Use of near-infrared spectroscopy to identify trends in regional cerebral oxygen saturation in horses

By

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Dedication

To my parents, who each in their own way have given me unwavering support and helped to make my dream a reality. To my mum especially; for always being there, for always "making a plan" and for always believing in me.

"Give a girl the right shoes and she can conquer the world"

Bette Midler

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List of Abbreviations

ABG	arterial blood gas
CA	cerebral autoregulation
CBF	cerebral blood flow
CNS	central nervous system
CO	cardiac output
CO_2	carbon dioxide
CPP	cerebral perfusion pressure
CRT	capillary refill time
CVR	cerebrovascular resistance
deoxyHb	deoxygenated haemoglobin
DSS	dorsal sagittal sinus
EEG	electroencephalography
Hb	haemoglobin
HR	heart rate
ICP	intracranial pressure
LAC	lactate
LED	light emitting diode

LPS	lipopolysaccharide
MAC	minimum alveolar concentration
MAP	mean arterial pressure
mm	mucous membranes
MD	microdialysis
NIRS	near-infrared spectroscopy
O_2	oxygen
OTAU	Onderstepoort Teaching Animal Unit
oxyHb	oxygenated Hb
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
$PbtO_2$	brain tissue oxygen tension
POCD	post operative cognitive decline
$PvCO_2$	venous partial pressure of carbon dioxide
PvO_2	venous partial pressure of oxygen
RP	recording period
RR	respiratory rate
rSO_2	regional cerebral oxygen saturation
SaO_2	arterial oxygen saturation

$SavO_2$	arteriovenous oxygen saturation
s.d.	standard deviation
s.e.	standard error
SjO_2	jugular bulb venous oxygen saturation
T	rectal temperature
TCD	transcranial doppler
VBG	venous blood gas

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Summary

USE OF NEAR-INFRARED SPECTROSCOPY TO IDENTIFY TRENDS IN REGIONAL CEREBRAL OXYGEN SATURATION IN HORSES

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This dissertation focuses on the use of near-infrared spectroscopy (NIRS) to identify trends in regional cerebral oxygen saturation (rSO_2) in both conscious and anaesthetised healthy horses.

A cerebral/somatic oximeter sensor recorded rSO_2 from the dorsal sagittal sinus of 6 healthy horses. Values for rSO_2, arterial and venous oxygen and carbon dioxide tensions (PaO_2, PvO_2, $PaCO_2$ and $PvCO_2$ respectively), along with arteriovenous oxygen saturations ($SavO_2$) were recorded in unsedated (recording period [RP] 1), sedated (RP2), and anaesthetised horses (RP3-5); and during recovery (RP6-8). During anaesthesia, horses were ventilated to achieve states of normo- (RP3), hyper- (RP4) and hypocapnoea (RP5).

Overall mean ± s.d. values for rSO_2, PaO_2, PvO_2, $PaCO_2$, $SavO_2$ and mean arterial pressure (MAP) varied significantly by RP. Significant decreases in rSO_2 were identified between RP1

and the post-anaesthetic periods however no significant differences in rSO_2 values were identified between RP1 and the intra-anaesthesia periods. Regional cerebral oxygen saturation was significantly correlated with PaO_2, PvO_2 and $SavO_2$. No correlation was identified between rSO_2 and lactate concentration.

This is the first study to identify trends in rSO_2 in horses using NIRS. A significant positive correlation was identified between rSO_2 and PvO_2, suggesting that alterations in cerebral oxygenation may be reflected in jugular PvO_2. Results of this study demonstrate that NIRS can be used during general anaesthesia to monitor trends in rSO_2 in healthy horses, and has the potential to alert clinicians to cerebral desaturation events when used in a clinical setting.

Keywords: *anaesthesia, cerebral oximeter, horse, hypercapnoea, near-infrared spectroscopy, neuromonitoring*

Chapter 1: General Introduction

Equine anaesthesia carries a high risk of mortality or serious morbidity, with the recovery period being a time of particular concern[1-6]. Recently, a UK-based enquiry into perioperative equine fatalities identified a mortality rate, defined as death within seven days of general anaesthesia, of 1.9%[3]; a significantly higher rate than that which is reported in humans (0.008%)[5] or dogs and cats (0.2%)[6]. Recovery from equine general anaesthesia is considered precarious and injuries sustained during this period account for 25-50% of anaesthetic-associated deaths[7]. Numerous factors are recognised as contributing to the quality of recovery including the duration of anaesthesia, type of anaesthetic agent used, temperament of the horse and degree of intraoperative hypotension[7].

Post-operative neurological complications are commonly encountered in human patients, particularly following cardiovascular surgery, and are an important cause of post-operative morbidity[8,9]. In children, neurologic outcome has surpassed mortality as the primary distinguishing feature of paediatric cardiac surgery, with long-term neurodevelopment impairment occurring in 21 to 69% of such patients[10,11]. These complications are thought to develop due to cerebral ischaemic hypoperfusion caused by reduced blood flow or haemodynamic impairment, thromboembolic events within the brain, or as a result of systemic inflammatory response syndrome[8,10,11].

In human medicine an association between intra-operative cerebral oxygen desaturation and adverse neurological outcomes, including delirium, disorientation and prolonged recoveries, has been identified[8,10,11,12]. As a result NIRS, a non-invasive neuromonitoring technique

which identifies trends in rSO_2, is being increasingly utilised to facilitate the early detection of haemodynamic abnormalities during general anaesthesia[12,13].

Currently, neuromonitoring techniques in equine medicine are vastly limited. Although the direct measurement of intracranial pressure has been reported in horses[14], the invasiveness of this procedure prohibits its use as a routine neuromonitoring tool. As alterations in cerebral haemodynamics may be a contributing factor to the complications associated with post-anaesthetic recovery in horses, use of a non-invasive neuromonitoring tool such as NIRS may prove to be beneficial.

The aims of this study were to determine whether NIRS can be used to measure rSO_2 in horses; detect changes in rSO_2 during periods of induced hypo- or hypercapnoea; and determine whether correlations exist between rSO_2 and PaO_2 and rSO_2 and PvO_2.

Chapter 2: Literature Review

2.1 GENERAL ANAESTHESIA OF HORSES

2.1.1 Mortality associated with general anaesthesia:

Equine general anaesthesia carries a high risk of mortality or serious morbidity, with the recovery period being a time of particular concern[1-6]. In an attempt to identify factors that may be associated with an increased risk of death during and within 7 days of general anaesthesia, a large-scale prospective epidemiological study investigating mortality associated with equine general anaesthesia was conducted[3]. The study involved 41, 824 cases of equine general anaesthesia from 62 veterinary centres in the United Kingdom over a 6-year period, and identified an overall perioperative mortality rate of 1.9%[3]; a significantly higher rate than that which is reported in humans (0.008%)[5] or small animals (0.2%)[6]. The most common cause of equine death reported during this enquiry was cardiac arrest (including post-operative cardiovascular collapse), accounting for 33% of all perioperative fatalities[3]. Limb fractures, myopathies, post-operative haemorrhage and respiratory complications were also recognised as causes of death occurring within the perioperative period[3].

In the study discussed above, central nervous system disturbances were responsible for 5.5% of fatalities[3]; the specific neurological conditions observed in affected horses however were not reported. In the equine veterinary literature, reports of neurological complications associated with the anaesthetic period are predominantly limited to case studies, the majority of which describe spinal cord pathology. To date, fracture of the cervical vertebra[15], spinal

cord myelomalacia[16] and cerebral necrosis[17,18] have all been reported as causes of mortality associated with general anaesthesia.

Over the past twenty years numerous risk factors have been identified as contributing to this increased incidence of equine perioperative fatalities. In adult horses an association between increasing age and risk of perioperative mortality has been reported, with horses older than 14 years being at the most risk[2-4]. The type of surgical procedure has also been shown to correlate with the likelihood of dying; with fracture repair, emergency exploratory laparotomy and caesarian-section carrying the highest risk[2-4]. Johnston *et al*. (1995) reported a significant positive association between surgical time and the likelihood of perioperative death[2]; most likely attributable to the cumulative effects of inadequate perfusion, hypoxaemia and acid-base derangements that are frequently encountered during prolonged periods of general anaesthesia[3].

2.1.2 Recovery from general anaesthesia:

Unlike humans and small animals, recovery from equine general anaesthesia necessitates standing which, in combination with the horses' natural flight instinct, makes this period a particularly precarious one. Injuries sustained during the recovery phase account for 25-50% of anaesthetic-associated deaths and can be attributed to horses attempting to rise before musculoskeletal coordination is adequate[7,19]. The aim of an ideal recovery is to achieve a slow and controlled return to consciousness so that appropriate and deliberate attempts to stand are made[19]. Young *et al*. (1993) found that recovery times had a strong influence on the type of recovery, in that the longer a horse took to stand, the better the quality of recovery[1]. Prolonged recoveries should be avoided however, as lengthy periods of recumbency will

increase the risk of developing postoperative complications such as myopathies and neuropathies which can further exacerbate a poor recovery[19].

Numerous factors are recognised as contributing to the quality of recovery including the duration of anaesthesia, temperament of the horse, degree of postoperative pain and type of anaesthetic agent used[7,19-21]. Isoflurane, a halogenated hydrocarbon commonly used to maintain general anaesthesia has been associated with a higher incidence (51%) of recovery delirium in adult humans when compared to halothane[7]. Comparative studies performed in horses however have shown no significant difference between isoflurane and halothane with regards to the quality of recovery, although a shorter time to sternal recumbency has been noted in isoflurane-anaesthetised horses[7,20,21].

