Two stereoselective pharmacokinetic studies were performed in Shetland ponies (Larenza et al. 2007, Larenza et al. 2008a). In the first study, ponies were anaesthetised with isoflurane and were then administered either 2.2 mg

$kg^{-1}$ racemic ketamine or 1.1 mg $kg^{-1}$ S(+)-ketamine intravenously (Larenza et al. 2007). The pharmacokinetic parameters of R(-)- and S(+)-ketamine did not significantly differ. But plasma concentrations of S(+)-norketamine were higher than plasma concentrations of R(-)-norketamine in the racemic group. This result suggests that in the presence of isoflurane, the metabolism of racemic ketamine is highly stereoselective. In the second study, 2.2 mg $kg^{-1}$ racemic ketamine or 1.1 mg $kg^{-1}$ S(+)-ketamine was administered intravenously in ponies previously sedated with xylazine (Larenza et al. 2008a). Plasma concentrations of S(+)-norketamine were statistically different between both groups at three different time-points. Distribution of ketamine was not enantioselective. The first-order elimination rate for S(+)-ketamine was higher, while elimination half-life and mean residual time were lower in the S(+)-ketamine group, when compared to the racemic ketamine group. These results suggest that S(+)-ketamine elimination might be favoured when given alone. In addition, ponies receiving the S(+)-isomer stood up faster than ponies receiving the racemic mixture.

### 2.3.4 Pharmacodynamics

#### 2.3.4.1 Molecular pharmacodynamics

The molecular pharmacodynamics of ketamine is complex. It interacts with multiple binding sites, including N-methyl D-aspartate (NMDA) and non-NMDA glutamate receptors, nicotinic and muscarinic cholinergic, monoaminergic and opioid receptors (Kohrs & Durieux 1998). In addition, interactions with voltage-dependent ion channels such as $Na^+$ and $Ca^{2+}$ channels have been described (Wong & Martin 1993). All of these interactions may play a role in ketamine's pharmacological and clinical properties, but NMDA receptor antagonism is responsible for most of the analgesic, amnestic, psychomimetic and neuroprotective effects of the agent (Kohrs & Durieux 1998).

The NMDA receptor (Figure 10) is an ionotropic receptor that is activated by glutamate, the most important excitatory neurotransmitter in the central nervous system (Kohrs & Durieux 1998). The channel is permeable to $Ca^{2+}$ and, to a lesser extent, to $Na^+$ and $K^+$. Glycine is an obligatory co-agonist and $Mg^{2+}$

inhibits the channel in a voltage-dependent manner. NMDA receptors are involved in the wind-up phenomenon and probably play a major role in the development of chronic pain (Woolf 1989, Ilkjaer et al. 1996, Kress 1997).
Ketamine binds to the phencyclidine receptor in the NMDA channel and thus inhibits non-competively the glutamate activation (Kohrs & Durieux 1998).

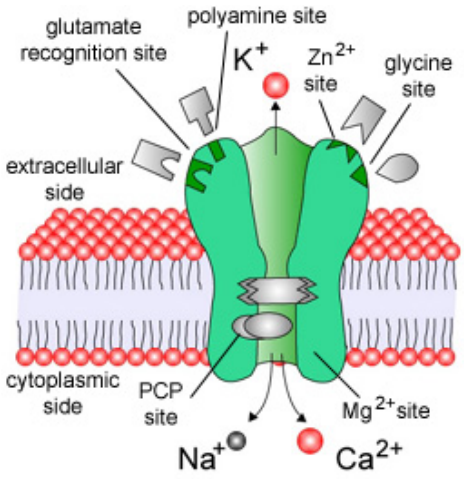



**Figure 10-** NMDA receptor (www.uochb.cz)

S(+)-ketamine has a three- to fourfold higher affinity for the phencyclidine binding site of the NMDA-receptor than the R(-)-enantiomer (Oye et al. 1992, Adams & Werner 1997, Kress 1997, Kohrs & Durieux 1998).
Non-NMDA glutamate receptors were demonstrated to be inhibited by ketamine (Gonzales et al. 1995). The effects are probably mediated through the glutamate/NO/cGMP system (Kohrs & Durieux 1998). Nitric oxide plays a role as neurotransmitter centrally and peripherically, and increases the intracellular cGMP production (Kress 1997). The NO synthase inhibition induced by ketamine may be involved in its analgesic effects (Gordh et al. 1995, Kohrs & Durieux 1998).
Ketamine also interacts with mu (μ), delta (δ) and kappa (κ) opioid receptors. Its affinity for these receptors is highest for μ, and lowest for δ receptor, and is 10 to 20 times less than for the NMDA channel. S(+)-ketamine has a two- to fourfold higher affinity to μ and κ opioid receptors than R(-)-ketamine (Kress 1997, Kohrs & Durieux 1998). The interaction of ketamine with opioid receptors seems not to be of major clinical importance (Kohrs & Durieux 1998), but a role

of κ opioid receptors in the occurrence of psychomimetic side effects seems to be established (Finck & Ngai 1982, Kohrs & Durieux 1998).

Ketamine also affects nicotinic and muscarinic acetylcholine receptors by inhibiting NMDA receptor-mediated acetylcholine release (Kress et al. 1997, Kohrs & Durieux 1998). In comparison to R(-)-ketamine, the S(+)-enantiomer shows a twofold stronger affinity for the muscarinic receptor (Kress et al. 1997). The affinity for this receptor is 10 to 20 times less than the binding to NMDA receptor (Kohrs & Durieux 1998). As seen for opioid receptors, emergence side effects during recovery phase may be partly related to the inhibition of the cholinergic transmission by ketamine (Kohrs & Durieux 1998).

Both ketamine enantiomers inhibit the neuronal uptake of noradrenaline, and S(+)-ketamine additionally inhibits the extraneuronal uptake (Adams & Werner 1997, Kress 1997).

Interactions with voltage-dependent ion channels have also been described (Kohrs & Durieux 1998). Inhibition of $Na^+$ channel function plays a role in the local application of ketamine, i.e. epidural injection (Kress 1997).

Gamma-amino-butyric receptors (GABA) are the most common inhibitory neurotransmitter in the central nervous system (Kohrs & Durieux 1998). Ketamine seems to have some effect on GABA receptors, but only in concentrations higher than those used for clinical purposes.

#### 2.3.4.2 Effects on the cardiovascular system

The cardiovascular activity of ketamine results of different indirect stimuli of the heart: sympathomimetic effects mediated within the central nervous system, inhibition of neuronal uptake of catecholamines by sympathetic nerve endings, direct vasodilatation of vascular smooth muscle and an inotropic effect on the myocardium (Muir & Hubbell 1988, Lin 2007).

Both, racemic ketamine and S(+)-ketamine, cause a dose dependent stimulation of heart rate and arterial blood pressures as well as an increase in cardiac output. These effects are caused by direct sympathomimetic activation (White et al. 1982, Adams & Werner 1997, Zielmann et al. 1997) and are particularly pronounced after the single use of ketamine. Studies in humans demonstrated that the administration of 2 mg $kg^{-1}$ racemic ketamine or 1 mg

$kg^{-1}$ S(+)-ketamine causes a comparable increase in heart rate and arterial blood pressure (Adams et al. 1992). In another study conducted by Doenicke et al. (1992a) in 30 healthy volunteers, no differences were found between racemic and S(+)-ketamine, as well. Different catecholamine levels were detected after administration of either racemic or S(+)-ketamine: epinephrine clearly increased after racemic ketamine administration, whilst only a slight variation occurred with the isomer (Doenicke et al. 1992b). However, norepinephrine levels similarly increased in both groups. The combined use with benzodiazepines prevented the centrally mediated sympathetic effects of ketamine on the heart. Therefore this combination is recommended in cardiac risk patients.

In horses, the clinical use of racemic ketamine was compared with S(+)-ketamine (Filzek et al. 2003). Horses were premedicated with xylazine and guaifenesin. Anaesthesia was induced with either 3 mg $kg^{-1}$ racemic ketamine or 2 mg $kg^{-1}$ S(+)-ketamine. Five repetitive xylazine/racemic ketamine, respectively xylazine/S(+)-ketamine doses were given intravenously to prolong anaesthesia. Both groups showed a decrease in heart rate, probably due to the administration of xylazine. In contrary to findings in humans, mean arterial blood pressure was higher in horses treated with S(+)-ketamine when compared to horses treated with the racemate.

At high doses (30 mg $kg^{-1}$), ketamine and its isomers induced a transient but significant cardiac depression for 15 minutes in dogs. Values returned to baseline values by 20 minutes after ketamine administration. No differences in the cardiopulmonary effects were noticed between ketamine or its isomers (Muir & Hubbel 1988).

Myocardial stimulation is associated with increased cardiac work and myocardial oxygen consumption (Lin 2007).

The direct effect of ketamine on the myocardium is controversial. The findings *in vivo* are not consistent with those reported *in vitro* (Graf et al. 1995, Adams & Werner 1997). Investigations with denervated heart preparations have demonstrated a dose-dependent depressant effect of ketamine on myocardial contractility (White et al. 1982, Lin 2007). On the isolated guinea pig heart, S(+)-ketamine had a significantly lesser cardiac depressant effect than did R(-)-ketamine (Graf et al. 1995). This finding was attributed to an increased

availability of catecholamines. However, *in vivo* the centrally acting effects of ketamine outweighed its depressing effect on the myocardium.

#### 2.3.4.3 Effects on the respiratory system

At usual clinical doses, ketamine has little effect on the respiratory system and causes minimal respiratory depression with only mild hypercapnia (Kohrs & Durieux 1998). In human patients suffering from asthma, ketamine is often used as an induction agent, because it reduces the airway resistance. In dogs, respiratory rate and minute volume decrease initially, but return to baseline values within 15 to 20 minutes (Haskins et al. 1985). Severe respiratory depression after an overdose has been reported in humans and felines (Lin 2007). If administered rapidly by intravenous injection, ketamine often induces a transient apnea. As it crosses the placental barrier (Hall & Clarke 1991b), respiratory depression may occur in the foetus (Adams & Werner 1997). Ketamine has a bronchodilatory effect (Reich & Silvay 1989), and pharyngeal and laryngeal reflexes are maintained (Lin 2007). Salivation and respiratory tract secretions increase after administration of ketamine. Care should always be taken to prevent airway obstruction or aspiration (Lin 2007). No differences between the isomers have been described in literature.

#### 2.3.4.4 Effects on the central nervous system (CNS)

Ketamine produces a dose-dependent central nervous system depression that leads to a "dissociative anaesthetic state" causing a catalepsy-like state, amnesia and profound analgesia. The mechanism of action suggests a functional and electrophysiological dissociation between the thalamocortical and the limbic systems (White et al. 1982, Kohrs & Durieux 1998). On molecular level, NMDA-receptor antagonism plays a major role in the mechanism of action of ketamine in the CNS.

In human patients, hallucinations and psychotomimetic reactions following ketamine anaesthesia may occur during recovery phase (Craven 2007). Mood alterations, extracorporeal experiences, floating sensations, illusions, and even delirium have also been described (White et al. 1982). A possible explanation

for the hallucinations and illusions is a ketamine-induced depression of the auditory and visual nuclei whereas the out-of-body experiences and floating sensations may be explained by the loss of skin and musculoskeletal sensations (White et al. 1982). When used in patients suffering from schizophrenia, ketamine may reactivate psychoses (Craven 2007). Therefore its use should be avoided in psychotic patients.

Ketamine has both pro- and anticonvulsant properties (Craven 2007). At low doses, ketamine may have anticonvulsant properties through antagonism of NMDA receptors (Reder et al. 1980). In some animal models, ketamine was effective in controlling recalcitrant seizures (Manno 2003). Some cases of human patients treated successfully in refractory status epilepticus have been described (Prüss & Holtkamp 2008, Varelas & Spanaki 2010). However, the occurrence of seizures in association with the administration of ketamine has been reported in dogs and cats (Mori et al. 1971, Lin 2007). Therefore, the risk-benefit-ratio of the use of ketamine in animals with a history of seizures or epilepsy should be assessed carefully.

Ketamine has been demonstrated to increase cerebral blood flow (CBF), intracranial pressure (ICP) and cerebrospinal fluid pressure as a result of cerebral vasodilatation and elevated systemic blood pressure (Lin 2007). However, the mechanism of action of ketamine on ICP is controversial. Some studies did not demonstrate any adverse effects of ketamine on CBF and ICP (Craven 2007). Most probably, differences in ventilation strategies may explain earlier findings (Schmidt et al. 2008). Independent of the pre-existing ICP, ketamine did not increase ICP when normocapnia was maintained with controlled ventilation (Pfenninger et al. 1984). In piglets with pre-existing intracranial hypertension, administration of ketamine caused an increase in both ICP and $pCO_2$ (Pfenninger et al. 1985). The ICP rise seems to be secondary to the hypercapnia. As a result of these studies it has been suggested that controlled ventilation should always be initiated when ketamine is administered to patients with intracranial disease (Lin 2007).

Neuroprotective and neuroregenerative effects of ketamine have been discussed recently. Ketamine, administered post-injury, has been demonstrated to have neuroprotective effects (Himmelseher et al. 1996). Post-ischemic administration of S(+)-ketamine had a positive influence on oxygen saturation

and cortical neuronal cell death after global forebrain ischemia (Proescholdt et al. 2001). In rat hippocampal neurons, neuroregenerative properties were demonstrated by S(+)-ketamine, but not by the racemate (Himmelseher et al. 1996).

#### 2.3.4.5 Analgesic effects

Sub-anaesthetic doses of ketamine produce a dose-dependent analgesia in humans and animals (Ryder et al. 1978, Finck & Ngai 1982, Arendt-Nielsen et al. 1996, Ilkjaer et al. 1996, Wagner et al. 2002). The degree of analgesia appears to be greater for somatic than for visceral pain (Haskins et al. 1985, Lin 2007).

In humans, rats and mice, the analgesic potency of S(+)-ketamine was reported to be twice that of the racemic mixture and four times that of the R(-)-isomer (Ryder et al. 1978, Mathisen et al. 1994, Arendt-Nielsen et al. 1996, Oye et al. 1992, Kohrs & Durieux 1998).

NMDA receptors are involved in hyperalgesic responses after tissue injuries. Hyperalgesia occurs when nociceptors are sensitised by an inflammatory response that reduces the threshold level of neuronal excitability. It is presumed that ketamine may be effective at reducing hyperalgesia (Lin 2007). *In vivo*, low doses of S(+)-ketamine applied before a high-frequency stimulation, blocked the long-term potentiation in the rat spinal cord (Benrath et al. 2005). The combined use with fentanyl induced a reduction of C-fibre evoked potentials and prevented long-term potentiation in pain pathways. In inhibiting central summation of pain in men, S(+)-ketamine was approximately twice as potent than ketamine racemate (Arendt-Nielsen et al. 1996). In horses, pre-emptive epidural ketamine reduced post-incisional pain (Rédua et al. 2002). In dogs undergoing forelimb amputation, peri-operative treatment with low doses of ketamine was associated with a slightly improved postoperative period, suggesting an analgesic effect (Wagner et al. 2002). An antinociceptive effect of ketamine, demonstrated as a depression of the nociceptive withdrawal reflex, was detected after a constant rate infusion in standing Shetland ponies (Knobloch et al. 2006, Peterbauer et al. 2008). In the study of Peterbauer et al. (2008), antinociceptive effects failed to be demonstrated after administration of

S(+)-ketamine. This lack of effect may be explained by the fact that S(+)-ketamine plasma levels after racemic ketamine administration were considerably higher than in ponies receiving S(+)-ketamine.

#### 2.3.4.6 Anaesthetic and post-anaesthetic emergence effects

Ketamine is a dissociative anaesthetic commonly used in humans and animals. It has also been reported to produce local anaesthesia (Hirota & Lambert 1996). In humans, it is clinically used for indications such as induction of anaesthesia in haemodynamically compromised patients, induction of anaesthesia in patients suffering from asthmatic disease, anaesthesia for short, painful surgeries such as dressing changes in burn patients, intramuscular induction in non-cooperative patients and children and supplementation of incomplete regional or local anaesthesia (Hempelmann & Kuhn 1997, Kohrs & Durieux 1998). In animals, ketamine has been shown to reduce requirements for inhalant anaesthetics, and therefore it is commonly used for balanced anaesthesia (Pypendop & Ilkiw 2005).

In rats and mice, S(+)-ketamine has a 1.5 to 3-fold greater hypnotic potency than the R(-)-isomer (Ryder et al. 1978, White et al. 1980). The calculated index of S(+)-ketamine is greater than that of racemic ketamine and the R-isomer (Marietta et al. 1977). In humans, S(+)-ketamine induced the same depth of anaesthesia with only half of the dose of racemic ketamine (Doenicke et al. 1992a). In cats, the S(+)-isomer has been proven to be advantageous over racemic ketamine in obtaining an identical depth of anaesthesia with only 60% of the racemic dose (Wiederstein & Auer 2003). In a study performed in dogs, the anaesthetic potency between racemic ketamine and S(+)-ketamine was 1:1.29 (Duque et al. 2008). Deleforge et al. (1991) demonstrated that S(+)-ketamine was three times more potent than the R(-)-isomer in dogs. In horses, only 2/3 of the racemic dose was necessary to maintain equivalent levels of anaesthesia with S(+)-ketamine (Filzek et al. 2003). In another study, horses were anaesthetised with either 2 mg $kg^{-1}$ racemic ketamine or 2 mg $kg^{-1}$ S(+)-ketamine in combination with diazepam (Rossetti et al. 2008). S(+)-ketamine was found to be advantageous over racemic ketamine by requiring less

anaesthetic agents during surgery and by showing a better overall recovery quality.

Emergence from ketamine anaesthesia is dose-dependent and can be complicated by the occurrence of unpleasant psychotomimetic reactions (Kohrs & Durieux 1998). Nausea and vomiting, alterations in mood state, amnesia, dissociative or out-of-body experiences, floating sensations, nightmares, illusions and delirium have been described in humans (White et al. 1982, Kohrs & Durieux 1998). Therefore, its combination with benzodiazepines is recommended. In volunteers, S(+)-ketamine was associated with a more rapid recovery of vigilance (White et al. 1985). Even if no difference in the frequency of dreams was observed, patients felt more comfortable after S(+)-ketamine (Doenicke et al. 1992a) and a larger proportion of patients would accept a repeat anaesthesia with S(+)-ketamine compared to racemic ketamine (85% vs. 65%) (White et al. 1980). In cats and horses, anaesthesia with S(+)-ketamine was also associated with an overall better recovery quality (Filzek et al. 2003, Larenza et al. 2008b, Rossetti et al. 2008, Larenza et al. 2009).

# 3. MATERIALS AND METHODS

## 3.1. Study design and animal selection

The present study was performed to support the data on clinical efficacy for the registration of S(+)-ketamine, a new medicinal product for use in horses. Originally, it was planned to compare a CRI of racemic ketamine with a CRI of S(+)-ketamine in isoflurane anaesthetised horses. Therefore, a pilot study comparing the effects on recovery quality of a CRI of racemic ketamine with a CRI of the single S(+)-isomer was performed in 10 horses (Larenza et al. 2009). The results of this preliminary study showed that recovery in horses anaesthetised with a CRI of ketamine racemate was of low quality, and the risk for the horses to get injured was considered too high for further evaluation. Therefore, it was decided to adapt the study design. Scientific advice prior to submission of the application for a marketing authorisation was thus requested from the Swiss Agency of Therapeutic Products (Swissmedic). After consultation with Swissmedic it was decided to compare the CRI of S(+)-

ketamine with the standard anaesthesia protocol from the University of Zurich using a CRI of medetomidine and racemic ketamine as an induction agent. Horses allocated to the Med group were therefore anaesthetised with racemic ketamine, whereas in horses allocated to the S-ket group, anaesthesia was induced with S(+)-ketamine as part of a completely new anaesthetic protocol.

The experiment was performed with the permission of the local Committee for Animal Experimentation. The study was conducted as a randomised, blinded, prospective clinical trial according to the Good Clinical Practice (GCP) guidelines. In total, 50 horses (16 mares, 19 stallions and 15 geldings), with a mean ± SD weight of 524 ± 97 (260 – 725) kg entered the study. Horses were aged between one and eighteen (7.7 ± 4.7) years. The horses were randomly allocated to two groups, containing 25 animals each: "Med group" and "S-ket group". Horses in Med group received a constant rate infusion (CRI) of medetomidine and horses in S-ket group received a CRI of S(+)-ketamine as part of a balanced anaesthetic regimen with isoflurane. The anaesthetist remained unaware of the group allocation.

Prior to anaesthesia induction, a careful physical examination was performed. Body weight, heart rate, respiratory rate, body temperature, mucous membrane colour, capillary refill time, pulse character and thoracic auscultation were assessed. Furthermore, haematocrit and plasma total proteins were analysed. Horses were classified according to the American Society of Anaesthesiologists' (ASA) physical status classification in ASA risk groups I to V. After careful clinical examination, they were included in the study if they matched following criteria: ASA I, II or III patients, horses undergoing elective surgery in every part of the body but the head or neck region, patients that were expected to recover from anaesthesia without assistance and patients with physiological heart arrhythmias only. On the other hand, patients allocated to ASA IV or V, horses with pre-anaesthetic pathological cardiovascular or haemodynamic disorders, pregnant mares, patients undergoing surgery of the head or neck region, horses with severe fractures or orthopaedic conditions that were expected to require assisted recovery, emergency patients or non-cooperative animals that required intramuscular sedation were excluded from the study.

### 3.2 Animal management and housing

Until anaesthesia induction, the horses were housed individually in conventional boxes at the stables of the Equine Clinic of the Vetsuisse Faculty in Zurich. Their owners were informed on the trial procedures and general anaesthesia risks and requested to sign a consent form. Immediately after surgery and until complete recovery of motor function, the horses were placed into a padded box until their release. During anaesthesia induction and recovery, the horses wore a protective helmet and bandages on their extremities. During the recovery phase, oxygen was provided through a nasal tube until achieving sternal position.

### 3.3 Animal feeding

The horses were fed according to their medical condition. Eight to sixteen hours before surgery, food was withheld to avoid the hazards of food aspiration after anaesthesia induction and to reduce the effects of intra-operative tympanism that may interfere with free movement of diaphragm and perturb breathing. As excessive fasting can encourage gut stasis and therefore increase the risk of post-anaesthetic colic (Hall & Clark 1991c), the horses were not starved for a longer time period. Free access to water was provided until half an hour prior to anaesthesia induction and after complete recovery from anaesthesia.

### 3.4 Treatments

The same anaesthetist, who was unaware of the group allocation, performed all anaesthetic episodes.

#### Anaesthesia Induction

A 14-gauge x 16 mm over the needle catheter (Secalon® T with flowswitch, Becton Dickinson AG, Basel, Switzerland) was placed into the jugular vein after skin desensitization with 2 mL mepivacaine 2%. Thirty minutes before anaesthesia induction, patients in both groups received an intravenous injection of 35'000 IU $kg^{-1}$ penicilline sodium (Penicilline Natrium Streuli 10 Mio UI ad us.

vet., G. Streuli & Co AG, Uznach, Switzerland), 7 mg $kg^{-1}$ gentamicine (Vetagent® ad us. vet., Injektionslösung, Veterinaria AG, Zurich, Switzerland) and 1 mg $kg^{-1}$ bodyweight flunixin meglumine (Fluniximin® ad us. vet., Injektionslösung, Dr. E. Graeub AG, Bern, Switzerland) and an intramuscular injection of 0.03 mg $kg^{-1}$ acepromazine (Prequillan® ad us. vet., Injektionslösung, Arovet AG, Zollikon, Switzerland). Patients were relocated to the surgical suite. All horses received an intravenous dose of 7 µg $kg^{-1}$ medetomidine (Domitor® ad us. vet., Injektionslösung, Pfizer AG, Zurich, Switzerland) as a slow bolus over 2 minutes. Approximately 7 to 10 minutes after medetomidine injection, and when sedative effects were achieved, the horses received 0.03 mg $kg^{-1}$ of intravenous midazolam (Dormicum® Ampullen, Roche Pharma AG, Reinach, Switzerland) followed by a rapid intravenous bolus of either 1.1 mg $kg^{-1}$ S(+)-ketamine (Keta-S® ad us. vet., Injektionslösung, Dr. E. Graeub AG, Bern, Switzerland) for patients in the S-ket group or 2.2 mg $kg^{-1}$ racemic ketamine (Ketasol ad us. vet., Injektionslösung, Dr. E. Graeub AG, Bern, Switzerland) for patients in the Med group.

To preserve blinding of the study, an assistant veterinarian prepared all induction agents, which were diluted with physiologic saline solution up to a final volume of 20 mL. After induction of anaesthesia, horses were intubated with a cuffed rubber-silicone tube and connected to a large animal anaesthesia machine via a large animal circle system. Anaesthesia was maintained with isoflurane (Isoflo® ad us. vet., Inhalationsanästhetikum, Abbott AG, Baar, Switzerland) in oxygen (3 - 5 L $min^{-1}$, Fi'O2 around 0.7) to an initial end-tidal concentration of 1.2%. They were then positioned on the surgical table in lateral or dorsal recumbency, depending on the surgical approach. A catheter was placed in the urinary bladder of all horses.

**Anaesthesia maintenance**

The lungs of the horses were mechanically ventilated to maintain the end-tidal carbon dioxide pressure between 45 and 55 mmHg. Isoflurane delivery was always adjusted by the same experienced anaesthetist to the minimal end-tidal concentration (FE'iso) required to prevent the horse from moving, muscle rigidity or nystagmus and to maintain corneal or blinking reflexes. If sudden

movements or nystagmus occurred during anaesthesia, a bolus of 0.1 to 0.5 mg $kg^{-1}$ thiopental (Pentothal®, Ospedalia AG, Hühnenberg, Switzerland) was administered intravenously. The total administered amount of thiopental was recorded.

An infusion of 10 mL $kg^{-1}$ $hour^{-1}$ of lactated Ringer's solution (RLS) (Ringer-Lactat Lösung, Fresenius Kabi AG, Stans, Switzerland) was delivered during the entire anaesthetic procedure through the jugular catheter. The patients in the S-ket group received a constant rate infusion (CRI) of 0.5 mg $kg^{-1}$ $hour^{-1}$ S(+)-ketamine and those in the Med group a CRI of 3.5 µg $kg^{-1}$ $hour^{-1}$ medetomidine through the jugular catheter. The RLS line was connected to a three-port stop cock which allowed for other supportive drug administration (i.e. dobutamine). This stop cock was connected to an extension tube which in turn was connected to two three-way stop cocks in parallel, one for the CRI delivery and the other one for lithium/thiopental administration. In order to avoid retrograde administration of solutions or drugs, unidirectional flow-valves were placed in between the above-mentioned stop cocks and extensions.

S(+)-ketamine and medetomidine CRIs were administered by use of a syringe infusion pump (Phoenix 700, Schoch electronics, Möriken, Switzerland), each via a 2 x 1.5-m-long (total volume: 6 mL) non-distensible extension tube connected to the intravenous catheter by the proximal three-way valve. To ensure accuracy in calculation of the infused volumes of S(+)-ketamine and medetomidine, the extension tubes were filled with 6 mL of drug solution and the initial volume contained in the syringe was recorded. At the end of the procedure, the infused volume of drug was calculated from the initial volume minus the volume that remained in the syringe. Medetomidine (1 mg $mL^{-1}$) and S(+)-ketamine (60 mg $mL^{-1}$) were diluted in physiologic saline (0.9% NaCl) solution to a working concentration of 0.1 mg $mL^{-1}$ and 14.3 mg $mL^{-1}$, respectively.