2.1.3 Hypoxaemia during general anaesthesia:

Despite the delivery of a high inspired oxygen (O_2) concentration during general anaesthesia, hypoxaemia (PaO_2 < 60 mmHg), is frequently reported[22]. The positioning of horses in either dorsal or lateral recumbency results in the development of atelectasis in the dependant lung regions, leading to inequalities between ventilation and perfusion, and consequently impaired arterial oxygenation[22-24]. As PaO_2 is one of the main determinants of oxygen delivery to the tissues[18], the development of hypoxaemia during anaesthesia will ultimately result in generalised tissue hypoxia and an increased risk of complications within the perioperative period.

2.2 CEREBRAL PERFUSION AND AUTOREGULATION

Cerebral autoregulation (CA) is a sensitive homeostatic mechanism which acts to maintain a near constant cerebral blood flow (CBF) despite alterations in cerebral perfusion pressure (CPP), through the control of cerebrovascular resistance (CVR)[25-27]. By doing so, this mechanism serves to appropriately match blood flow and oxygen delivery with the metabolic demands of the central nervous system (CNS)[28]. Cerebral perfusion pressure is defined as the difference between MAP at the level of the arterial circle of the brain and intracranial pressure (ICP), and is determined by CBF and CVR. Consequently, any increase in ICP will result in a decrease in CBF, unless accompanied by a compensatory increase in MAP or decrease in CVR[28].

2.2.1 Effects of hypercapnoea on cerebral perfusion and autoregulation:

Hypercapnoea ($PaCO_2$ > 50 mmHg) is frequently encountered during equine general anaesthesia, predominantly due to the respiratory depression and subsequent hypoventilation caused by the inhalation agents used for maintenance. Studies conducted in both humans and dogs have shown that exposure to moderate hypercapnia ($PaCO_2$ = 60-75 mmHg) results in enhanced cardiovascular performance, as determined by an increase in cardiac output (CO) and arterial blood pressure, thought to be caused by an increased stimulation of the sympathetic nervous system[29]. A similar response has been reported in horses anaesthetised with halothane, whereby exposure to moderate and severe levels of hypercapnia ($PaCO_2$ = 82.6 mmHg and 110 mmHg respectively) resulted in a marked degree of haemodynamic stimulation[19].

Hypercapnoea however, has frequently been associated with the impairment of CA, attributable to the potent vasodilatory effects of CO_2 on the cerebral vasculature[25]. McCullouch *et al*. (2000) reported that CA was readily impaired at clinically relevant levels of hypercapnoea ($PaCO_2$ > 50 mmHg) in anaesthetised healthy humans; the extent of impairment however was dependant on the anaesthetic maintenance agent used[25].

2.2.2 Effects of hypocapnoea on cerebral perfusion and autoregulation:

Induction of hypocapnoea through hyperventilation was widely used in humans with acute brain injury as a means of reducing ICP[30] and has been reported to restore cerebral autoregulatory function[26]. Recently however, several deleterious effects associated with hypocapnoea have been highlighted; the most prominent being cerebral hypoperfusion[30]. Not only does hypocapnoea result in a respiratory alkalosis, leading to arterial vasoconstriction and a subsequent decrease in CBF, it also increases the cerebral metabolic requirement for oxygen, ultimately resulting in regional cerebral ischaemia[30]. Even short exposure (20 minutes) to moderate hypocapnia ($PaCO_2$ = 27-32 mmHg) has been reported to produce critical reductions in regional brain tissue oxygenation and has been associated with impaired psychomotor function and long-term neurologic abnormalities[30].

2.2.3 Effect of isoflurane on cerebral autoregulation:

Regardless of the concentration, the use of inhalation agents will decrease CO and mean arterial pressure (MAP), resulting in systemic hypotension which may interfere with the cardiovascular reflexes responsible for maintaining CPP[28]. McPherson *et al*. (1988) reported that the cerebral autoregulatory response to decreased CPP in dogs was preserved at the

minimum alveolar concentration (MAC) of isoflurane (1.3%) however was abolished at twice the MAC[31]. In the presence of diminished CA and inappropriate CPP, cerebral ischaemia is likely and may therefore be associated with an increased risk of perioperative complications.

2.3 NEUROMONITORING

2.3.1 The importance of neuromonitoring during general anaesthesia:

Over the past decade numerous studies have demonstrated an association between intra-operative cerebral oxygen desaturation and adverse neurological outcomes, including post-operative cognitive decline (POCD) or dysfunction, delirium, disorientation and prolonged recoveries[8,12]. It has also been reported that low cerebral oxygen saturation during surgery leads to prolonged ICU and hospital stays[8,32,33]. Consequently, non-invasive techniques to monitor brain and cerebral circulation are being increasingly utilised to facilitate early detection of electrophysical or haemodynamic abnormalities and thus aid in reducing the incidence of post-operative neurological complications.

Post-anaesthetic cerebral necrosis has been described in horses and although rare, should be considered an important cause of perioperative mortality[17,18]. In all reported cases, neurological signs developed within 7 days of anaesthesia; and included ataxia, hypermetria, central blindness, head pressing, recumbency and seizures[17,18]. All horses were subsequently euthanised due to the severity of clinical signs[17,18]. Histopathological examination identified diffuse acute necrosis of the cerebral cortices, type II Alzheimer astrocytosis, and neuronal necrosis consistent with ischaemia[17,18]. The anatomical distribution of the lesions, in association with their histopathological characteristics was consistent with global cerebral

hypoxia, and the severe neurological signs observed in the post-anaesthetic phase correlated well with the pathological findings[17]. In the case series reported by McKay *et al*. (2002), anaesthesia records were available for 4 of the 5 cases. All 4 of those cases encountered variable periods of hypoxaemia and hypercapnoea during general anaesthesia, leading the authors to postulate that the cerebral neuronal ischaemic damage was attributable to episodes of hypotension, hypoxaemia, hypercapnoea or reduced cardiac output[18]. Alternatively, in the case described by Spadavecchia *et al*. (2001), PaO_2 was maintained above 70 mmHg and $PaCO_2$ < 60 mmHg, thereby suggesting hypoxaemia and hypercapnoea did not play a role in the development of cerebral ischaemia[17]. In man, neurological complications observed in relation to general anaesthesia are often associated with concurrent brain pathology[34], thus it was proposed that the affected pony may have had a pre-existing, but clinically silent, microscopic brain lesion[17]. Although the precise mechanism for the development of post-anaesthetic cerebral necrosis in horses remains unknown, it is likely attributable to alterations in cerebral blood flow and perfusion. Consequently, adopting a neuromonitoring protocol for horses undergoing general anaesthesia may provide important information regarding cerebral oxygenation and reduce the risk of developing neurological sequelae.

2.3.2 Types of neuromonitoring:

The ideal neurophysiological monitor should be non-invasive and provide real-time, continuous, objective and rapid assessment of cerebral perfusion and function[12,35,36]. Neuromonitoring should allow early detection of problems associated with cerebral blood flow and neuronal function, thus facilitating appropriate interventions and correction of the neurophysiological imbalance, before the effects become irreversible[12,13,37]. By doing so the overall duration of hospitalisation may be reduced, and clinical outcomes improved[8,9,38].

When periods of cerebral desaturation are encountered, the primary therapeutic aim is to improve cerebral oxygenation. In human cases this is generally achieved by: 1) increasing arterial blood pressure and improving cardiac output; 2) inducing hypercapnoea; 3) the administration of blood products, particularly if Hb concentration is inadequate; and 4) increasing the final inspired O_2 concentration[39]. If resolution of cerebral desaturation fails to occur in response to these initial interventions, reduction of cerebral metabolic requirement for O_2 is attempted through the administration of propofol, a potent suppressor of neuronal metabolism[40].

2.3.2.1 Invasive neuromonitoring:

Multimodal invasive neuromonitoring, including the measurements of ICP, CBF and brain tissue oxygen tensions ($PbtO_2$), along with the use of cerebral microdialysis, is becoming increasingly popular in human intensive care units, particularly in cases of traumatic brain injury (TBI)[41,42].

Invasive monitoring of ICP via the placement of an external ventricular drain is considered to be the “gold-standard” neuromonitoring technique for human patients with TBI[41,43,44]. Not only does this method facilitate the continuous and accurate measurement of ICP, it has the additional advantage of enabling drainage of CSF if deemed necessary[43,44]. A number of other ICP monitoring devices are currently available for use in human medicine including fibre optic devices, strain gauge devices and pneumatic sensors, all of which can be positioned within different intracranial anatomical locations[43,44].

Monitoring CBF plays an important role in human neurosurgical practice by providing an opportunity to identify significant flow alterations before they result in potentially irreversible consequences[45]. Following implantation of a specialised microprobe within the brain parenchyma or vasculature, thermal diffusion can then be used to continuously and quantitatively monitor regional CBF[46,47]. The direct monitoring of $PbtO_2$ involves placement of a microcatheter or probe into the cerebral white matter, and is used extensively to predict cerebral ischaemia in human adults with severe TBI[48,49].