**Anaesthesia monitoring**

A 18-gauge cannula (Surflo® IV Catheter 22G x 1", Terumo®, Medical Solution GmbH, Cham, Switzerland) was inserted into a facial artery and connected via a non-distensible extension tube filled with heparinized saline to an electronic pressure transducer. The transducer was placed and zeroed at the level of the

shoulder for horses placed in dorsal recumbency or at the level of the sternal manubrium for horses placed in lateral recumbency. Systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressures were obtained. Oxygen saturation ($SpO_2$) was obtained via a pulse oximetry infrared probe placed around the tongue. A lead II electrocardiogram (ECG) was displayed, and heart rate (HR) was calculated. Inspired oxygen fraction ($Fi'O_2$), end-tidal isoflurane concentration (FE'iso) and end-tidal carbon dioxide partial pressure ($PE'CO_2$) were obtained from a side-stream gas sampler plugged to a port placed at the bifurcation of the Y-piece of the breathing system. Respiratory rate (RR) was calculated from the capnogram. All data was continuously measured with a monitor (Datex S5, Helsinki, Finland) and recorded every 5 minutes. Prior to each anaesthetic episode, the monitor was calibrated with a standardized calibration gas according to the manufacturers' instructions. Values for peak inspiratory pressure were obtained from a manometer placed within the ventilator.

Arterial blood samples were anaerobically collected into syringes containing heparin and analysed immediately. Arterial blood pH, partial pressure of oxygen ($PaO_2$), partial pressure of carbon dioxide ($PaCO_2$), bicarbonate concentration ($HCO_3^-$), total carbon dioxide concentration ($TCO_2$), base excess (BE), haemoglobin concentration (Hb) and haemoglobin oxygen saturation ($SaO_2$) were assessed by use of a blood gas machine (i-STAT analyzer and G3+ cartridges, Axon Lab AG, Baden-Dättwil, Switzerland).

The degree of impairment of alveolar oxygen exchange was estimated with the $PaO_2/Fi'O_2$ index.

**Treatment of intra-operative hypotension/hypertension and hypoxaemia**

Dobutamine (Dobutrex®, Eli Lilly S.A., Geneva, Switzerland) was diluted in a saline (NaCl) solution plus 5% glucose to a final concentration of 1 mg $mL^{-1}$. After anaesthesia induction, an initial dobutamine infusion was started at a dose rate of 0.01 mg $kg^{-1}$ $hour^{-1}$ and subsequently adjusted to keep a target mean arterial pressure (MAP) between 70 and 100 mmHg.

If MAP was below 70 mmHg, the dobutamine infusion rate was increased by 0.01 mg $kg^{-1}$ $hour^{-1}$ every 5 minutes until target MAP was reached. In contrast, if MAP was above 100 mmHg, the infusion rate was reduced by 0.01 mg $kg^{-1}$

hour$^{-1}$ every 5 minutes, or discontinued if the rate was already 0.01 mg kg$^{-1}$ hour$^{-1}$, until target MAP was reached. At the end of the procedure, the total dose rate of dobutamine was calculated.

If intra-operative $PaO_2$ fell below 70 mmHg, salbutamol (Ventolin® Dosier-Aerosol, GlaxoSmithKline AG, Münchenbuchsee, Switzerland) was delivered through the endotracheal tube at a dose of 2 µg kg$^{-1}$ every 20 minutes until a $PaO_2$ ≥ 70 mmHg was achieved. The total dose of salbutamol to provide a $PaO_2$ ≥ 70 mmHg was calculated at the end of the procedure.

**Cardiac output (CO) and cardiac index (CI)**

Cardiac output was measured 20, 45, 85, 150 and 210 minutes after anaesthesia induction, if the length of anaesthesia allowed for it, using the lithium dilution technique (LiDCO) (Linton et al. 2000). This method involved injecting the indicator, lithium chloride, into a catheter inserted into the jugular vein and measuring the diluted concentration of lithium in a blood sample obtained from a peripheral arterial site using a sensor that is selective for lithium. The housing for the sensor included inlet and outlet ports. The inlet port was attached to the catheter inserted in the transverse facial artery via a 3-way valve, and the outlet port attached via tubing to a disposable blood collection bag. The tubing between the sensor and collection bag passed through a flow regulator pump. When the pump was activated, arterial blood passed through the sensor at a constant rate (4 mL min$^{-1}$). This sensor consisted of a flow through cell housing a lithium-selective electrode. When the lithium-selective membrane was in contact with blood, the voltage across it was related to plasma lithium concentration by the Nernst equation. This voltage was measured by use of an isolated amplifier, digitized on-line, and analysed with a LiDCO cardiac monitor. To measure cardiac output via this technique, the LiDCO cardiac monitor required the input of the sensor constant, the injection dose of lithium chloride, haemoglobin concentration and serum sodium concentration of each horse. When the baseline voltage recorded from the lithium electrode was stable, the injection dose was placed into an extension set attached to the jugular catheter. A subsequent volume (10 mL) of heparinized saline (0.9% NaCl) solution was then injected rapidly to flush in the lithium chloride. A dose rate of lithium chloride was adjusted to achieve an ideal

lithium concentration between 0.2 and 0.8 mM (1.5 mL of lithium chloride 1.5 molar). Thus, an optimal adequacy of measurement was guaranteed. The information generated by the sensor and transmitted to the LiDCO monitor generated a concentration time curve for lithium. The obtained area under the curve (AUC) after the first peak represented the stroke volume (SV). Cardiac output (CO, L $min^{-1}$) was calculated as SV x HR. The cardiac index (CI) was calculated by using 2 formulas:

- **CI (mL $min^{-1}$ $kg^{-1}$) = [CO/BW] x 1000**

where bodyweight (BW) was measured in kilograms

- **CI (L $min^{-1}$ $m^{-2}$) = CO/BSA**

where body surface area (BSA) = 10.5 x body weight $(g)^{2/3}$ x $10^{-4}$ ($m^2$)

**Anaesthesia recovery**

Twenty minutes before the end of the surgical procedures, 0.1 mg $kg^{-1}$ morphine (Morphin HCl Sintetica, Sintetica SA, Mendrisio, Switzerland) was administered intramuscularly for postoperative analgesia. After surgical procedure, the ventilator was switched off and the patients were left to recover spontaneous respiration. Each horse was placed into a padded recovery box and oxygen was delivered through the nostrils (10 L $min^{-1}$). The whole recovery period was video recorded for further analysis. At the first sign of swallowing and/or nystagmus, the endotracheal tube was removed, post anaesthetic sedation administered (both groups: medetomidine 2 µg $kg^{-1}$, IV) and the time recorded. Times to achieve sternal and standing positions were recorded. After complete recovery from anaesthesia, the patients were transferred to their respective boxes in the clinic.

By use of the video recordings, the quality of anaesthesia recovery was evaluated by four experienced anaesthetists (all Dipl ECVAA) of the Anaesthesia Section of the Equine Department of the Vetsuisse Faculty of Zurich, by using a modified score recovery quality system (numeric rating score, NRS) for horses (Table 1).

**Table 1-** Description of the numeric rating score (NRS) used for assessing the quality of the recovery phase (adapted from Mama et al. 1996)

| Score | Description |
|---|---|
| 1 | Excellent<br>Quiet, coordinated efforts (1 - 2) to achieve sternal and standing position. No or light ataxia once standing. Calm. |
| 2 | Good<br>Quiet, slightly uncoordinated efforts (1 - 2) to achieve sternal or standing positions. Mild ataxia once standing. Calm. |
| 3 | Fair<br>Multiple (≥ 3) quiet attempts to achieve sternal and standing positions. Mild to considerable ataxia once standing. Calm. |
| 4 | Moderate<br>Multiple (≥ 3) uncoordinated attempts to achieve sternal and standing positions. Moderate excitement. |
| 5 | Poor<br>Unable to stand up two hours after extubation. Horse required additional sedation because of severe excitement. |

In addition, a visual analogue scale (VAS) from 0 mm (best possible recovery) to 100 mm (worst possible recovery) was used.

### 3.5 Statistical methods

The NCSS 2004 software package (Kaysville, Utah, USA) was used to perform the statistical evaluation.

All data was analysed for normal distribution with the Shapiro-Wilk W test. The significance of difference between groups for parametric data was assessed with Student's *t*-tests and for non-parametric variables by using the Mann-Whitney U and Kruskal-Wallis tests. Proportions were evaluated with Chi-Square tests. For all tests $p < 0.05$ was considered the minimum level of statistical significance.

For intra-anaesthetic continuous response variables, which included heart rate, respiratory rate, arterial blood pressures, end-tidal concentration of isoflurane, end-tidal carbon dioxide partial pressure, inspired oxygen fraction, cardiac output, cardiac index and arterial blood gas values ($PaO_2$, $PaCO_2$, $SaO_2$, pH), all available measurements were included in the statistics, accepting that therefore animals would not be represented equally in the dataset.

Recovery scores (VAS and NRS) obtained from the 4 observers were averaged to obtain a single score for each horse. For each group, recovery quality parameters were associated with the length of infusions and with the times to achieve the sternal and standing positions by use of the Pearson correlation coefficient (*r*) and linear regression analysis, and significance was tested by use of the Student *t* test.

Parametric data is presented as mean ± standard deviation and non-parametric data is presented as median, range, interquartile ranges (box plots) or by use of descriptive statistics.

## 4. RESULTS

### 4.1 Study animals and pre-anaesthesia examination

Animal details

A total of 50 horses were enrolled in the study. All horses were allocated to two groups of 25 individuals each. Most of them (70%) were warmblood horses. The rest of the population consisted in Quarter horses (8%), thoroughbreds (6%), Freibergers (4%), ponies (4%), Pure Spanish Horses (2%), Palominos (2%), Arabian horses (2%) and Friesian horses (2%).

Demographics of horses are detailed in the tables below (Tables 2a & b).

**Table 2a-** Demographics of horses allocated to Med group (F = female, M = male, MC= male castrated)

| CRF | Age (years) | Weight (kg) | Breed | Sex | Surgery |
|---|---|---|---|---|---|
| 1 | 17 | 725 | Irish Warmblood | MC | neurectomy |
| 3 | 3 | 455 | CH Warmblood | M | castration |
| 4 | 2 | 420 | Thoroughbred | M | castration |
| 8 | 8 | 555 | CH Warmblood | F | arthroscopy |
| 9 | 2 | 420 | CH Warmblood | MC | arthroscopy |
| 10 | 6 | 500 | Quarter Horse | F | arthroscopy |
| 13 | 18 | 610 | Selle Français | MC | sarcoid |
| 14 | 7 | 625 | Holstein | MC | arthroscopy |
| 18 | 8 | 550 | Hanoverian | MC | arthroscopy |
| 21 | 7 | 515 | Freiberger | F | arthroscopy |
| 22 | 1 | 260 | CH Warmblood | F | plate removal |
| 24 | 6 | 520 | Hanoverian | F | osteophyte removal |
| 26 | 1 | 370 | CH Warmblood | M | castration |
| 28 | 14 | 620 | German Warmblood | MC | arthroscopy |
| 29 | 13 | 460 | Warmblood | M | castration |
| 30 | 3 | 480 | CH Warmblood | M | castration |
| 34 | 8 | 580 | CH Warmblood | F | arthroscopy |
| 35 | 1 | 295 | Quarter Horse | M | castration |
| 37 | 3 | 480 | CH Warmblood | M | castration /chip |
| 39 | 9 | 670 | CH Warmblood | MC | arthroscopy/ tendovaginoscopy |
| 44 | 3 | 350 | Icelandic Pony | M | castration |
| 45 | 5 | 500 | Quarter Horse | M | arthroscopy |
| 46 | 5 | 620 | Freiberger | F | fetlock/arthroscopy |
| 47 | 15 | 410 | Pony | F | wound flush |
| 49 | | 660 | Holstein | F | desmotomy |
| Mean | | 506 | | | |
| SD | | 118 | | | |
| Median | 6 | | | | |
| Min | 1 | | | | |
| Max | 18 | | | | |

**Table 2b-** Demographics of horses allocated to S-ket group (F = female, M = male, MC= male castrated)

| CRF | Age (years) | Weight (kg) | Breed | Sex | Surgery |
|---|---|---|---|---|---|
| 2 | 2 | 520 | CH Warmblood | M | arthroscopy/castration |
| 5 | 18 | 500 | Russian Horse | MC | splint bone resection |
| 6 | 4 | 620 | Selle Français | M | castration/hernia |
| 7 | 9 | 540 | CH Warmblood | M | castration |
| 11 | 4 | 630 | German Warmblood | MC | sequester |
| 12 | 5 | 440 | Arabian Horse | M | castration |
| 15 | 11 | 630 | Trakehner | F | neurectomy |
| 16 | 9 | 510 | PRE | M | neurectomy |
| 17 | 10 | 580 | Holstein | MC | arthroscopy |
| 19 | 6 | 600 | Selle Français | M | castration |
| 20 | 3 | 465 | Friesian | M | castration |
| 23 | 9 | 430 | Quarter Horse | F | Tenovaginoscopy/ desmotomy |
| 25 | 10 | 540 | CH Warmblood | M | castration |
| 27 | 9 | 380 | Iceland | F | exostosis |
| 31 | 3 | 520 | CH | M | castration |
| 32 | 13 | 545 | CH | MC | arthroscopy |
| 33 | 6 | 500 | Thoroughbred | MC | arthroscopy |
| 36 | 15 | 580 | Irish Warmblood | MC | neurectomy |
| 38 | 8 | 570 | CH Warmblood | F | joint flush |
| 40 | 13 | 650 | Oldenburg | MC | arthroscopy/neurectomy |
| 41 | 11 | 600 | Italian Warmblood | F | arthroscopy |
| 42 | 12 | 575 | CH Warmblood | F | arthroscopy |
| 43 | 3 | 500 | Poland Warmblood | M | arthroscopy/castration |
| 48 | 13 | 590 | Oldenburg | MC | neurectomy |
| 50 | 5 | 550 | Thoroughbred | F | arthrodesis |
| Mean | | 542 | | | |
| SD | | 67 | | | |
| Median | 9 | | | | |
| Min | 2 | | | | |
| Max | 18 | | | | |

There were no differences between groups in regard to distribution of sex (Table 3) ($p$ = 0.83), ASA grading (Table 4) ($p$ = 0.19), age ($p$ = 0.13; Med group: 6 [1 – 18] years; S-ket group: 9 [2 – 18] years) and body weight ($p$ = 0.18; Med group: 506 ± 118 kg; S-ket group: 542 ± 67 kg).