Cerebral microdialysis has been used to monitor human patients suffering from TBI for over a decade, providing valuable information regarding brain metabolism and local brain chemistry[42,50]. The technique involves surgical placement of a microdialysis catheter into the cortical surface, usually within the area of injury, via a 1 mm corticectomy[42]. Sterile artificial cerebrospinal fluid (CSF) is then perfused at a rate of 0.3µl/min and samples collected on an hourly basis, facilitating the monitoring of short-term metabolic changes and long-term trends[42,50].

The use of these neuromonitoring techniques provide direct assessment of cerebral haemodynamic parameters and have been associated with improved outcomes in human patients following severe head injury[48]. Given the requirement for surgical placement of monitoring probes within different anatomical regions of the central nervous system however, the clinical use of such invasive neuromonitoring devices in horses is considered unfeasible.

2.3.2.2 Non-invasive neuromonitoring:

In human medicine, electroencephalography (EEG) is used to measure the spontaneous electrical activity of the cerebral cortex, allowing detection of changes in cerebral and neuronal perfusion[12,13,38,51]. Not only does EEG have a low sensitivity for the detection of cerebral ischaemia (false negative rate of 40.6%) but also the interpretation of an EEG trace requires a trained neurophysiologist[12,36]. Due to these limitations, EEG is unlikely to be used routinely in veterinary medicine.

Transcranial Doppler (TCD) ultrasonography is another commonly used modality to assess cerebral perfusion in human patients. By continuously and non-invasively measuring changes in the velocity of blood flow through both the intracranial arteries and veins, TCD is not only able to instantly recognise alterations in cerebral blood flow, but also allows the detection of clinically significant microemboli[12,13,36,38,51]. However, due to the anatomical structure of the horse's skull it is not possible to adequately image the intracranial vessels, therefore rendering TCD of very limited use in equine medicine.

Similar to EEG and TCD, near-infrared spectroscopy (NIRS) provides real-time, continuous information regarding cerebral perfusion, in a non-invasive manner[8,52]. By measuring the concentrations of oxygenated haemoglobin (oxyHb) and deoxygenated haemoglobin (deoxyHb), regional cerebral oxygen saturation (rSO_2) is determined[37,52,53]. An rSO_2 value greater than 20% below the baseline is indicative of cerebral hypoperfusion and should prompt immediate intervention to improve cerebral oxygenation, and thus prevent cerebral injury[8,12,13,36,51,53].

2.4 NEAR-INFRARED SPECTROSCOPY

2.4.1 General introduction:

Near-infrared spectroscopy was first introduced in 1977 as a means of monitoring tissue oxygenation, and was first reported for use as a neuromonitoring tool during congenital heart surgery in 1991[10,54]. There are three main principles on which NIRS relies in order to derive an rSO_2 value: 1) most living tissues, with the exception of haemoglobin (Hb) and cytochrome oxidase, are relatively transparent to infrared light in the range close to the visual spectrum (700-1000 nm); 2) the absorbance spectrum of Hb is dependant on it's oxygenation status (deoxyHb absorbs less infrared light than oxyHb)[55,56]; and 3) the Beer-Lambert law, which states *"the transmission of light through a solution is a logarithmic function of the density or concentration of the absorbing molecules in the solution, the path length of light through the solution and the specific extinction coefficient for the material at a given wavelength"*[10,55-57]. Therefore, by applying the principles of light transmission (where the degree of change in light absorption is related to the change in concentration of the chromophore within the tissue illuminated), a ratio of oxyHb to total Hb can be calculated. This ratio is shown as a percentage value and is reflective of rSO_2[10,56].

2.4.2 INVOS® 5100C Cerebral/Somatic Oximeter:

The INVOS® 5100C Cerebral/Somatic Oximeter utilises a disposable sensor which incorporates a light emitting diode (LED) and two light-collecting optodes (Figure 1)[55,58]. These optodes are set at fixed distances from the LED: the proximal detector, set at 3 cm, receives a reading from the extracerebral tissue whereas the distal detector, set at 4 cm,

receives a reading from the cortical and intracerebral structures[51,55]. Measurement of the absorption ratios at two different sites allows for the removal of the extracranial contribution via a subtraction algorithm, thus giving a mean value for cerebral saturation[10,52,54,55]. In order to generate an rSO_2 value, cerebral oximetry makes several assumptions. Not only is it assumed that 75% of the cerebral blood flow is venous (25% arterial) and that this ratio is unchanging,[8,59] but also that the degree of light scatter is constant throughout the measurement period and therefore can effectively be ignored[55].

Light absorption data is collected continuously fifteen times per second for a total of fifty samples (3.3 sec). These values are averaged to determine the new rSO_2 value and this number is then displayed on the monitor (Figures 2 and 3)[45]. Due to the significant intra-individual variability and the assumptions made by the cerebral oximeter, NIRS is deemed more reliable when monitoring trends of oxygen saturation, rather than absolute values[12,55,59]. An rSO_2 value $< 50\%$ or a decrease of $\geq 20\%$ compared to the baseline value has been shown to be consistent with cerebral hypoperfusion, and indicates the need for intervention[12,13].

2.4.3 Potential role for near-infrared spectroscopy in equine general anaesthesia:

Traditionally, neuromonitoring has been primarily used in human patients undergoing cardiothoracic surgeries, those suffering from traumatic head injury or for bedside monitoring of critically ill preterm infants[10,13,39]. More recently however, the use of NIRS has been advocated in patients undergoing non-cardiac abdominal surgery on the basis that all general anaesthetics will produce cardiovascular depression, thereby potentially exposing patients to inadequate cerebral perfusion[39]. Undoubtedly cardiothoracic surgeries are rarely performed in

horses; exploratory abdominal surgery however is common, and it is in this regard that NIRS may be beneficial in equine patients.

Endotoxaemia is encountered in approximately 29% of horses presenting with acute intestinal disease, and the likelihood of this complication is increased in horses undergoing an exploratory laparotomy[60]. Cerebral hypoperfusion has been reported in response to administration of lipopolysaccharide (LPS) and is attributable to a transient decrease in CBF and endothelial dysfunction[61]. In young lambs, administration of LPS resulted in a 30% reduction in CBF, in the absence of hypoxaemia and hypercapnoea, and is thought to be associated with cerebral vasoconstriction mediated by tumour necrosis factor alpha[61]. Vasodilation of the systemic vasculature also occurs following the release of endotoxin, resulting in a decrease in mean arterial pressure and systemic vascular resistance. This hypotension is frequently exacerbated by hypovolaemia and dehydration, resulting in a decrease in CPP[62]. In addition, exposure to endotoxin can cause significant impairment of cerebral autoregulation leading to a profound reduction in CBF, particularly in the presence of inappropriate CPP[63]. Therefore, the use of NIRS should be considered in horses with endotoxaemia as they are potentially at an increased risk of cerebral desaturation during general anaesthesia.

2.5 THE VENOUS DRAINAGE SYSTEM OF THE BRAIN

The anatomy and haemodynamics of the equine brain lends itself to the possibility of using near-infrared spectroscopy to determine rSO_2. The venous drainage system involves a number of endothelial-lined sinuses enclosed within the dura mater and are divided into dorsal and ventral systems[64]. The dorsal system includes the dorsal sagittal sinus (DSS), rectus sinus,

transverse sinus, dorsal petrosal sinus and temporal sinus (Figures 4 and 5). The dorsal sagittal sinus lies within the *falx cerebri* and receives numerous veins directly from the cerebral hemispheres[65]. As it runs caudally, it joins the rectus sinus which is formed from *corporis callosi major* and the *cerebri magna* veins, before terminating and splitting into the transverse sinus[64]. The transverse sinus lies in a transverse plane and joins the dorsal petrosal sinus before entering the temporal meatus and joining the temporal sinus[64]. The dorsal venous sinus system then exits the cranial cavity via the *emissaria foramen retroarticularis* vein which ultimately communicates with the external jugular vein[64,66].

Chapter 3: Use of Near-infrared Spectroscopy to Identify Trends in Regional Cerebral Oxygen Saturation in Horses

3.1 INTRODUCTION

It is widely accepted that equine general anaesthesia carries a high risk of mortality or serious morbidity, with the recovery period being a time of particular concern[1-6]. A UK-based epidemiological multicentre study of perioperative equine fatalities identified an overall mortality rate of 1.9%[3]; a significantly higher rate than what is reported in small animals (0.2%)[6]. Unlike humans and small animals, recovery from equine general anesthesia necessitates standing, making this period particularly precarious. Although there is currently no evidence supporting hypoxaemia as a risk factor for equine peri-anaesthetic mortality[3], it is possible that alterations in cerebral haemodynamics may contribute to the degree of disorientation and incoordination experienced during recovery. Hence the use of non-invasive neuromonitoring tools may be beneficial.

Post-operative neurological complications are commonly encountered in human patients[8,32,38]. Over the past decade, numerous human studies have demonstrated an association between intra-operative cerebral oxygen (O_2) desaturation and adverse neurological outcomes[12,38]. Consequently, techniques to monitor cerebral circulation are being increasingly utilised to facilitate early detection of haemodynamic alterations.

Near-infrared spectroscopy (NIRS) is frequently used intra-operatively in human patients, and provides real-time, continuous data regarding cerebral perfusion[32]. By using light optical spectroscopy to measure the concentrations of oxygenated and deoxygenated haemoglobin

(Hb) within the cerebral vascular bed, regional cerebral oxygen saturation (rSO_2) can be determined[12,52].