**Table 3-** Distribution of sex (F = female, M = male, MC= male castrated)

| Group | F | M | MC | Total |
|---|---|---|---|---|
| **Med group** | 9 (36%) | 9 (36%) | 7 (28%) | 25 (100%) |
| **S-ket group** | 7 (28%) | 10 (40%) | 8 (32%) | 25 (100%) |
| **Total** | 16 (32%) | 19 (38%) | 15 (30%) | 50 (100%) |

**Table 4-** Distribution of ASA grading (I=normal healthy patient; II=patient with mild systemic disease; III=patient with severe systemic disease)

| | I | II | III | Total |
|---|---|---|---|---|
| **Med group** | 6 (24%) | 16 (64%) | 3 (12%) | 25 (100%) |
| **S-ket group** | 8 (32%) | 17 (68%) | 0 (0%) | 25 (100%) |
| **Total** | 14 (28%) | 33 (66%) | 3 (6%) | 50 (100%) |

No differences between groups in regard to distribution of surgery types were detected ($p$ = 0.18; "IV": invasive orthopaedic procedures, "NIV": minimally invasive orthopaedic or dermatological procedures, "Neu": neurectomies and "Ctr": castrations) (Table 5). If two procedures were performed simultaneously, horses were allocated to the group considered to cause more nociceptive stimulation (i.e. castration and non-invasive arthroscopy allocated to "Ctr").

**Table 5-** Distribution of surgery types (IV=invasive orthopaedic procedures; NIV=minimally invasive orthopaedic or dermalogical procedures; Neu= neurectomies; Ctr= castrations)

| | IV | NIV | Neu | Ctr | Total |
|---|---|---|---|---|---|
| **Med group** | 4 (16%) | 12 (48%) | 1 (4%) | 8 (32%) | 25 (100%) |
| **S-ket group** | 5 (20%) | 6 (24%) | 5 (20%) | 9 (36%) | 25 (100%) |
| **Total** | 9 (18%) | 18 (36%) | 6 (12%) | 17 (34%) | 50 (100%) |

Baseline heart rate (HR) ($p$ = 0.96), body temperature (T°C) ($p$ = 0.20), haematocrit (Hto) ($p$ = 0.72) and total proteins (TP) ($p$ = 0.42) obtained before pre-medication were also not different between groups. There was only a trend for differences in respiratory frequencies RR ($p$ = 0.06).

**Table 6a-** Pre-clinical examination performed in horses allocated to Med group

| CRF | HR | RR | T | Hto | TP | Pulse | ASA |
|---|---|---|---|---|---|---|---|
| 1 | 28 | 8 | 38 | 41 | 62 | strong | II |
| 3 | 36 | 8 | 37.9 | 31 | 58 | strong | II |
| 4 | 36 | 12 | 37.8 | 35 | 60 | good | II |
| 8 | 40 | 16 | | 34 | 57 | good | II |
| 9 | 32 | 12 | 38 | 23 | 62 | strong | II |
| 10 | 40 | 14 | 37.7 | 40 | 66 | strong | II |
| 13 | 34 | 12 | 37.9 | 29 | 63 | strong | II |
| 14 | 33 | 10 | 38 | 35 | 62 | strong | II |
| 18 | 32 | 12 | 37.9 | 36 | 62 | strong | II |
| 21 | 40 | 12 | 37.9 | 34 | 62 | strong | II |
| 22 | 38 | 10 | 37.8 | 38 | 60 | strong | II |
| 24 | 44 | 15 | 37.9 | 33 | 64 | good | III |
| 26 | 40 | 12 | 37.8 | 38 | 67 | normal | I |
| 28 | 32 | 12 | 37.9 | 30 | 60 | strong | II |
| 29 | 48 | 18 | 37.7 | 42 | 68 | normal | I |
| 30 | 40 | 18 | 37.9 | 36 | 68 | strong | I |
| 34 | 36 | 12 | | 37 | 69 | strong | II |
| 35 | 56 | 16 | 38.6 | 30 | 52 | strong | I |
| 37 | 36 | 8 | 37.9 | 30 | 67 | strong | I |
| 39 | | | 38.1 | 40 | 60 | strong | II |
| 44 | 38 | 12 | 37.9 | | | strong | I |
| 45 | | | | 26 | 52 | | III |
| 46 | 32 | 14 | 38 | 28 | 68 | strong | II |
| 47 | 32 | 8 | 37.5 | 25 | 70 | strong | III |
| 49 | 32 | 12 | | 28 | 58 | strong | II |
| Mean | | | | 33.3 | 62.4 | | |
| SD | | | | 5.3 | 4.9 | | |
| Median | 36 | 12 | 37.9 | | | | |
| Min | 28 | 8 | 37.5 | | | | |
| Max | 56 | 18 | 38.6 | | | | |

**Table 6b-** Pre-clinical examination performed in horses allocated to S-ket group

| CRF | HR | RR | T | Hto | TP | Pulse | ASA |
|---|---|---|---|---|---|---|---|
| 2 | 36 | 10 | 37.9 | 29 | 67 | normal | II |
| 5 | 36 | 12 | 38 | 26 | 52 | strong | II |
| 6 | 36 | 16 | 37.8 | 31 | 62 | normal | I |
| 7 | 32 | 16 | 37.8 | 43 | 61 | strong | I |
| 11 | 30 | 8 | 37.9 | 39 | 64 | strong | II |
| 12 | 36 | 16 | 37.7 | 40 | 60 | normal | I |
| 15 | 36 | 12 | 37.8 | 29 | 64 | strong | II |
| 16 | 40 | 12 | 37.8 | 33 | 64 | strong | II |
| 17 | 32 | 8 | 37.4 | 43 | 70 | strong | II |
| 19 | 36 | 8 | 37.7 | 38 | 58 | strong | I |
| 20 | 36 | 12 |  | 35 | 58 | strong | I |
| 23 | 46 | 12 | 37.7 | 36 | 65 | strong | II |
| 25 | 36 | 8 | 37.7 | 38 | 58 | strong | I |
| 27 | 38 | 8 | 37.8 | 33 | 60 | strong | II |
| 31 | 44 | 16 |  | 36 | 70 | strong | I |
| 32 | 40 | 8 |  | 31 | 63 | strong | II |
| 33 | 32 | 8 | 38 | 34 | 59 | strong | I |
| 36 | 44 | 10 | 38.1 | 36 | 58 | strong | II |
| 38 | 36 | 10 | 38.2 | 27 | 64 | strong | II |
| 40 | 32 | 10 | 37.9 | 31 | 64 | strong | II |
| 41 | 36 | 8 | 38 | 33 | 56 | strong | II |
| 42 | 32 | 10 | 37.9 | 34 | 60 | strong | II |
| 43 | 32 |  | 38 | 33 | 66 | normal | II |
| 48 | 36 | 8 | 37.2 | 27 | 58 | strong | II |
| 50 | 48 | 16 |  | 30 | 50 | strong | II |
| Mean |  |  |  | 33.8 | 61.2 |  |  |
| SD |  |  |  | 4.7 | 4.8 |  |  |
| Median | 36 | 10 | 37.8 |  |  |  |  |
| Min | 30 | 8 | 37.2 |  |  |  |  |
| Max | 48 | 16 | 38.2 |  |  |  |  |

### 4.2 Anaesthesia

No serious adverse event occurred during the whole anaesthetic episode.

Median CRI times were not significantly different between groups (Med group: 117 [70 - 231] minutes; S-ket group: 115 [77 – 239] minutes) ($p$ = 0.90).

The distribution of horses in lateral or dorsal recumbency was not different ($p$ = 0.57) between groups (Table 7).

**Table 7-** Distribution of recumbency during anaesthesia.

| Group | dorsal recumbency | lateral recumbency | Total |
|---|---|---|---|
| **Med group** | 13 (52%) | 12 (48%) | 25 (100%) |
| **S-ket group** | 15 (60%) | 10 (40%) | 25 (100%) |
| **Total** | 28 (56%) | 22 (44%) | 50 (100%) |

In both groups, the same proportions of horses had an Esmarch's bandage for surgical haemostasis ($p$ = 0.75).

Median intra-anaesthetic end-tidal concentrations of isoflurane (FE'iso) required to maintain an adequate plane of anaesthesia were similar between groups (both groups: 1% ($p$ = 0.91) (Figure 11).

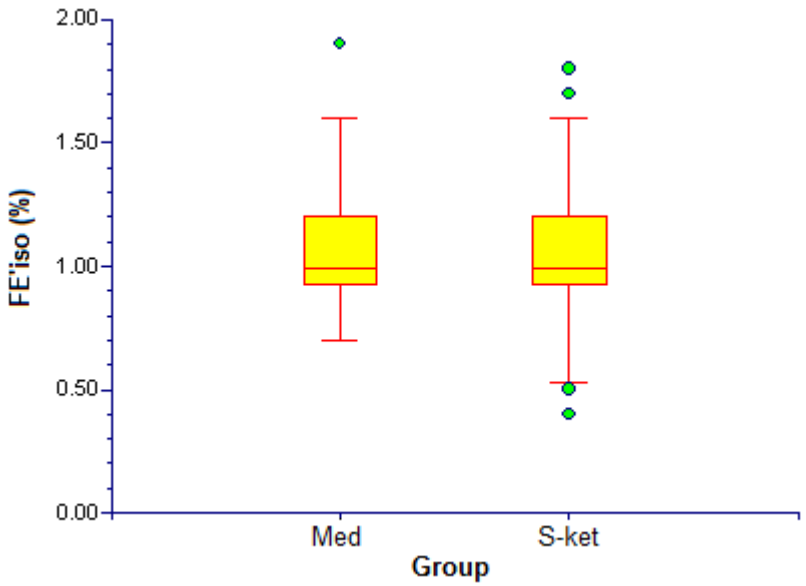



**Figure 11**- Intra-anaesthetic end-tidal concentrations of isoflurane (FE'iso) required to maintain an adequate plane of anaesthesia obtained in horses anaesthetised with isoflurane and a CRI of medetomidine (Med; n = 25) or S(+)-ketamine (S-ket; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median and ◆ the outliers. No significant differences between groups ($p$ = 0.91).

However, horses in S-ket group required significantly higher ($p$ = 0.03) thiopental doses to maintain an adequate anaesthetic depth than horses in Med group (Med group: 0 [0 – 0.38] mg $kg^{-1}$; S-ket group: 0.1 [0 – 0.61] mg $kg^{-1}$).

Haemodynamic and respiratory data

Overall, median intra-anaesthetic heart rates (HR) were not significantly different between both groups (Med group: 32 [23 - 51] beats $min^{-1}$; S-ket group: 32 [24 - 89] beats $min^{-1}$) ($p$ = 0.07) (Figure 12). However, during the first 15 minutes of anaesthesia, heart rates were significantly higher in Med group than in S-ket group, whereas 60, 70, 75, 80 and 90 minutes after begin of anaesthesia, horses in S-ket group experienced significantly higher heart rate values than horses in Med group. Evaluation of the individual plots showed that in total 7 horses experienced individual heart rates above 50 beats $min^{-1}$ (Med: n = 1; S-ket: n = 6).

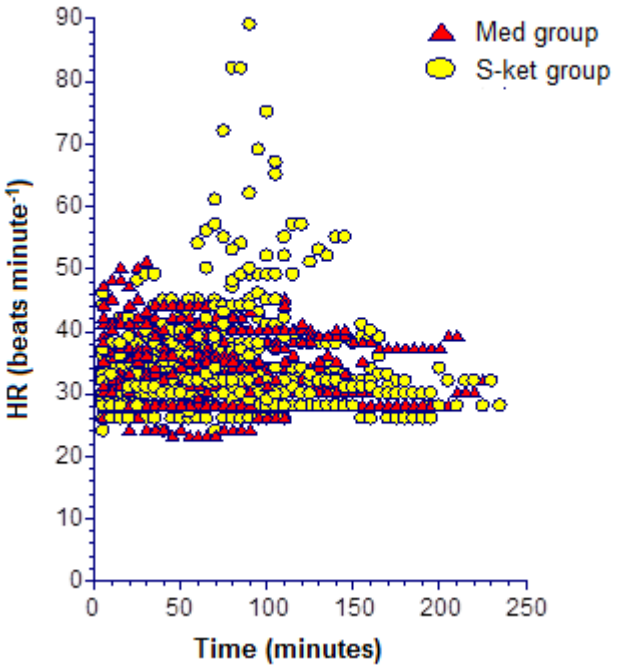



**Figure 12-** Intra-anaesthetic heart rates (HR) over time obtained from horses anaesthetised with isoflurane and a constant rate infusion of medetomidine (Med group) or S(+)-ketamine (S-ket group) (n decreasing with time) ($p$ = 0.07).

The median dose of dobutamine necessary to maintain the target mean arterial blood pressure (MAP) of 70 – 100 mmHg (Med-group: 0.015 [0.001 – 0.055] mg $kg^{-1}$ $hour^{-1}$; S-ket group: 0.018 [0.004 – 0.066] mg $kg^{-1}$ $hour^{-1}$, during the whole anaesthetic episode was not different between groups ($p$ = 0.31) (Figure 13).

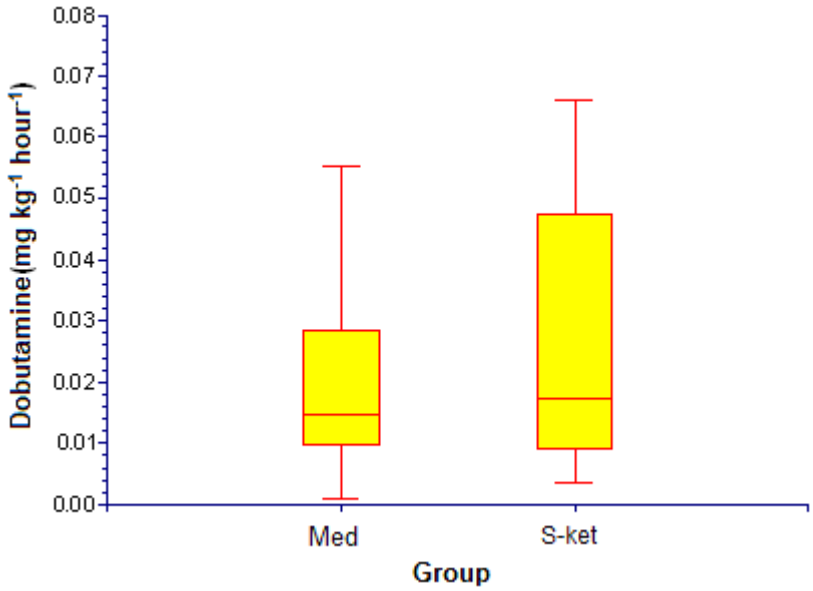



**Figure 13-** Intra-anaesthetic dobutamine dose necessary to maintain a target MAP of 70-100 mmHg obtained from horses anaesthetised with isoflurane and a constant rate infusion of medetomidine (Med; n = 25) or S(+)-ketamine (S-ket; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median. No significant differences between groups ($p$ = 0.31).

Mean arterial blood pressure (MAP) (Med group: 83 [42 - 139] mmHg; S-ket group: 83 [51 - 133] mmHg) and diastolic arterial blood pressure (DAP) (Med group: 70 [36 - 137] mmHg; S-ket group: 69 [41 – 121] mmHg) were not different in both groups ($p$ = 0.13 and 0.12, respectively) (Figures 14 & 15). Systolic arterial blood pressure was significantly higher in Med group (110 [59 - 162] mmHg) than in S-ket group (106 [60 - 156] mmHg) ($p$ = 0.02) (Figure 16).

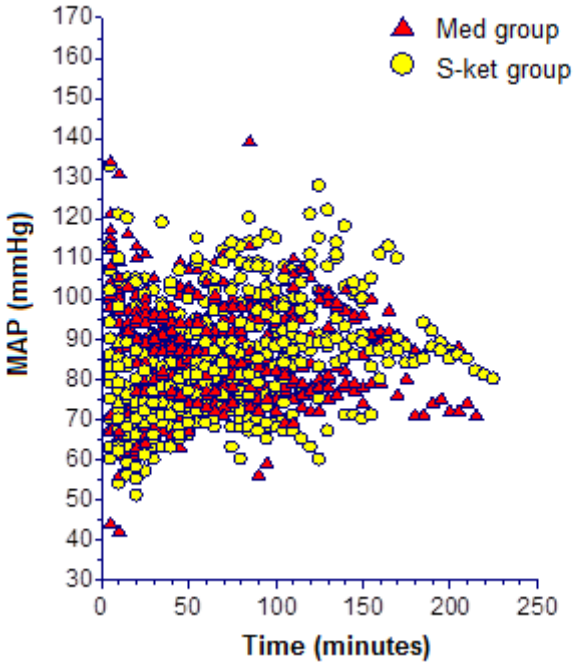



**Figure 14-** Mean arterial blood pressure (MAP) over time measured in horses anaesthetised with isoflurane and a CRI of medetomidine (Med group) or S(+)-ketamine (S-ket group) (n decreasing with time) ($p$ = 0.13).

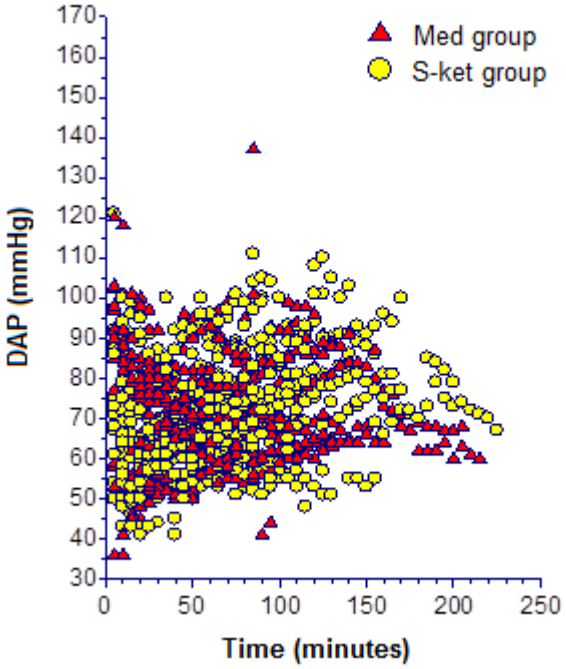



**Figure 15-** Diastolic arterial blood pressure (DAP) over time measured in horses anaesthetised with isoflurane and a CRI of medetomidine (Med group) or S(+)-ketamine (S-ket group) (n decreasing with time) ($p$ = 0.12).

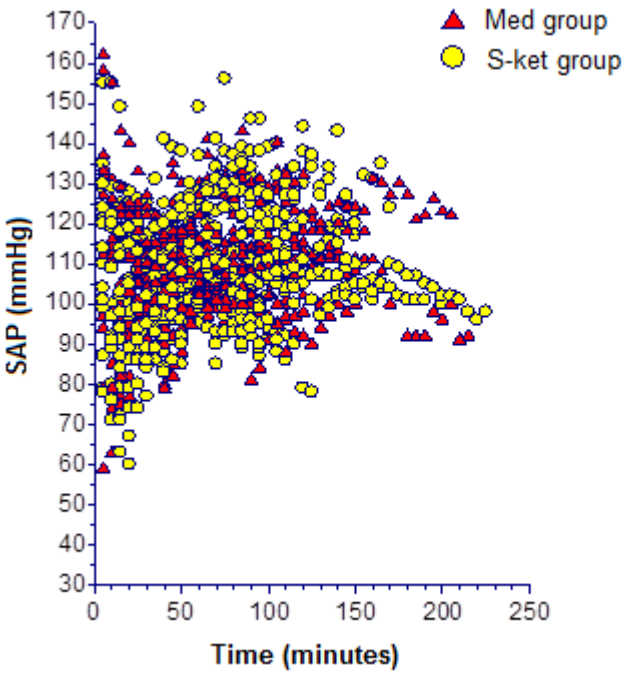



**Figure 16-** Systolic arterial blood pressure (SAP) over time measured in horses anaesthetised with isoflurane and a CRI of medetomidine (Med group) or S(+)-ketamine (S-ket group) (n decreasing with time) ($p$ = 0.02).

Values obtained for cardiac output (CO) in Med group ranged between 12.38 and 79 L $min^{-1}$, and in S-ket group between 16.31 and 59.84 L $min^{-1}$.

The median cardiac index (CI = cardiac output indexed to body surface) (L $min^{-1}$ $m^{-2}$) was significantly higher ($p < 0.01$) in S-ket group (4.47 [2.6 – 8.5] L $min^{-1}$ $m^{-2}$) compared with Med group (3.93 [2.3 – 9.9] L $min^{-1}$ $m^{-2}$) (Figure 17).

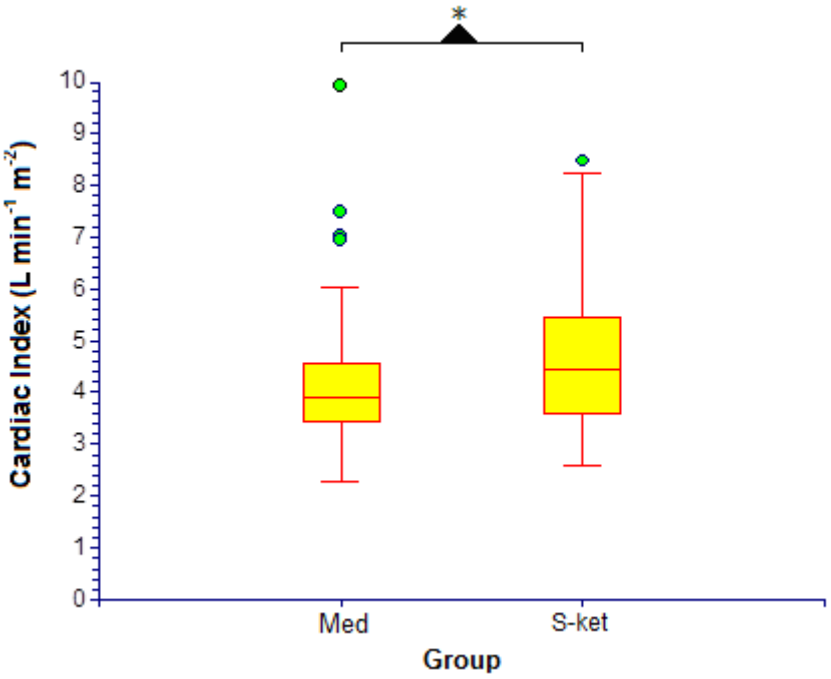



**Figure 17-** Cardiac Index (L $min^{-1}$ $m^{-2}$) obtained from horses anaesthetised with isoflurane and a CRI of medetomidine (Med) or S(+)-ketamine (S-ket). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median and ● the outliers. *Cardiac Index was significantly higher in S-ket group ($p$ < 0.01)

Similarly, the median CI (mL $min^{-1}$ $kg^{-1}$) in which the weight of the patient, but not the BSA is taken into account, was significantly higher in S-ket group (59 [32 – 109] mL $min^{-1}$ $kg^{-1}$) than in Med group (52 [31 – 123] mL $min^{-1}$ $kg^{-1}$) ($p$ < 0.01) (Figure 18).

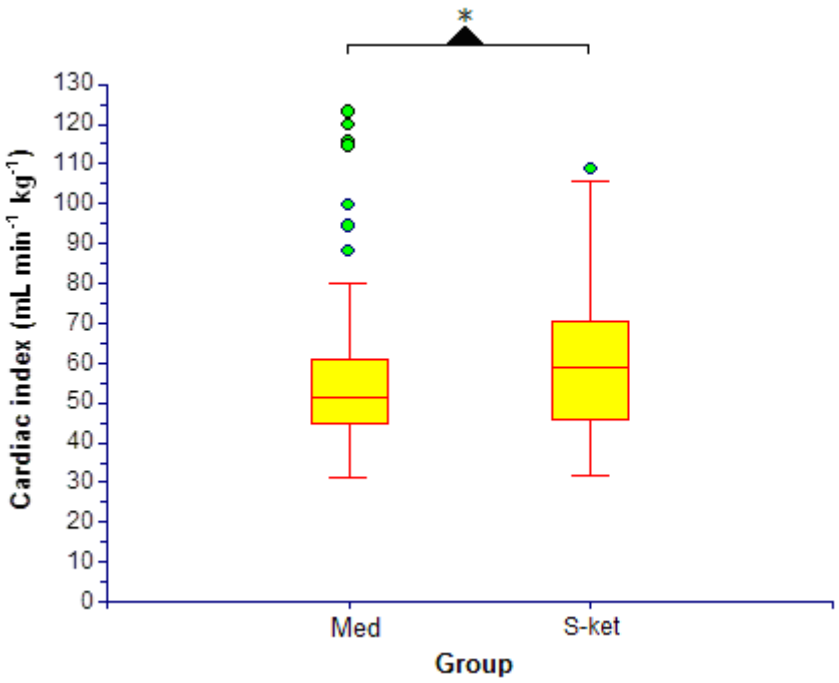



**Figure 18-** Cardiac Index (mL $min^{-1}$ $kg^{-1}$) obtained from horses anaesthetised with isoflurane and a CRI of medetomidine (Med) or S(+)-ketamine (S-ket). Boxes represent the 25th interquartile range, bars the 75th interquartile range, the horizontal line the median and ◦ the outliers. *Cardiac Index was significantly higher in S-ket group ($p < 0.01$).

Overall, horses in both groups showed a similar degree of respiratory acidosis evidenced by $PaCO_2$ values above 45 mmHg. In 3 horses, 2 allocated to Med group and 1 allocated to S-ket group, that were all positioned in dorsal recumbency, $PaO_2$ was below 70 mmHg at 15 minutes after anaesthesia induction (54.9, 66.2 and 64 mmHg, respectively) and required salbutamol administration. The $PaO_2$ values at 30 minutes after anaesthesia induction were 124, 188 and 103 mmHg, respectively and no further salbutamol had to be given. Overall, $PaO_2$ values were significantly lower for horses in S-ket group than in Med group ($p < 0.01$). The ratio of the partial pressure of oxygen in arterial blood to the inspired oxygen fraction ($PaO_2/Fi'O_2$) showed in both groups mean values above 200 mmHg at every time point but only horses allocated to Med group showed mean $PaO_2/Fi'O_2$ values above 300 mmHg at every sampling time. This difference was found to be statistically significant ($p < 0.01$). Still, both groups had $PaO_2$ values above 100 mmHg at all times. Although the statistical test detected

significant differences for pH values, on average, both groups had values within the expected normal range (7.24 – 7.46).

Results of arterial blood gases obtained 15, 30, 60, 90, 120, 150, 180, 210 and 240 after anesthesia induction are detailed below (Table 8).