Currently, neuromonitoring techniques available for use in equine medicine are limited. The direct measurement of intracranial pressure has been reported[14]; however the invasiveness of this procedure prohibits its use as a routine neuromonitoring tool in horses.

3.2 OBJECTIVES

The objectives of this study were to determine whether NIRS could be used to: 1) assess trends in rSO_2 in clinically healthy conscious and anaesthetised horses; 2) detect changes in rSO_2 during periods of induced hypo- or hypercapnoea; and 3) determine whether a correlation exists between rSO_2 and venous oxygen tensions (PvO_2).

3.3 HYPOTHESES

We hypothesised that NIRS would be able to detect trends in rSO_2 in conscious and anaesthetised horses; detect changes in rSO_2 during periods of induced hypo- or hypercapnoea, and that there would be a positive correlation between rSO_2 and PvO_2.

3.4 MATERIALS AND METHODS

3.4.1 Horses:

Six healthy adult Nooitgedacht mares (age range 12-16 years, median 13 years; weight range 400-486 kg, median 432.5 kg) were selected from the Onderstepoort University Teaching Animal Unit (OTAU) for inclusion in this study. To eliminate any potential confounding associated with age, middle-aged mares were selected for this study. The researchers did not individually select the mares; rather appropriately aged mares were allocated to the study by OTAU based on their availability for the data collection period.

During hospitalisation mares had free access to hay with the exception of the 12 h period preceding general anaesthesia, and water was offered *ad libitum*. The study was approved by the Animal Use and Care Committee of the Faculty of Veterinary Science, University of Pretoria.

3.4.2 Blood sample collection and analyses:

Horses were sedated with xylazine[a] (0.2 mg/kg bwt, IV) and an 18 G over-the-wire arterial catheter[b] was placed in the left carotid artery under ultrasonographic guidance. Retrograde catheterisation of the left external jugular vein was performed using a 14 G, 13 cm catheter[c] placed with the tip positioned close to the bifurcation of the vein, at the angle of the mandible. Blood was collected and stored in heparinised blood gas syringes[d] at room temperature, for arterial and venous blood gas analyses[e] (ABG and VBG respectively). Analyses were corrected for body temperature and performed within 15 minutes of collection. Plasma lactate

concentrations ([LAC]) were measured from blood samples collected into sodium fluoride/potassium oxalate tubes, using a hand-held lactate monitor[e].

3.4.3 rSO_2 measurements:

Cerebral rSO_2 values were obtained using the INVOS® 5100C Cerebral/Somatic Oximeter[f] which utilises a disposable, self-adhesive sensor[g]. A dedicated sensor was used for each of the 6 horses and disposed of following completion of data collection. To obtain cerebral rSO_2 values the sensor was placed directly onto clean, shaved pigmented skin, and positioned on midline, over the region of the DSS (Figure 6). A 12 x 8 cm rectangle of black duct tape was placed over the sensor to prevent external light interference.

The INVOS® 5100C automatically collected light absorption data fifteen times per second, for a total of fifty samples, before averaging them to determine the new rSO_2 value. This result was then displayed on the monitor and updated every 3.3 sec. (Figure 7)

3.4.4 General anaesthesia:

All horses were premedicated with romifidine[h] (50 µg/kg, IV). General anaesthesia was induced using ketamine[i] (2.2 mg/kg, IV) and diazepam[j] (10 mg/horse, IV), and the horses were positioned in right lateral recumbency. A 26 mm internal diameter silicone tube was used for endotracheal intubation, connected to a large animal circle circuit. Anaesthesia was maintained using inhalant isoflurane[k] delivered in 5 L/kg/min O_2, at an expired concentration close to the minimum alveolar concentration (MAC) value of 1.2 reported for horses[68]. Positive pressure ventilation[l] was initiated using a constant peak inspiratory pressure of 20 cm

H_2O and varying respiratory rate (RR) to obtain states of normo- ($PaCO_2$ = 40-50 mmHg), hyper- ($PaCO_2$ = 50-60 mmHg) and hypocapnoea ($PaCO_2$ = 25-35 mmHg) as determined by capnography and confirmed by ABG analysis. Direct arterial blood pressure[m], electrocardiogram[m] and heart rate (HR) were measured and displayed continuously throughout the anaesthetic period, along with the expired isoflurane concentration[m] and the expired CO_2 tension[m]. Intravenous isotonic crystalloid fluids[n] were administered at a rate of 5 ml/kg/h, along with a dobutamine[i] constant rate infusion (1 µg/kg/min, IV) if the MAP dropped below 60 mmHg.

In preparation for recovery, mechanical ventilation and isoflurane were discontinued, and horses were allowed to breathe spontaneously. Romifidine[g] (0.01 mg/kg bwt, IV) was administered as soon as nystagmus was noted. Oxygen was administered via the endotracheal tube (15 L/min) until attempts to sternal recumbency were made. Horses recovered without assistance and were extubated once standing.

3.4.5 Experimental procedures:

Horses were assigned to either Group A or B (n = 3, respectively), based on predetermined blinded randomisation conducted by the principal investigator. Whilst under general anaesthesia, those horses in Group A were hypoventilated first, followed by a period of hyperventilation; whereas those in Group B were hyperventilated first.

The study consisted of 8 recording periods (RPs), each of 10 minutes duration (Table 1). Physical examinations were performed at the beginning (t = 1 min) and end (t = 10 min) of each recording period, and HR, pulse quality, capillary refill time, mucous membrane colour,

RR and temperature were recorded (Appendix 1). Venous and arterial blood samples were collected at t = 1, 5 and 10 min for both VBG and ABG analyses, and at t = 1 min for measurement of [LAC]. Cerebral rSO_2 values were automatically recorded by the cerebral oximeter once every 5-6 sec, for the duration of each RP.

The cerebral oximeter was equilibrated for up to 5 min prior to each recording period, whereby rSO_2 values were displayed but not recorded, to ensure adequate signal strength was achieved and maintained.

3.4.6 Data analysis:

Data were assessed for normality using descriptive statistics, histograms and the Anderson-Darling test for normality. Normally distributed data were presented as mean ± standard deviation (s.d.). Mean rSO_2 values were calculated over the 30 second period in which blood was collected for VBG and ABG evaluation, to use for statistical analysis. Linear mixed effects models were used to estimate the effects of RP and time for rSO_2, PaO_2, PvO_2, $PaCO_2$, $PvCO_2$, $SavO_2$ and MAP. The horse was included as a random effect and fixed effects included RP, dobutamine administration and the interaction between RP and dobutamine administration. Bonferroni correction was used for *post hoc* pairwise comparisons. Variance components analysis was used to estimate the influence of dobutamine administration on rSO_2 values, and to evaluate the repeatability of rSO_2 measurements. Correlations between rSO_2 and PaO_2; rSO_2 and PvO_2; rSO_2 and $PaCO_2$; rSO_2 and $PvCO_2$; rSO2 and $SavO_2$; as well as between rSO_2 and [LAC] were assessed using the Pearson's correlation coefficient. All statistical analyses were performed using commercially available statistical software[o,p]. Statistical significance was set at $P < 0.05$.

3.5 RESULTS

The mean rSO_2 values recorded for each horse during RP1-8 is shown in Table 2. The highest overall mean rSO_2 value was recorded during RP4 (60.0 ± 6.9%), whereas the lowest was recorded during RP7 (40.7 ± 6.8%) (Figure 8). Similarly, the highest overall mean PvO_2 value was recorded during RP4 (64.7 ± 12.0 mmHg), and the lowest was recorded during RP7 (27.7 ± 3.6 mmHg).

Overall means for rSO_2, PaO_2, PvO_2, $PaCO_2$, $SavO_2$ and MAP varied significantly by RP ($P < 0.001$) (Table 3). Significant differences in rSO_2 were identified between RP1 and the post-anaesthetic periods (RP6-8) ($P < 0.001$). No significant differences in rSO_2 values were identified between periods of normo- (RP3), hyper- (RP4) and hypocapnoea (RP5); or between RP1 and RP3, RP4 and RP5. Both PaO_2 and PvO_2 were significantly increased during RP3, RP4 and RP5 ($P < 0.001$), with PaO_2 values differing significantly between those periods ($P < 0.05$). Compared to the standing horse (RP1), $SavO_2$ increased significantly during the anaesthetic periods (RP3-5) ($P < 0.001$), and significantly decreased during RP7 ($P < 0.05$). Mean $PaCO_2$ and $PvCO_2$ values recorded during periods of hyper- and hypocapnoea (RP4 and RP5) differed significantly from RP1 as well as from those recorded following recovery (RP7 and RP8) ($P < 0.001$). Mean arterial pressures were significantly lower during general anaesthesia compared to RP1 and RP2 ($P < 0.001$).

All horses received dobutamine, for a period of time ranging between 15-60 min during general anaesthesia. Dobutamine administration had a significant effect on MAP values ($P < 0.01$) whilst the interaction between RP and dobutamine administration was significant for rSO_2 ($P < 0.01$), PaO_2 ($P < 0.001$) and PvO_2 ($P < 0.001$).