**Table 8-** Mean ± SD values for arterial blood gases obtained at 15, 30, 60, 90, 120, 150, 180, 210 and 240 minutes after anaesthesia induction in horses anaesthetised with isoflurane and receiving a constant rate infusion of medetomidine (Med group) or S(+)-ketamine (S-ket group).

| Time (min) | Group | n | pH | PaCO2 (mmHg) | PaO2 (mmHg) | SaO2 (%) | PaO2/Fi'$O_2$ (mmHg) |
|---|---|---|---|---|---|---|---|
| 15 | Med | 25 | 7.35±0.04 | 50.7±7.1 | 197±107 | 98.8±2.4 | 333±141 |
| | S-ket | 25 | 7.38±0.04 | 49±5.5 | 143±74 | 98.6±2.2 | 261±126 |
| 30 | Med | 24 | 7.35±0.04 | 52.3±5.9 | 223±87 | 99.7±0.5 | 359±139 |
| | S-ket | 25 | 7.37±0.04 | 51.4±5.6 | 186±86 | 99.4±1.1 | 309±149 |
| 60 | Med | 25 | 7.35±0.04 | 54.6±5.9 | 254±93 | 99.8±0.4 | 378±127 |
| | S-ket | 25 | 7.36±0.04 | 53.3±4.5 | 191±68 | 99.6±1.2 | 302±118 |
| 90 | Med | 22 | 7.35±0.04 | 56.4±6.2 | 243±100 | 99.7±0.6 | 365±133 |
| | S-ket | 23 | 7.36±0.03 | 54.5±6.3 | 194±81 | 99.6±0.6 | 296±129 |
| 120 | Med | 11 | 7.35±0.03 | 56.6±5.6 | 248±110 | 99.3±1.4 | 366±172 |
| | S-ket | 13 | 7.36±0.03 | 55.6±7.9 | 221±115 | 99.3±1.5 | 330±178 |
| 150 | Med | 7 | 7.35±0.04 | 55.1±3.7 | 264±98 | 99.6±0.7 | 400±144 |
| | S-ket | 8 | 7.36±0.03 | 58.3±9.8 | 199±94 | 99.4±1.3 | 313±155 |
| 180 | Med | 3 | 7.33±0.05 | 58.4±8.3 | 243±115 | 99.9±0 | 379±213 |
| | S-ket | 4 | 7.37±0.04 | 58.1±8.0 | 228±92 | 99.8±0.4 | 360±157 |
| 210 | Med | 2 | 7.29±0.06 | 69.2±9.1 | 196±126 | 99.7±0.2 | 302±256 |
| | S-ket | 0 | | | | | |
| 240 | Med | 1 | 7.27 | 73 | 181 | 99.5 | 152 |
| | S-ket | 0 | | | | | |
| **All** | **Med** | **120** | **7.35±0.04** | **54.4±6.9** | **232±98** | **99.5±1.3** | **359±140** |
| | **S-ket** | **123** | **7.37±0.04** | **52.9±6.6** | **186±85** | **99.5±2.5** | **300±137** |
| | ***p*** | | **< 0.01** | **0.11** | **< 0.01** | **0.98** | **< 0.01** |

Median inspired oxygen fraction ($Fi'O_2$) values were not significantly different between groups (Med group: 65 [40 - 94]%; S-ket group: 63 [38 - 97]%) ($p$ = 0.14) (Figure 19).

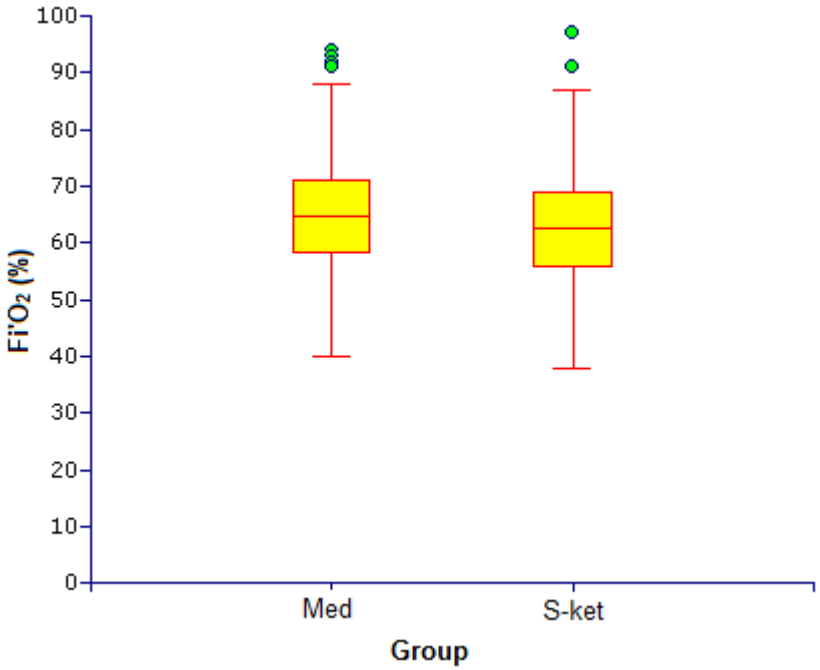



**Figure 19-** Inspired oxygen fraction ($Fi'O_2$) obtained from horses anaesthetised with isoflurane and a CRI of medetomidine (Med group; n = 25) or S(+)-ketamine (S-ket group; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median and ◦ the outliers. No significant differences between groups ($p$ = 0.14).

In Med group, median values for $PaO_2/Fi'O_2$ were not different for patients in dorsal recumbency (356 [59 - 664] mmHg) compared with lateral recumbency (365 [149 - 570] mmHg ($p$ = 0.26). In S-ket group, median values of $PaO_2/Fi'O_2$ were lower for patients in dorsal recumbency (185 [88 - 537] mmHg) when compared with lateral recumbency (413 [148 - 591] mmHg) ($p$ < 0.01). Median values of $PaO_2/Fi'O_2$ were not significantly different between groups for patients in lateral recumbency ($p$ = 0.95), but were significantly lower for patients in dorsal recumbency allocated to S-ket group ($p$ < 0.01) (Figure 20).

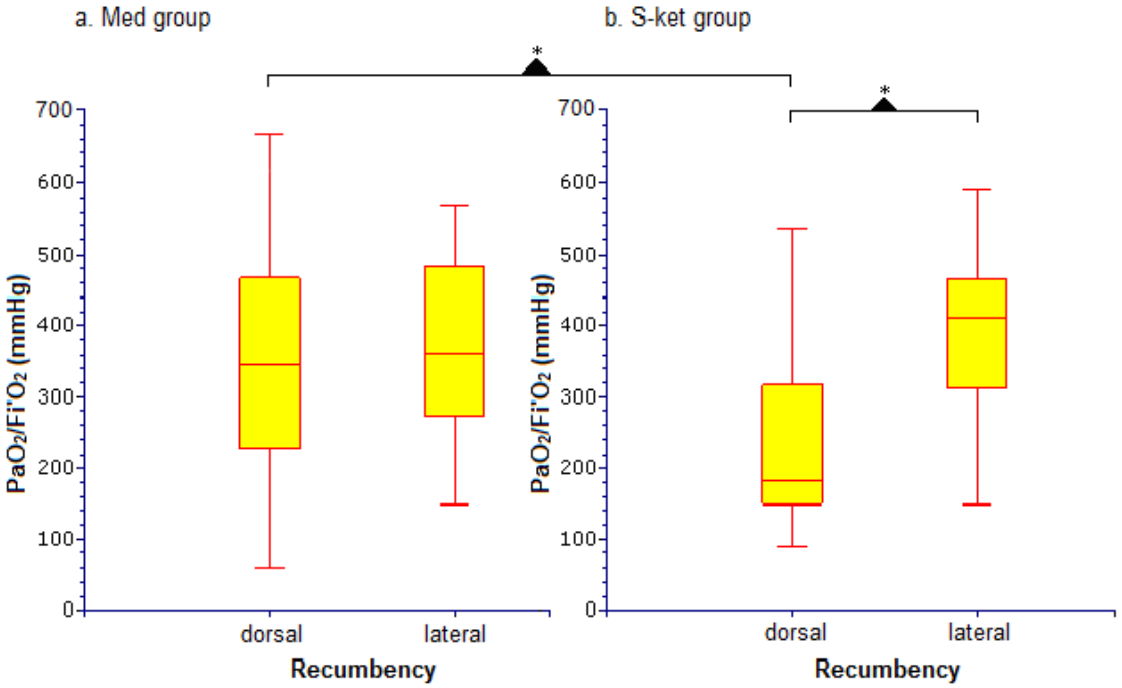



**Figure 20-** $PaO_2/Fi'O_2$ (mmHg) obtained from horses anaesthetised with isoflurane and a CRI of medetomidine (Med group) or S(+)-ketamine (S-ket group). Boxes represent the 25th interquartile range, bars the 75th interquartile range, the horizontal line the median. *In horses anaesthetised in dorsal recumbency $PaO_2/Fi'O_2$ was significantly higher in Med group ($p < 0.01$). In S-ket group horses in dorsal recumbency had significantly lower $PaO_2/Fi'O_2$ than horses in lateral recumbency ($p < 0.01$).

Throughout anaesthesia, median respiratory rate (RR*)* values were similar between groups (Med group: 7 [2 - 20] breaths $min^{-1}$; S-ket group: 7 [3 - 17] breaths $min^{-1}$) ($p = 0.32$). However, median values obtained for end-tidal carbon dioxide partial pressure ($PE'CO_2$) (Med group: 48 [34 - 64] mmHg; S-ket group: 45 [30 - 64] mmHg) were significantly different between groups ($p < 0.01$).

### 4.3 Anaesthesia recovery

Horses allocated to S-ket group regained sternal recumbency significantly faster (38 [8 - 75] minutes) compared with those allocated to Med group (48 [10 - 72] minutes) ($p = 0.01$) (Figure 21). Subsequently, horses allocated to S-ket group stood up significantly faster (50 [18 - 91] minutes) than those allocated to Med group (60 [20 - 99] minutes) ($p = 0.02$) (Figure 22).

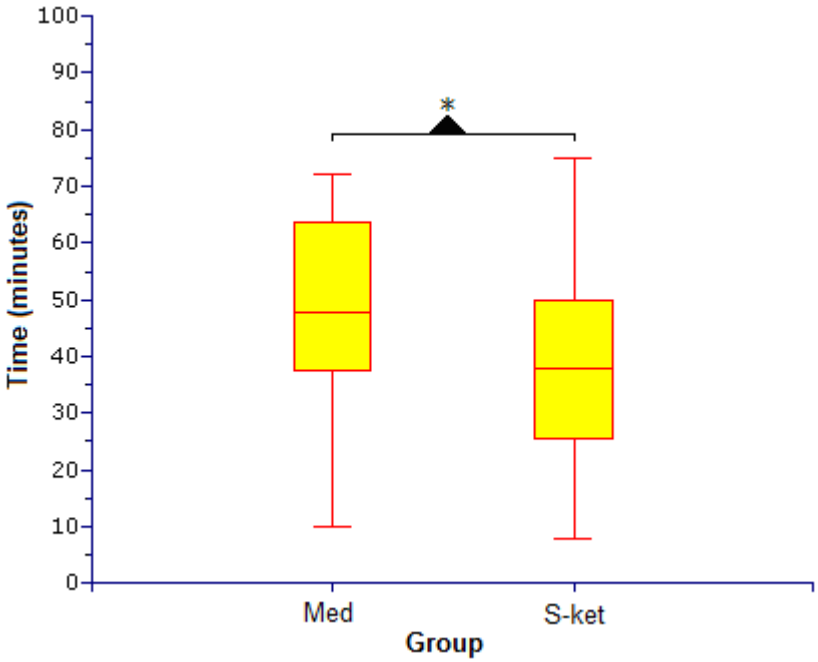



**Figure 21-** Time to achieve sternal position in horses after anaesthesia with isoflurane and a CRI of medetomidine (Med group; n = 25) or S(+)-ketamine (S-ket; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median. *Horses in S-ket group regained significantly faster sternal position ($p$ = 0.01).

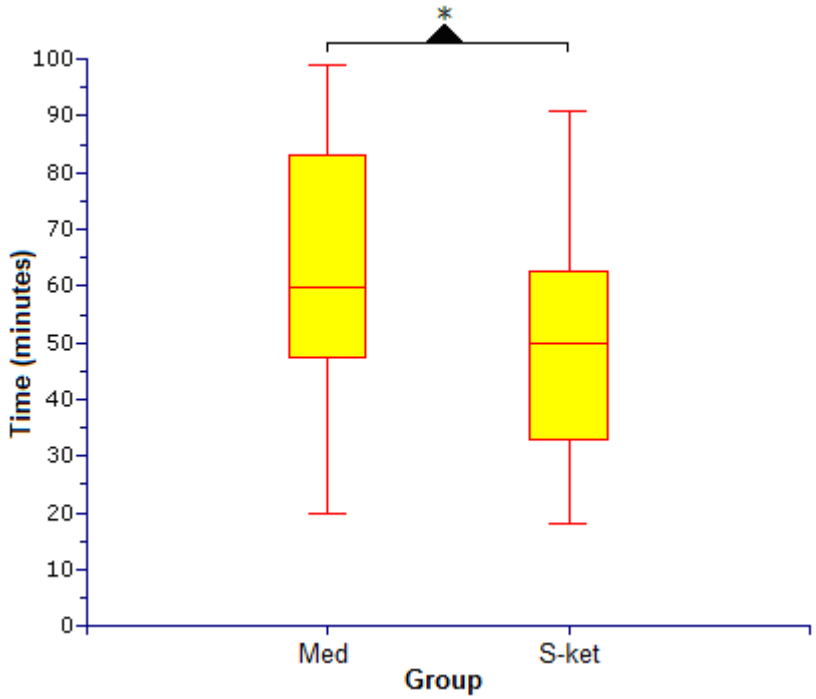



**Figure 22-** Time to achieve standing position in horses after anaesthesia with isoflurane and a CRI of medetomidine (Med group; n = 25) or S(+)-ketamine (S-ket group; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median. *Horses in S-ket group regained significantly faster standing position ($p$ = 0.02).

Median recovery visual analogue scale (VAS) values were statistically significantly lower for horses in Med group (14.5 [4 - 89] mm) when compared with horses in S-ket group (31.75 [1.5 – 93.25] mm) ($p$ = 0.01) (Figure 23).

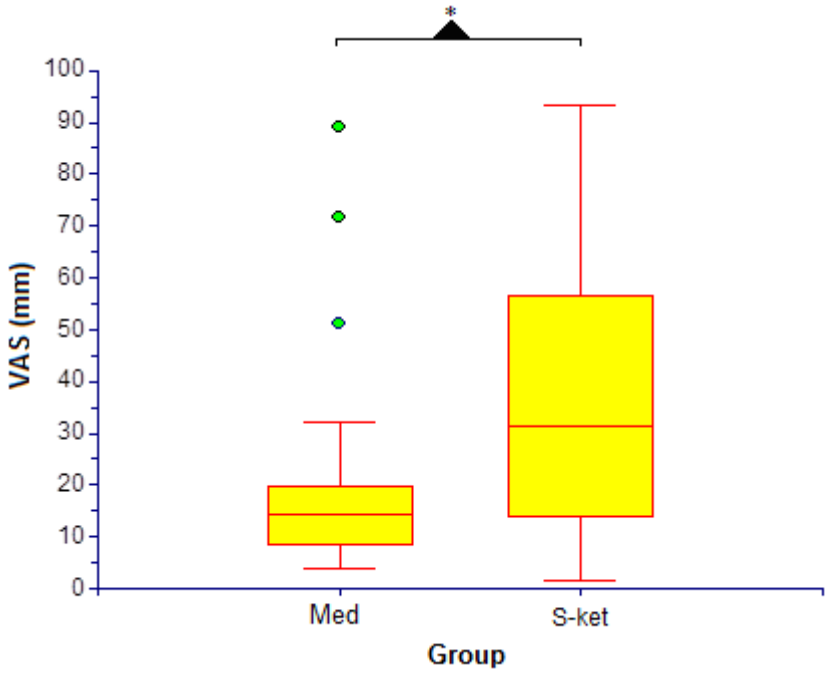



**Figure 23-** Visual analogue scale (VAS; 0 mm = best possible recovery; 100 = worst possible recovery) obtained from the evaluation of the anaesthesia recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (Med group; n = 25) or S(+)-ketamine (S-ket group; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median and ● the outliers. *VAS was significantly better in Med group ($p$ = 0.01).

Median recovery numeric rating score (NRS) (1 = excellent, 2 = good, 3 = fair, 4 = moderate, 5 = poor) was also significantly lower for patients in Med group (1.25 [1 - 5]) than for patients in S-ket group (2.25 [1 - 5]) ($p$ < 0.01) (Figure 24). One horse in each group was scored as "poor" (NRS=5). The horse allocated to Med group required additional sedation.

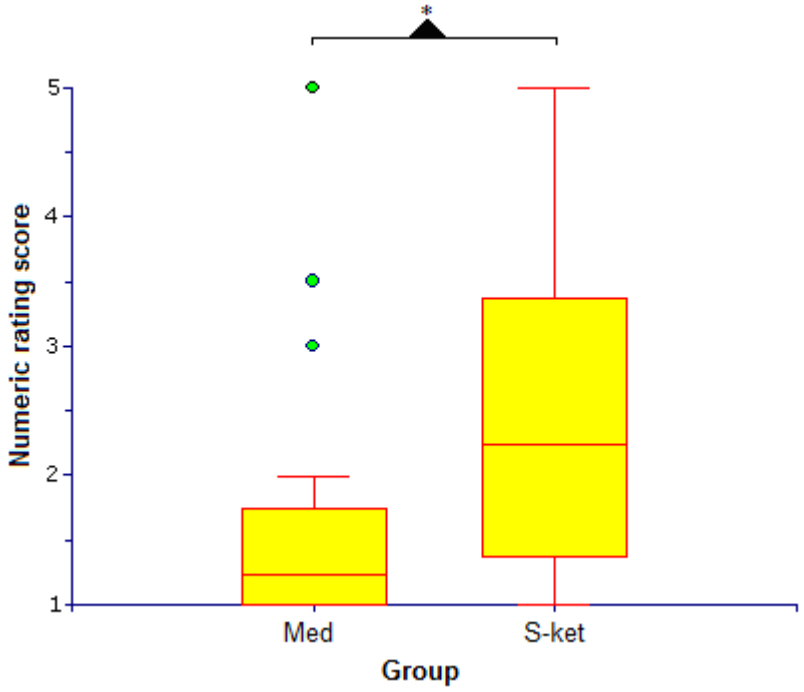



**Figure 24-** Numeric rating score (1 - 5; 1 = excellent; 5 = poor) obtained from the evaluation of the anaesthesia recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (Med group; n = 25) or S(+)-ketamine (S-ket group; n = 25). Boxes represent the 25$^{th}$ interquartile range, bars the 75$^{th}$ interquartile range, the horizontal line the median and ◦ the outliers. *NRS score was significantly better in Med group ($p < 0.01$).

There was a correlation between the duration of the infusion and the quality of anaesthesia recovery for horses in S-ket group ($p = 0.02$), but not for horses in Med group ($p = 0.52$) (Figure 25).

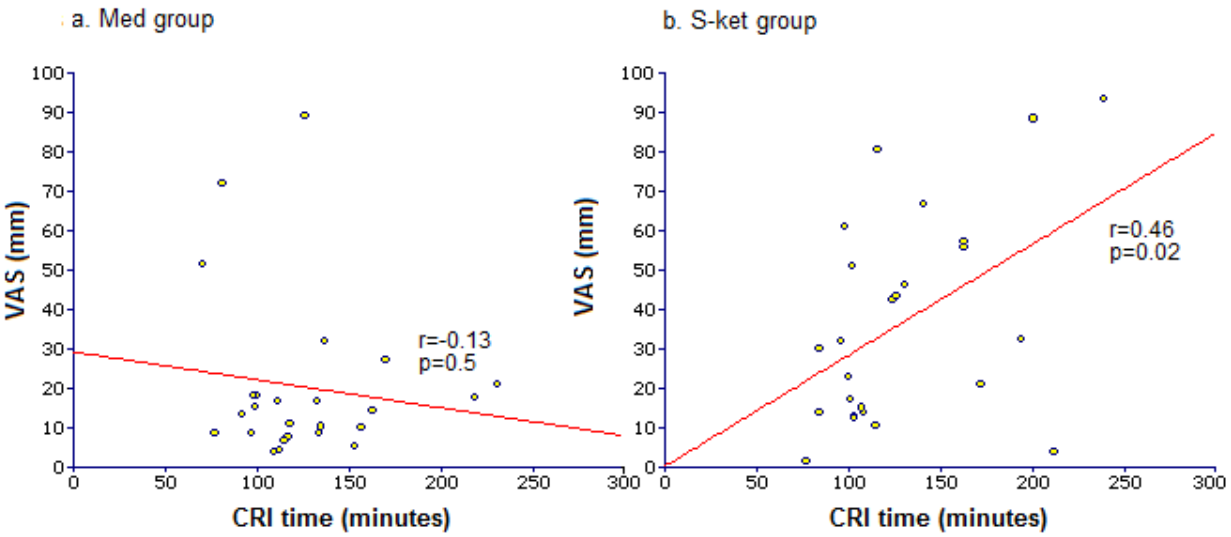



**Figure 25-** Visual analogue scale values (VAS) obtained from the evaluation of anaesthesia recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (a. Med group; n = 25 horses) or S(+)-ketamine (b. S-ket group; n = 25 horses) Data for VAS were plotted over duration of CRI and linear correlation (straight line) and Pearson correlation coefficient (r) were calculated. Only VAS values of horses allocated to S-ket group had a strong correlation with the length of the CRI.

When plotted over time to regain sternal and standing position, horses allocated to both groups showed a significant negative correlation with anaesthesia recovery VAS (Figures 26 & 27). The faster horses regained sternal and standing position the poorer recovery quality was (Med group: $p < 0.01$; S-ket group: $p = 0.01$ respectively $p = 0.04$).

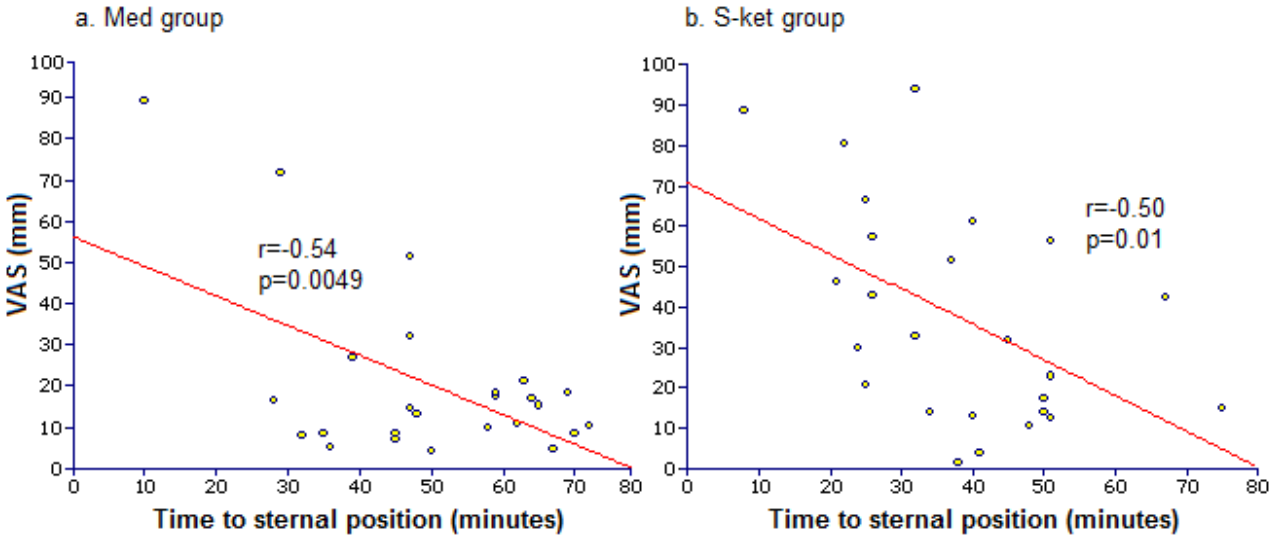



**Figure 26-** Visual analogue scale values (VAS) obtained from the evaluation of the anaesthetic recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (a. Med group: n = 25) or S(+)-ketamine (b. S-ket group; n = 25). Data for VAS were plotted over time to regain sternal position and linear correlation (straight line) and Pearson correlation coefficient (r) were calculated. VAS values of horses allocated to both groups had a negative correlation with time to sternal position.

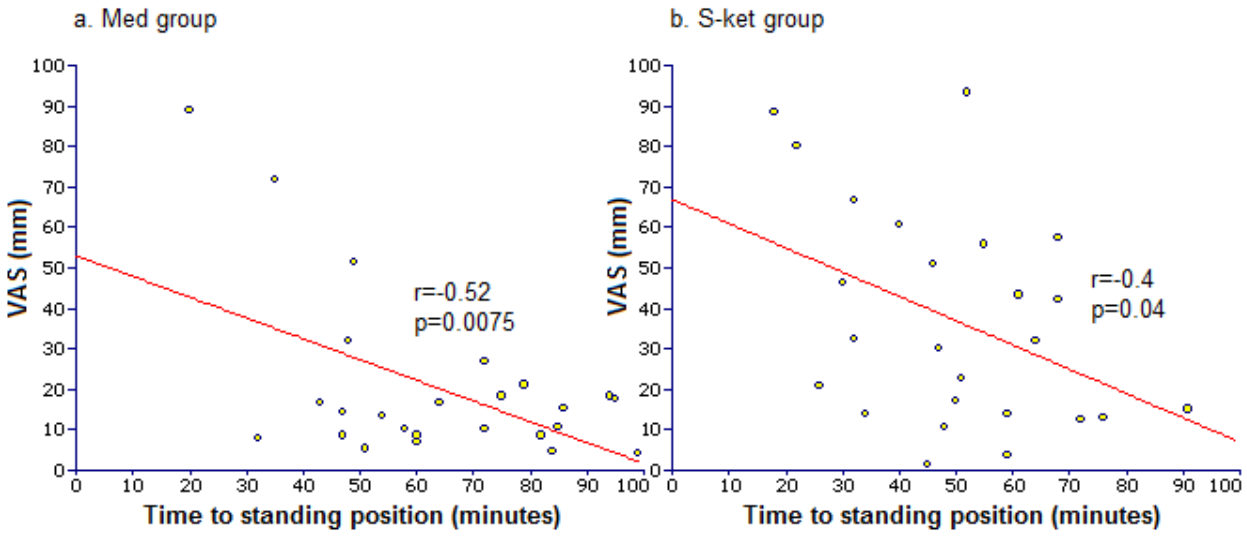



**Figure 27-** Visual analogue scale values (VAS) obtained from the evaluation of the anaesthesia recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (a. Med group; n = 25) or S(+)-ketamine (b. S-ket group; n = 25). Data for VAS were plotted over time to regain standing position and linear correlation (straight line) and Pearson correlation coefficient (r) were calculated. VAS values of horses allocated to both groups had a negative correlation with time to standing position.

There were no correlations between the amount of thiopental administered and the mean VAS values (Med group: $p$ = 0.40; S-ket group: $p$ = 0.48) (Figure 28).