The largest proportion of variation in rSO_2 values was due to each individual horse (33%), the RP (29%), and how individual horses responded to the different RP (18%). Dobutamine administration accounted for 13% of the variability in rSO_2 values and varied by RP (administration was limited to RP3, RP4 and RP5). Variance components analysis also estimated that only 6.6% of the variability in rSO_2 was attributed to unmeasured variables such as sensor positioning.

Significant positive correlations were identified between rSO_2 and PaO_2 ($r = 0.448$, $P < 0.001$) (Figure 9); rSO_2 and PvO_2 ($r = 0.512$, $P < 0.001$) (Figure 10); rSO2 and $SavO_2$ ($r = 0.469$, $P < 0.001$) (Figure 11); and rSO_2 and $PaCO_2$ ($r = 0.198$, $P < 0.05$) (Figure 12). No significant correlations were identified between rSO_2 and either $PvCO_2$ ($r = 0.056$, $P = 0.51$) or [LAC] ($r = -0.257$, $P = 0.08$) (Figures 13 and 14 respectively).

3.6 DISCUSSION

Near-infrared spectroscopy has previously been used in equine medicine to monitor tissue oxygenation during wound healing, muscle oxygenation, and the haemodynamics of the pedal circulation[69-71]; however to the authors' knowledge, this is the first study demonstrating that NIRS can be used to identify trends in regional cerebral oxygen saturation in horses.

For the purpose of this study, the cerebral oximeter sensor was placed on midline over the region of the DSS, rather than dorso-laterally over the frontal cortex as it is in humans, in a bid to limit inter-subject variations in rSO_2 values associated with sensor positioning. The DSS forms part of the dorsal venous drainage system of the brain, and receives numerous veins directly from both cerebral hemispheres[65]. Therefore, because of its close association

with the cerebrum, as well as its proximity to an easily accessible region of the skull, the DSS was considered an appropriate anatomical location from which to try and record rSO_2 values.

A normal baseline rSO_2 value in an adult human is considered to be approximately 70%, which is considerably higher than those recorded in 4 out of 6 horses in this study (Table 2); however values ranging from 45-90% have been reported in human patients[51,59]. The reason for the lower rSO_2 values observed in the majority of horses may be attributable to the fact that horses have an increased skin and skull thickness compared to humans, as these factors have been shown to have a major effect on the intensity of infra-red light detected, and hence the calculated rSO_2 value[72].

The influence of pigmented skin on NIRS recordings should also be considered, despite receiving little attention in the medical literature. Near-infrared spectroscopy relies on the principle that light, at a wavelength 650 – 1000 nm, passes readily through skin and subcutaneous tissue however is absorbed by chromophores such as Hb, cytochrome oxidase and melanin[73]. Given this phenomenon, it would seem that the degree of skin pigmentation would have a significant effect on the reported rSO_2 value, in that the darker and more pigmented the skin, the more the NIRS signal would be impeded. Wassenaar *et al*. (2005) reported a loss of signal when using NIRS on human patients with very dark skin; however the sample size was deemed too small to quantify the effect[73]. Alternatively, in a study performed on calf skulls, no significant difference in NIRS signal was observed when the probe was placed over black or white skin, nor was there a detectable difference in rSO_2 values when the skin was removed from the skull[74]. In the present study, NIRS was used to identify trends in rSO_2, rather than absolute values. Consequently, each mare acted as her own

control, thereby eliminating any potentially confounding effect caused by differences in skin pigmentation.

An rSO_2 value less than 50% or a relative decrease of ≥ 20% compared to pre-induction baseline values is considered clinically significant, and has been associated with focal cerebral ischaemia in human patients[12,36,51]. Decreasing rSO_2 values are thought to reflect shifts in the delicate balance between cerebral O_2 supply and demand, suggestive of cerebral hypoxia; and indicate the need for interventions aimed at improving cerebral perfusion[33,53]. Similarly, keeping rSO_2 values ≥ 75% of the pre-induction baseline value has been associated with a decreased incidence of post-operative neurological outcomes and shorter hospital stays[12,33].

In the present study, 4 out of 6 horses had pre-induction baseline rSO_2 values < 50%, and all horses except for Horse 6 had a ≥ 20% decrease in baseline rSO_2 during the period immediately post-recovery (RP7) (Table 2). Despite this, each horse recovered uneventfully, taking a minimum of 1 attempt (Horses 1, 2, 4, 5 and 6) and a maximum of 3 attempts to stand (Horse 3). Obvious neurological deficits were not observed in any horse during the immediate post-anaesthetic period, or the 24 h following recovery which could suggest that horses are able to tolerate lower levels of cerebral oxygen saturation without gross deleterious consequences. It is currently unknown at which rSO_2 value cerebral oxygen desaturation occurs in horses, hence there are no guidelines to indicate a need for intervention as there is in human medicine. Correlating recovery scores and post-anaesthetic neurological status with intra-operative and recovery rSO_2 values was beyond the scope of this study, however should be considered in the future to provide information that may be used to devise equine-appropriate intervention protocols.

The significant differences identified between baseline and post-anaesthetic rSO_2 values are most likely attributable to a combination of three key factors: 1) impairment of pulmonary gas exchange occurring as a result of general anaesthesia and lateral recumbency; 2) differences in the oxygen saturation of Hb (SaO_2) during those recording periods; and 3) an increase in tissue metabolic rate during the recovery period.

It is widely accepted that general anaesthesia and recumbency results in ventilatory compromise in horses, predominantly due to ventilation/perfusion (V/Q) mismatch; however anaesthetic agent-induced respiratory depression, restricted thoracic expansion of the dependant lung region and a decrease in functional residual lung capacity are also contributing factors[24,75-77]. In recumbent horses the non-dependant lung is preferentially ventilated, resulting in maldistribution of inspired gas, whereas the dependant lung is more adequately perfused, even if intermittent positive pressure ventilation is used in combination with oxygen-rich gas[76-78]. Consequently, inappropriate perfusion of ventilated alveoli occurs, along with increasing differences in alveolar-arterial oxygen partial pressure gradients and subsequent right-to-left intrapulmonary shunting[75-77].

In this study, despite being positioned in lateral recumbency, arterial oxygen tensions and Hb oxygen saturations remained above 200mmHg and 99% respectively, for the duration of the anaesthetic period, mostly likely due to the administration of 100% oxygen. Following discontinuation of mechanical ventilation and endotracheal administration of 100% oxygen however, mean PaO_2 values were observed to decrease from 340.8 ± 76.0 mmHg to 62.3 ± 22.2 mmHg (Table 1). Hypoxaemia (PaO_2 < 60 mmHg) occurs frequently in the immediate post-anaesthetic period regardless of body position or PaO_2 recorded during anaesthesia, as a result of the abrupt change in fraction of inspired oxygen (FiO_2), from 1 to 0.21[76,79].

Moreover, hypoventilation occurring as a result of residual anaesthetic agent-induced CNS depression, will contribute to the lowered PaO_2 values seen during recovery[76].

The primary aim of oxygen supplementation during the initial recovery phase is to maximise Hb saturation, as delivery of oxygen to the tissues is largely dependant on Hb concentration and saturation[80,81]. In the present study, despite the administration of O_2 at 15 L/min, SaO_2 decreased markedly from 99.8% during RP5, to 93.4% and 95.7% during RPs 6 and 7 respectively. Unsurprisingly, a similar decrease in $SavO_2$ was also noted during the recovery periods, with the lowest value being recorded during RP7 (63.8 ± 7.3%). As Hb saturation is an important factor in determining rSO_2, the decrease in Hb oxygen saturation observed during the initial recovery period (RPs 6 and 7) is likely to have played a role in the generation of lower rSO_2 values.

Near-infrared spectroscopy relies on the principle that the absorbance spectrum of Hb is dependent on its oxygenation status, whereby deoxygenated Hb absorbs less infrared light than oxygenated Hb[55]. Therefore, a positive correlation between rSO_2 and $SavO_2$ was to be expected. As $SavO_2$ is calculated using the ratio 25% SaO_2:75% SvO_2 however, it is not surprising that a stronger correlation was not reported, given that the cerebral oximeter sensor was placed over a venous sinus, through which the venous component of blood is > 75%.

It has been well described in human medicine that whole-brain metabolism decreases substantially during general anaesthesia[82,83]. Sokoloff (1981) reported that local energy metabolism in cerebral tissue is closely linked to the degree of functional activity[84]; therefore it can be surmised that the less neuronal activity present, the lower the metabolic requirement. Regional cerebral metabolic requirement, defined as the product of regional cerebral blood

flow and cerebral arterio-venous oxygen content difference, has been reported to decrease by up to 51% during isoflurane anaesthesia[83]. As a consequence of reduced cerebral metabolism, regional oxygen extraction will decrease proportionately, resulting in increased PvO_2, $SavO_2$ and subsequently rSO_2 values, as was demonstrated during RPs 3, 4 and 5 (Figure 1). Alternatively, during recovery from general anaesthesia the metabolic rate of tissues, along with oxygen extraction at the tissue level increases[76], which is likely to have contributed to the decrease in PvO_2 and rSO_2 values observed during RPs 6 and 7 (Figure 1).

Cerebral autoregulation is an important homeostatic mechanism which acts to minimise alterations in CBF following changes in CPP, through vasomotor effectors that control cerebrovascular resistance[25]. As a result, this regulatory function ensures that blood flow and oxygen delivery are adequately matched with the metabolic demands of the cerebral tissue[28].

Both hypercapnoea and the use of inhaled anesthetic agents, including isoflurane, are reported to have a direct, dose-dependant dilatory effect on the cerebral vasculature resulting in an increase in CBF yet impaired CA[25,85]. Interestingly however, in a study performed on healthy horses, Brosnan *et al*. 2011 demonstrated that CA was preserved during isoflurane anaesthesia, suggesting that inhalation anaesthetic dose effects on CA are considerably different to those previously reported in humans[28]. This preservation of CA may therefore explain why no significant differences were observed between baseline (RP1) rSO_2 values and those recorded during general anaesthesia (RPs 3, 4 and 5).

Hypercapnoea ($PaCO_2$ > 50 mmHg) is frequently encountered during equine general anaesthesia, predominantly due to the respiratory depression and subsequent hypoventilation caused by inhalation agents used for maintenance[86]. Although originally thought to be

detrimental, mild to moderate hypercapnoea ($PaCO_2$ = 50-65 mmHg) has been shown to improve arterial blood pressure and cardiac output due to an increased CO_2-induced sympatho-adrenal response[29,68,86]. Khanna et al. (1995) also reported a significant increase in both Hb and haematocrit during periods of moderate and severe hypercapnoea ($PaCO_2$ = 75 - 85 mmHg and > 95 mmHg respectively), in response to increased sympathetic nervous system activity[29], which may result in improved O_2 delivery to tissues. As expected, during the period of induced hypercapnoea (RP4) a trend of increasing rSO_2 values was observed, compared to those measured in the awake, standing horse (RP1). However, this increase did not reach statistical significance, most likely due to the fact that only a mild state of hypercapnoea was achieved (mean 55.5 ± 3.3 mmHg), as well as the small study population.

Rectal temperatures were measured during each recording period at the same time in which blood was collected for arterial and venous blood gas analyses to allow for temperature correction. During RP1, 2 and 8 the rectal temperatures of all 6 horses were within the range of 36.5 - 38.1°C. During the anaesthetic periods (RP3-5) recorded rectal temperatures ranged from 35.6 - 37.5°C; and from 35.1 - 36.6°C during the recovery periods (RP6-7) (Appendix 2).

A decrease in body temperature causes a shift of the oxygen-haemoglobin dissociation curve to the left, resulting in decreased release of oxygen bound to haemoglobin and a subsequent decrease in oxygen delivery to the tissues[86]. Haemoglobin-oxygen saturation remains high however, despite a decrease in PO_2[86]. Calculated values for $SavO_2$ are therefore not affected by temperature, however, temperature-corrected values for PaO_2, PvO_2, $PaCO_2$ and $PvCO_2$ will differ slightly compared to those reported at 37°C, as was observed in the current study (Table 4).

In dogs, severe hypothermia (T = 29.08°C) has been shown to result in marked decreases in CBF, MAP, and cerebral metabolic rate of O_2, along with an increase in CVR[87]; all of which will affect calculated rSO_2 values. In the present study only mild hypothermia was observed, with a minimum rectal temperature of 35.1°C being recorded during the recovery period. It is possible however, that even this small decrease in temperature may have contributed to the lower rSO_2 values recorded during this time, as a consequence of altered CBF and CVR.

Results of this study are in agreement with what is reported in humans, whereby rSO_2 values are significantly correlated with venous oxygen tensions[88]. In human medicine it is generally accepted that the venous portion of blood predominates in the cerebral tissue and as a result rSO_2 values are largely reflective of venous oxygenation[8,38,59]. Jugular bulb venous oxygen saturations (SjO_2) have been used to provide global estimates of cerebral oxygen extraction in human medicine, and a significant correlation between SjO_2 and rSO_2 values was identified in children with congenital cardiac abnormalities[89]. Similarly, significant correlations between central venous oxygen saturations, obtained from the right atria or superior vena cava, and rSO_2 have also been reported[90]. As jugular venous oxygen tensions and rSO_2 values are measurements of regionally different entities, close agreement between the 2 parameters is not necessarily expected. Given their relationship however, it is feasible to expect that a change in jugular PvO_2 will be paralleled by a change in rSO_2, as was observed in the present study (Figure 8).

All horses included in the study received the positive inotropic agent dobutamine, for a period of time ranging between 15-60 min during general anaesthesia, in order to maintain MAP above 60 mmHg; however despite this, MAP values measured during RPs 3, 4 and 5 were significantly lower than those recorded during RPs 1 and 2 (Table 1). This can be explained

through the dose-dependant hypotension secondary to peripheral vasodilation, and decrease in cardiac output, caused by the administration of isoflurane[29,68]. The administration of dobutamine, a β_1-adrenoreceptor agonist, has been shown to have little direct effect on cerebral haemodynamics; however increasing MAP through the use of a β-sympathomimetic will result in an increase in CPP[91]. As demonstrated by Brosnan et al. (2011), CA is preserved in horses during isoflurane-anaesthetic thus CBF remains constant, despite an increase in CPP[28]. Therefore, it is unlikely that the administration of dobutamine will influence rSO_2 values recorded during general anaesthesia.

The presented results of this study should be evaluated in light of a number of potential limitations. Firstly, there is no "gold-standard" for the validation of rSO_2 values. In human medicine there is also a lack of a "gold-standard"; instead, measurements of jugular bulb oxygen saturations have been used as a means of assessing global cerebral oxygenation, and to compare with rSO_2 values for the purpose of cerebral oximeter validation[89,92]. Attempts to recreate this method were made in the present study by catheterising the external jugular vein in a retrograde manner, in order to sample venous blood from as close to the brain as possible.

Secondly, the subtraction algorithm used by the cerebral oximeter to calculate rSO_2 was designed for human patients and is based on the assumption that 75% of cerebral blood flow is venous[8,89]. In the present study the sensor was placed over the region of the DSS through which venous blood flow is greater than 75%, potentially invalidating the algorithm.

It is generally accepted that the thickness of a horse's skin and skull is greater than that of a human. Okada and Delpy (2003) reported that superficial tissue (skin and skull) and cerebrospinal fluid thickness had a significant effect on the intensity of light detected by the

spectrophotometer; that is, the thicker the skull the less near-infrared light detected and the lower the calculated rSO_2 value[72]. Therefore, if the horses' skull is thicker than that of a human at the level of the sensor, increased extracerebral attenuation of near-infrared light may occur, resulting in the recording of lower rSO_2 values.

The small sample size used in this study is also considered to be a limitation. However, a 2-sample t test performed *post hoc* demonstrated that a sample size of 6 was adequate to detect a difference of at least 10% in rSO_2 values between recording periods, for a statistical test with a minimum of 80% power. Also, in a number of other reports describing the use of NIRS in horses, a sample size of no more than 6 was used[69-71].

3.7 CONCLUSION

In conclusion, this study demonstrates that near-infrared spectroscopy can be used to identify trends in regional oxygen saturation in horses. A positive correlation between rSO_2 and PvO_2 was also identified, suggesting that changes in regional cerebral oxygenation may be reflected in PvO_2 obtained from the jugular vein. Future studies involving larger numbers of horses need to be performed, to determine: 1) the usefulness of NIRS as a neuromonitoring tool in horses and 2) whether rSO_2 values recorded during general anaesthesia influences a horse's recovery.

Figures and Tables

Figure 1: The INVOS® 5100C Cerebral/Somatic Oximeter disposable sensor incorporates a light-emitting diode (white arrow) and two light-collecting optodes (black arrows). The optodes are set at a fixed distance of 3 cm and 4 cm from the light-emitting diode in order to collect readings from the extracerebral tissue, as well as the cortical and intracerebral structures respectively.