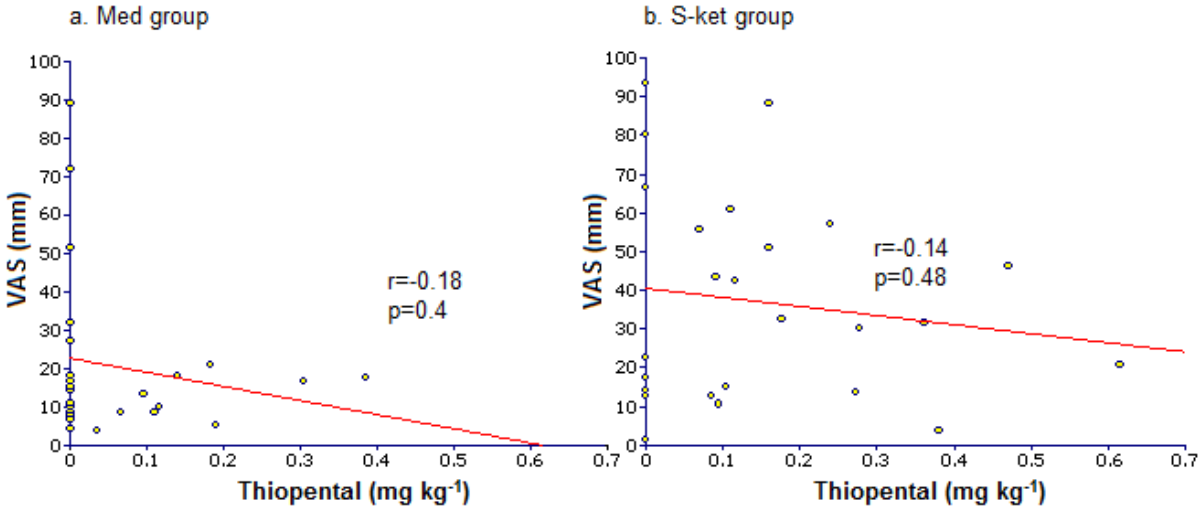



**Figure 28-** Visual analogue scale values (VAS) obtained from the evaluation of the anaesthesia recovery quality by 4 observers. Horses were anaesthetised with isoflurane and a CRI of medetomidine (a. Med group; n = 25) or S(+)-ketamine (b. S-ket group; n = 25). Data for VAS were plotted over thiopental dose necessary to maintain an adequate anaesthetic depth. Linear correlation (straight line) and Pearson correlation coefficient (r) were calculated. No correlation could be detected.

## 5. DISCUSSION

The aim of the present study was to compare the effects on MAC and cardiopulmonary function of a constant rate infusion of S(+)-ketamine with a constant rate infusion of medetomidine during isoflurane anaesthesia in horses and the resulting recovery duration and quality. Fifty horses undergoing elective surgical procedures in every part of the body except the neck and the head regions were randomly allocated to receive S(+)-ketamine CRI or medetomidine CRI during isoflurane anaesthesia. The identity of the treatment was blinded for the anaesthetist. There was no difference concerning the effects on MAC, but cardiac index was slightly better with S(+)-ketamine. On the other hand, recovery quality was better following medetomidine.

Eight to sixteen hours before anaesthesia, food was withheld to avoid the

hazards of food aspiration after anaesthesia induction and to reduce the effects of intra-operative tympanism that may interfere with free movement of diaphragm and perturb breathing.

Anaesthesia was induced with either 2.2 mg $kg^{-1}$ racemic ketamine (Med group) or 1.1 mg $kg^{-1}$ S(+)-ketamine (S-ket group). In a previous study performed in horses sedated with xylazine, 2/3 of the racemic dose was given for S(+)-ketamine (Filzek et al. 2003). In the present study, only half of the racemic dose was administered, as S(+)-ketamine has approximately twice the anaesthetic potency of racemic ketamine (Ryder et al. 1978, White et al. 1980, Kohrs & Durieux 1998). Preliminary studies in our clinic have shown that this is also the case in horses (unpublished data).

Horses in both treatment groups required similar concentrations of isoflurane to maintain anaesthesia. However, horses receiving S(+)-ketamine CRI required significantly higher doses of thiopental as a rescue medication to achieve an adequate surgical anaesthetic depth than horses receiving medetomidine CRI. This indicates that horses allocated to Med group had a more stable anaesthesia than horses allocated to S-ket group. The strong sedative effects of medetomidine might have resulted in a more profound hypnosis in comparison to S(+)-ketamine which resulted in a reduced need for incremental thiopental in this group. Overall, median intra-anaesthetic heart rates (HR) were not different between both groups. However, during the first 15 minutes of anaesthesia, horses in Med group showed significantly higher heart rates than horses in S-ket group. The loading dose of racemic ketamine in Med group may be responsible for this result. In a study comparing the effects of repetitive boli of racemic and S(+)-ketamine, mean heart rate was also significantly higher with racemic ketamine (Filzek et al. 2003). Twenty minutes after the beginning of anaesthesia, heart rate slightly decreased in horses allocated to Med group. Sixty, 70, 75, 80 and 90 minutes after the beginning of anaesthesia, horses in S-ket group showed significantly higher values than horses in Med group. Evaluation of the individual plots showed that in total 7 horses experienced individual heart rates above 50 beats $min^{-1}$ (Med: n = 1; S-ket: n = 6). These high heart rate values mainly occurred during surgery, suggesting intraoperative sympathomimetic stimulation. This stimulation might either be induced by

S(+)-ketamine directly (White et al. 1982, Adams & Werner 1997, Zielmann et al. 1997) or be a result of surgical stimulation. Especially in one horse undergoing surgical castration and allocated to S-ket group, intra-anaesthetic heart rates increased over 80 beats $min^{-1}$ at time points 80 and 90, immediately after requiring a rescue dose of thiopental to maintain an adequate surgical anaesthetic plane. It remains unclear whether this increase was the consequence of direct sympathomimetic stimulation of S(+)-ketamine, of intraoperative nociception or of both. On the other hand, medetomidine decreases heart rate and blunt responses to surgical stimulation to a certain extent (Ringer et al. 2007), which certainly helped to reduce the incidence of tachycardia in this group.

The cardiac index (CI), the main indicator of muscular perfusion in horses (Lee et al. 1998), was significantly higher in S-ket group than in Med group. Therefore muscular perfusion was better with S(+)-ketamine. The CI values observed in Med group were similar to those reported in a previous study in which a similar anaesthetic regime was used (Ringer et al. 2007). The CI values for horses in S-ket group were comparable with those reported in mechanically ventilated horses anaesthetised with isoflurane alone after sedation with romifidine and induction of anaesthesia with a combination of ketamine and diazepam (Blissitt et al. 2008).

Alpha2-adrenoceptor agonists usually induce an initial increase in arterial blood pressure followed by a longer lasting decrease. In this study, a dobutamine infusion was given to all horses and the dosages were adjusted to keep a target mean arterial blood pressure between 70 and 100 mmHg. Horses in both groups required the same dose of dobutamine to maintain this target MAP. Mean arterial blood pressure was not statistically different throughout the whole anaesthetic episode.

In anaesthetised horses, hypoxemia, hypercapnia and acid-base disturbances often occur as a result of impaired pulmonary gas exchange such as hypoventilation, ventilation-perfusion mismatch and right-to-left vascular shunts (Schatzmann 1995, Kalchofner et al. 2009). Lung atelectasis, leading to poor oxygenation of the non-oxygenated blood arriving to the lungs, often occurs (Schatzmann 1995). In the present study, overall

arterial $PaCO_2$ levels were in the same range in both groups. This reflected the pattern of mechanical ventilation which aimed at keeping the end-tidal carbon dioxide pressure between 45 and 55 mmHg. $PaO_2$ values were statistically significantly lower in S-ket group than in Med group whilst median $Fi'O_2$ values were not different between both groups. However, mean $PaO_2$ values were above 100 mmHg in both groups and as the mean $SaO_2$ values remained above 99%, the difference is not considered clinically relevant.

The degree of impairment of pulmonary gas exchange can be assessed by the ratio of the partial pressure of oxygen in arterial blood ($PaO_2$) to the inspired oxygen fraction ($PaO_2/Fi'O_2$) (Rice et al. 2007). In humans, values below 300 mmHg are associated with acute lung injury, and values below 200 mmHg are associated with acute respiratory distress syndrome (Bernard et al. 1994). On average, horses in the S-ket group had significantly lower $PaO_2/Fi'O_2$ values than horses in the Med group. In the Med group, all mean values were above 300 mmHg at any measured time point. Horses in S-ket group showed mean values below 300 mmHg, namely 261 ± 126 mmHg 15 minutes after the beginning of anaesthesia and 296 ± 129 mmHg 90 minutes after the beginning of anaesthesia. These values indicate that venous admixture was not severe. In S-ket group, horses placed in dorsal recumbency had significantly lower $PaO_2/Fi'O_2$ values than horses placed in lateral recumbency. This finding is not surprising, as $PaO_2$ partially depends on the position during recumbency (Hall et al. 1968). Nyman and Hedernstierna (1989) showed that horses in dorsal recumbency had a greater shunt than horses in lateral recumbency. Surprisingly, this issue was not demonstrated in Med group, even if the lowest value of 59 mmHg was found in this group. This value may be explained by the dorsal recumbency of a heavy horse (725 kg). However, the $PaO_2/Fi'O_2$ values for patients in dorsal recumbency were significantly lower in the S-ket group than in the Med group, whereas values for patients in lateral recumbency were similar between both groups. It seems that venous admixture was enhanced in horses given S(+)-ketamine only when placed in dorsal position. The difference in $PaO_2/Fi'O_2$ values cannot be explained by any known pharmacodynamic property of medetomidine or ketamine. It might be attributed to slightly insufficient artificial ventilation in individual horses. In

both groups, values for respiratory frequency lied within the reference range of mechanically ventilated horses. Nevertheless values for pH were statistically different in both groups, but were within the normal values expected in anaesthetised horses (Schatzmann 1995). This is more likely attributable to a slight difference in artificial ventilation than to a difference caused by the different drugs.

Recovery is a very critical phase of equine anaesthesia and is often associated with fractures, articular dislocations, trauma and dehiscence of surgical wounds (Clark-Price et al. 2008). Therefore, the quality of anaesthesia recovery is very important, and it is a great concern in equine anaesthesia to achieve a quiet and coordinate recovery phase. Acepromazine was administered prior to anaesthesia induction. This drug has a long duration of action (Auckburally & Flaherty 2009) and may still exert sedative effects in the recovery phase, and might therefore help to smooth the process. It depresses the central nervous system, and therefore the need for other anaesthetic drugs can be reduced. Finally it has vasodilative effects improving muscle perfusion, and should therefore help to reduce the risk of development of post-anaesthetic myopathy (Auckburally & Flaherty 2009). As additional sedation in the early postoperative period improves recovery (Santos et al. 2003), medetomidine was administered to all horses after extubation.

Balanced anaesthesia with isoflurane and a CRI of medetomidine leads to good recovery quality in ponies and horses (Kalchofner et al. 2006, Bettschart-Wolfensberger & Larenza 2007). It has been compared to other common balanced anaesthesia regimes like for example isoflurane-lidocaine, and was found to lead to better and smoother recovery phases (Ringer et al. 2007). Racemic ketamine CRI's were associated with poor recovery quality and an increased risk of self-injuries (Spadavecchia et al. 2002, Larenza et al. 2009), but the use of S(+)-ketamine showed significantly better recoveries (Larenza et al. 2009).

In the present study, recovery time was compared between both groups. Horses in S-ket group regained sternal position faster than horses allocated to Med group. Furthermore, horses in S-ket group stood up faster than

horses in Med group. Recovery quality was evaluated by four qualified anaesthetists, unaware of both the treatment identity and the appreciation of the other observers. VAS values were lower (and thus of better quality) in patients receiving a CRI of medetomidine than in those receiving S(+)-ketamine. The visual analogue scale (VAS) is a model used to measure a characteristic or attitude that ranges across a continuum of values and cannot be measured directly. VAS is very subjective, and therefore some caution is required when handling such data, and score rankings are preferred by many researchers (Gould et al. 2001). NRS values of 1, 2 and 3 are commonly considered as recoveries of adequate quality, whereas scores of 4 and 5 stand for inadequate recoveries. In one horse of each group, anaesthesia recovery was considered as poor (NRS 5). The horse allocated to Med group even required additional sedation to allow for a safe recovery. This particular horse was already very nervous at the clinical pre-anaesthesia examination. The thoroughbred with poor recovery in S-ket group (CRF 50) was admitted for an arthrodesis and anaesthesia lasted for up to 4 hours. The temperament of the horse, the very long duration of anaesthesia, postoperative pain or a combination of these factors may explain this bad recovery. Withehair et al. (1993) demonstrated that the temperament of the individual horse influences recovery significantly. In 7 horses, 2 in Med group and 5 in S-ket group, the observers were at odds with each other concerning recovery quality. Whilst some anaesthetists considered the recovery phase as good to moderate (NRS 2 to 3), and thus as adequate, the other observers estimated the nature of recovery as inadequate by allocating a NRS score of 4. Therefore, the results for recovery quality in the individual horses may be judged with caution. However, when comparing all scores between groups, horses allocated to Med group showed significantly lower NRS scores than horses in S(+)-ket group.

Duration of anaesthesia is negatively associated to the quality of recovery (Young & Taylor 1993). This was confirmed in S-ket group, but not in Med group. In a previous study, this correlation was demonstrated in horses receiving racemic ketamine, but not in patients receiving S(+)-ketamine (Larenza et al. 2009). Because of the small number of animals involved and

the relatively short durations of anaesthesia in this prior study, these results may be judged with caution.

Horses are flight animals and therefore tend to stand up straight after anaesthesia. If horses try to stand up while the remaining isoflurane is not completely eliminated from some body tissues, ataxia and incoordination may develop, increasing the risk of self-injuries. Thus, a longer recovery phase is associated with an improved quality of recovery (Whitehair et al. 1993, Santos et al. 2003). In the present study, horses that took longer to stand up also showed better recovery quality on average. This is in contrast to the findings of Larenza et al. (2009), where horses receiving S(+)-ketamine recovered faster than horses receiving racemic ketamine, but quality of recovery was also significantly better in the S(+)-ket group. In this study, the psychomimetic effects following racemic ketamine dominated recovery characteristics, despite the longer recoveries with this drug.

In the present study, S(+)-ketamine CRI and medetomidine CRI during isoflurane anaesthesia provided acceptable cardiopulmonary function during anaesthesia in horses. Cardiac index was higher in S-ket group, but minimal (and thus dangerous) values recorded were not different between the groups. Both groups required the same concentrations of isoflurane to maintain anaesthesia, but more thiopental to deepen anaesthesia had to be used with S(+)-ketamine. Horses recovered faster with S(+)-ketamine, but the quality of recovery was better with medetomidine. In S-ket group, the longer the duration of infusion, the worse anaesthesia recovery was. Therefore, when using a S(+)-ketamine CRI in patients undergoing long lasting anaesthesia, special care must be taken to prevent the occurrence of injuries during recovery.

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