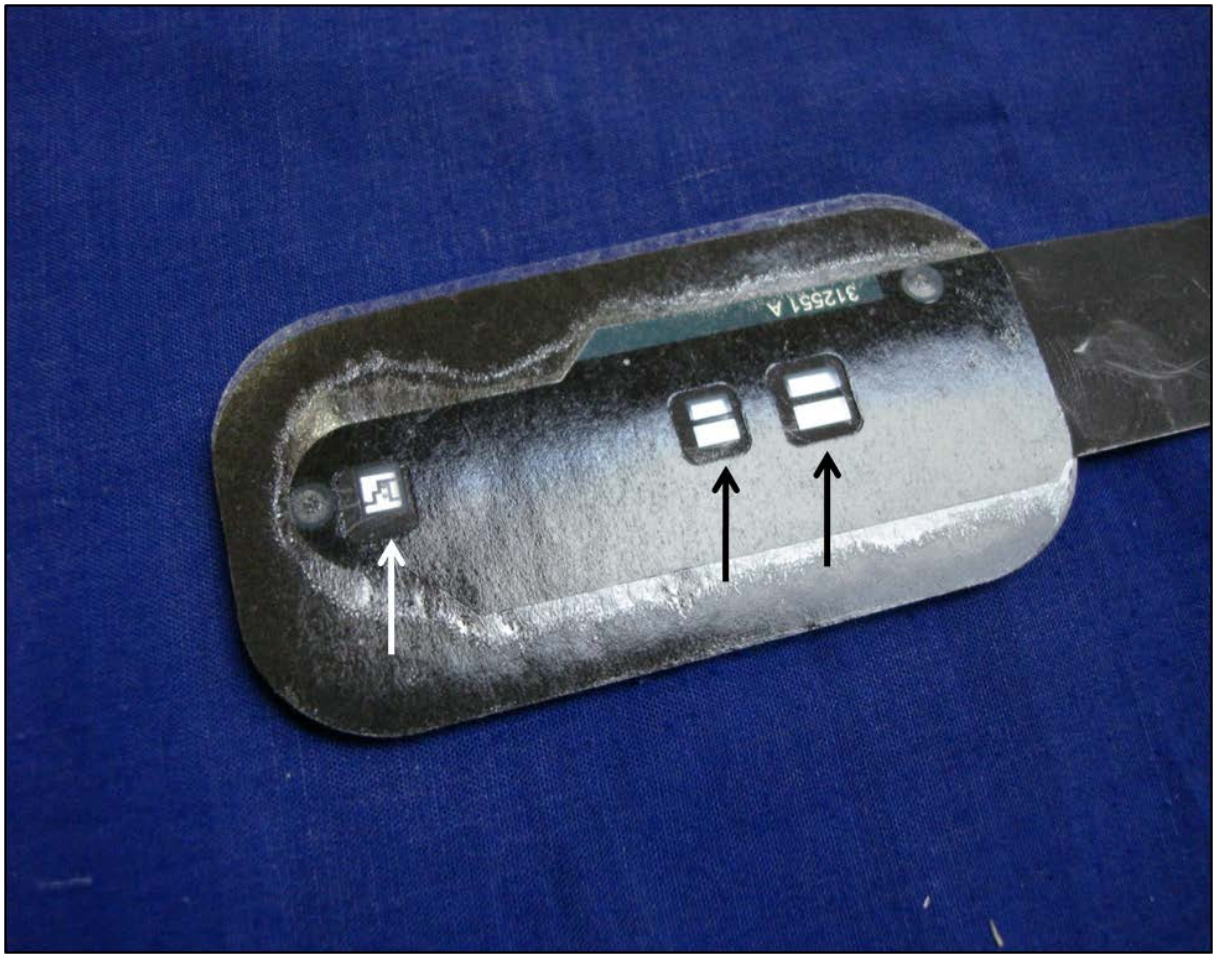
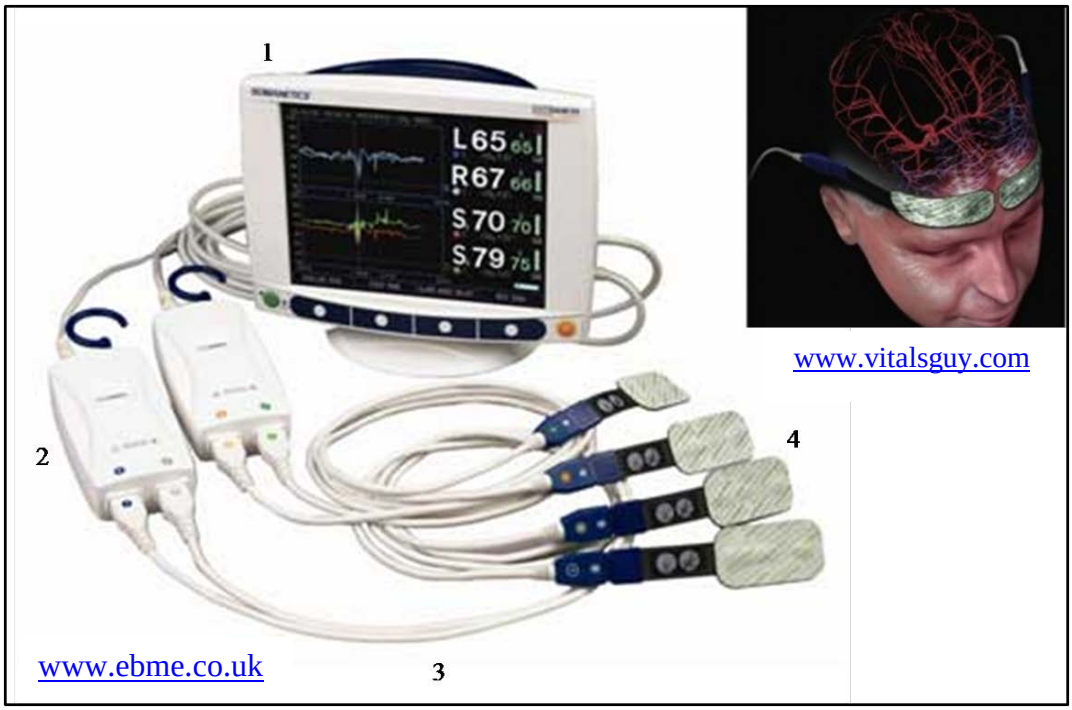


Figure 2: The INVOS® 5100C Cerebral/Somatic Oximeter system consists of a display monitor, 2- or 4-channel preamplifier, sensor cables and disposable sensors. The monitor is capable of displaying rSO_2 values (arrows) obtained from a maximum of 4 sensors at any one time. Generally 2 cerebral sensors are utilised in human patients, positioned to receive readings from the left and right cerebral cortices (inset); with the somatic (S) sensors being used concurrently if required.



1) INVOS® 5100C Cerebral/Somatic Oximeter monitor
2) 4-channel pre-amplifier
3) Sensor cables
4) Disposable adult sensors

Figure 3: The 2-channel INVOS® 5100C Cerebral/Somatic Oximeter monitor displaying both the updated rSO_2 value (white block arrow) and baseline rSO_2 value (black arrow). The monitor also displays the trend in recorded rSO_2 (blue arrow) and the near-infrared signal strength (white arrow).

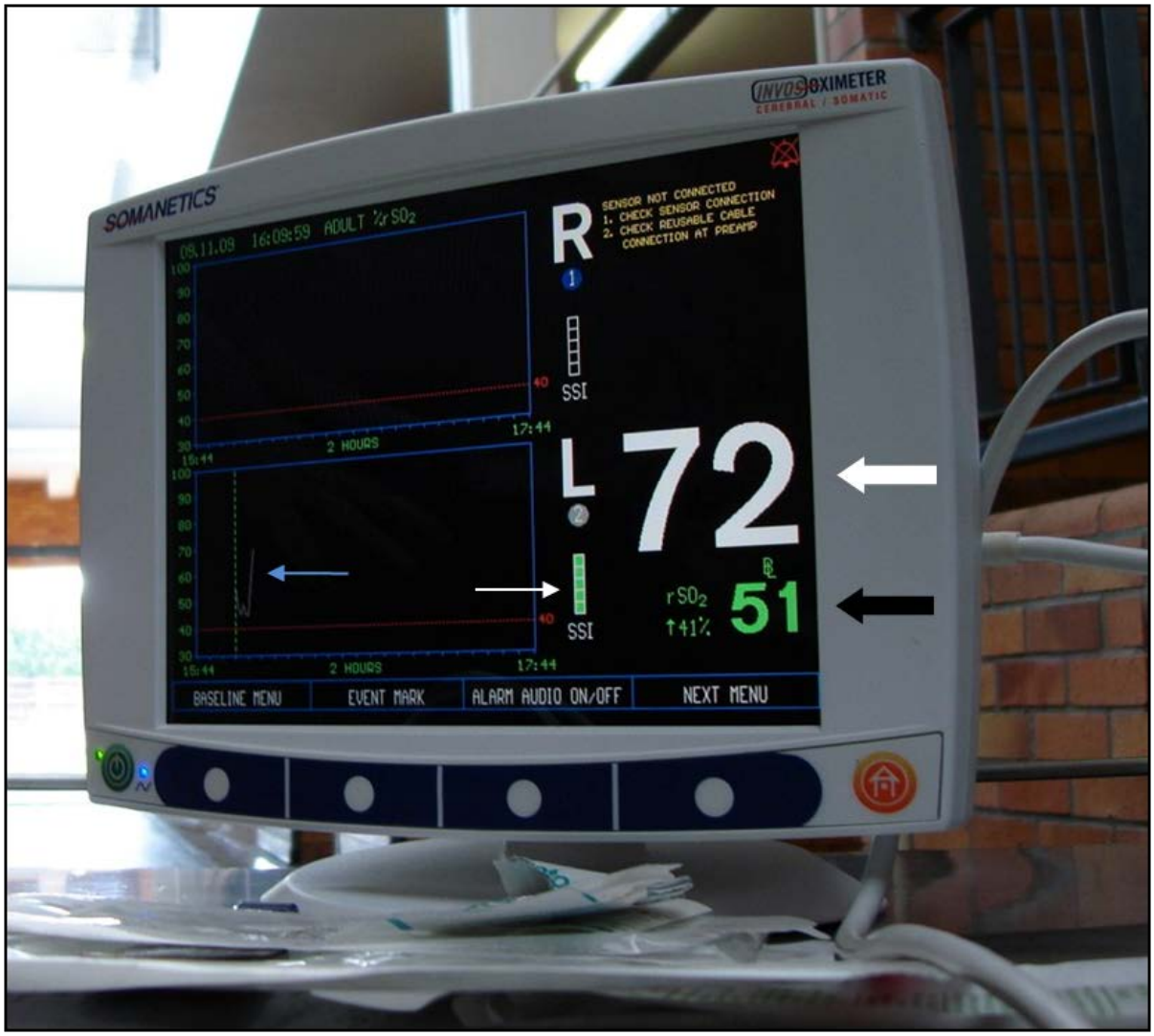
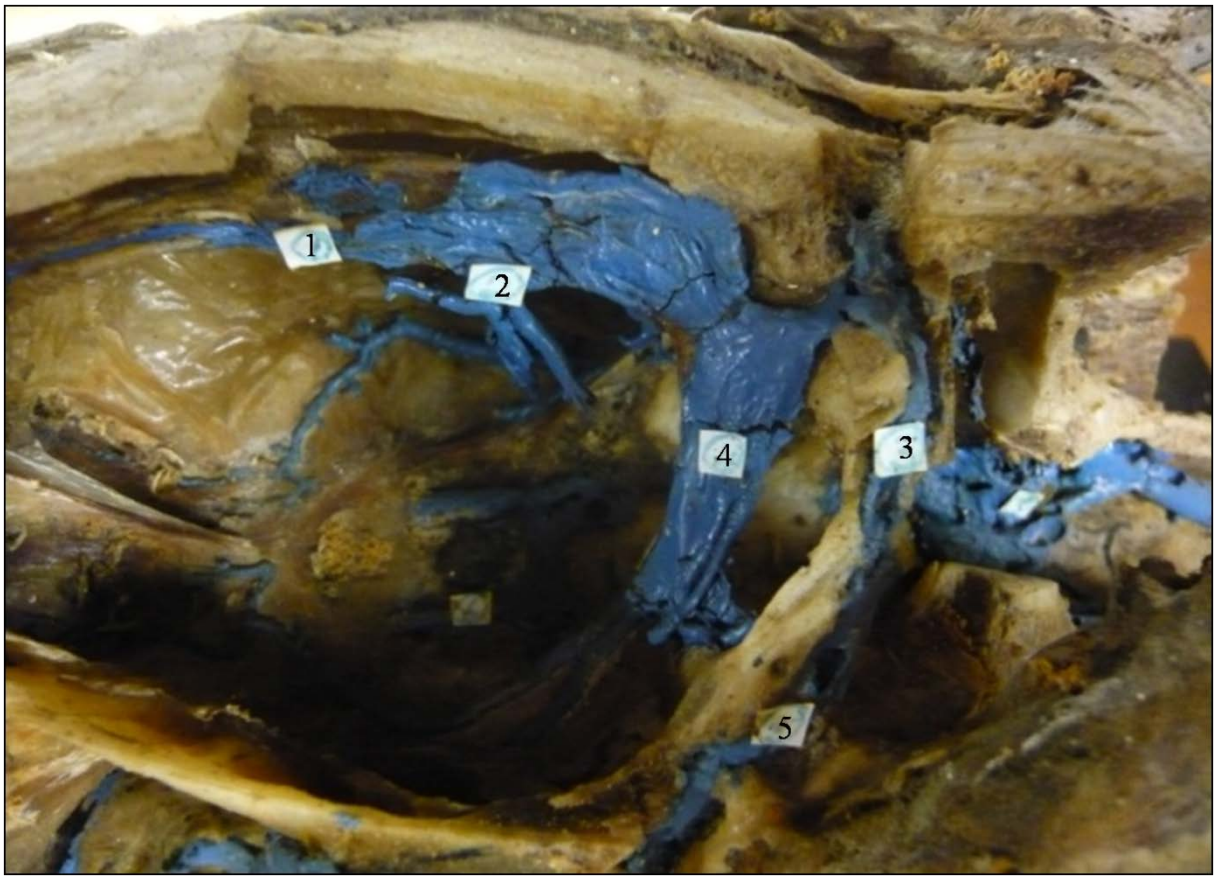


Figure 4: An equine cadaver skull demonstrating the anatomical location of the dorsal venous sinuses of the dura mater (black box) along with their subsequent communication with the veins of the head. The frontal bone, zygomatic process, body of the maxilla and the brain have been removed to allow visualisation of the vascular structures.

Figure 5: Close-up photograph of the area outlined in Figure 4 demonstrating the anatomy of the dorsal venous sinuses of the dura mater. As the dorsal sagittal sinus runs caudally, it joins the rectus sinus before terminating and splitting into the transverse sinus. The transverse sinus lies in a transverse plane and joins the dorsal petrosal sinus before entering the temporal meatus and joining the temporal sinus.



1) Dorsal sagittal sinus
2) Rectus sinus
3) Transverse sinus
4) Dorsal petrosal sinus
5) Temporal sinus

Figure 6: Positioning of the INVOS® 5100C Cerebral/Somatic Oximeter sensor on a standing horse in relation to the dorsal sagittal sinus. a) The sensor is placed on midline, 1cm above the zygomatic process (red solid line). The red dashed lines demonstrate the position of the 2 light-collecting optodes (inset, arrow), which receive absorption data from the cerebral and extra-cerebral tissue. b) Anatomy of the dorsal venous sinus system of the brain. The solid orange line indicates the position of the oximeter sensor. Modified from Kramer *et al.* (2007)[46] © 2007 by The American College of Veterinary Surgeons.

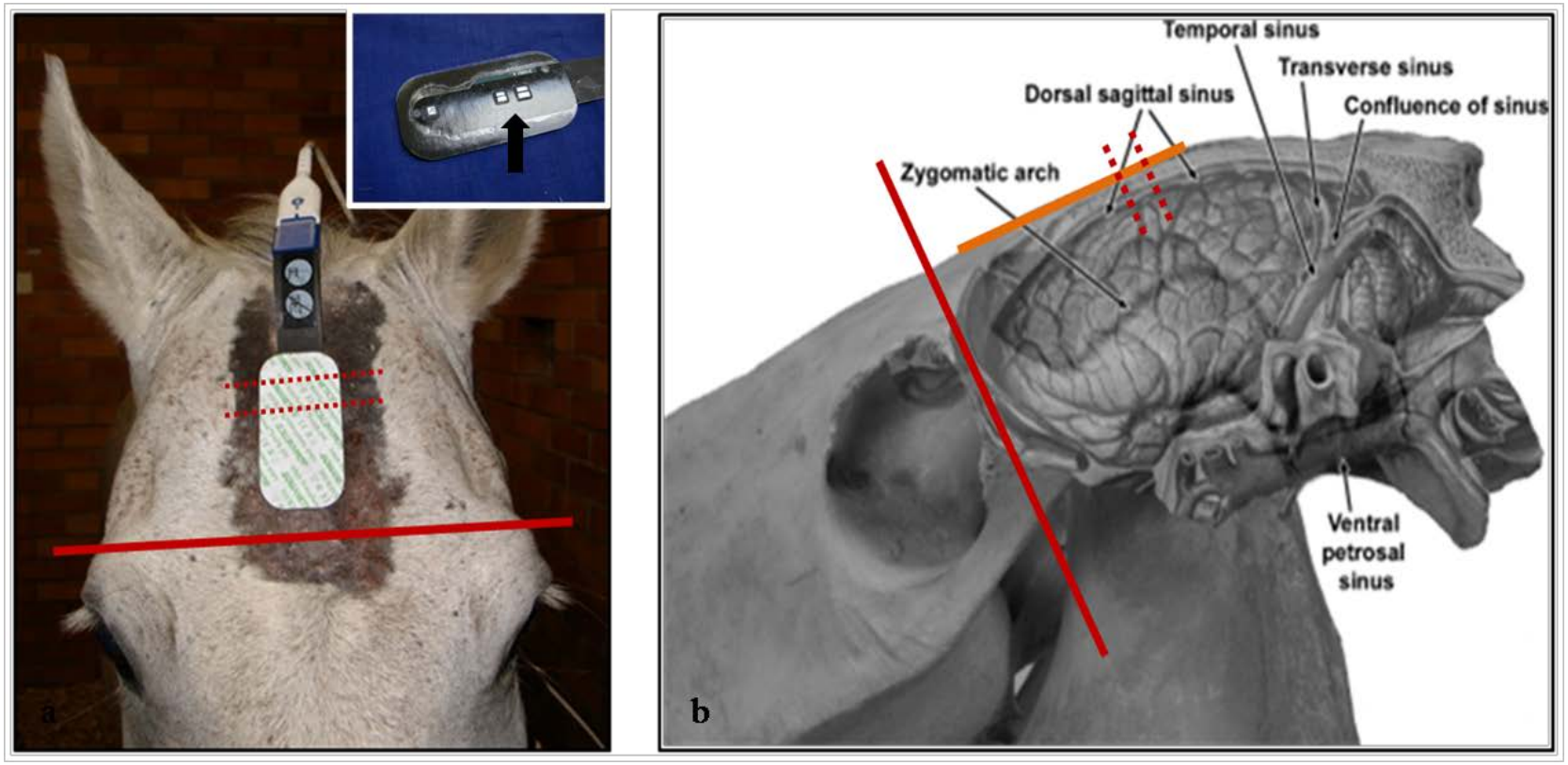


Figure 7: The INVOS® 5100C Cerebral/Somatic Oximeter being used in a horse under general anaesthesia. The 2-channel oximeter monitor allows for the concurrent use of cerebral (C) and somatic (S) sensors however for the purpose of this study, only the cerebral sensor was used. The updated rSO_2 values are displayed on the upper half of the monitor, corresponding with the cerebral sensor (C) channel. Note how the sensor has been covered with black duct tape to limit external light interference.

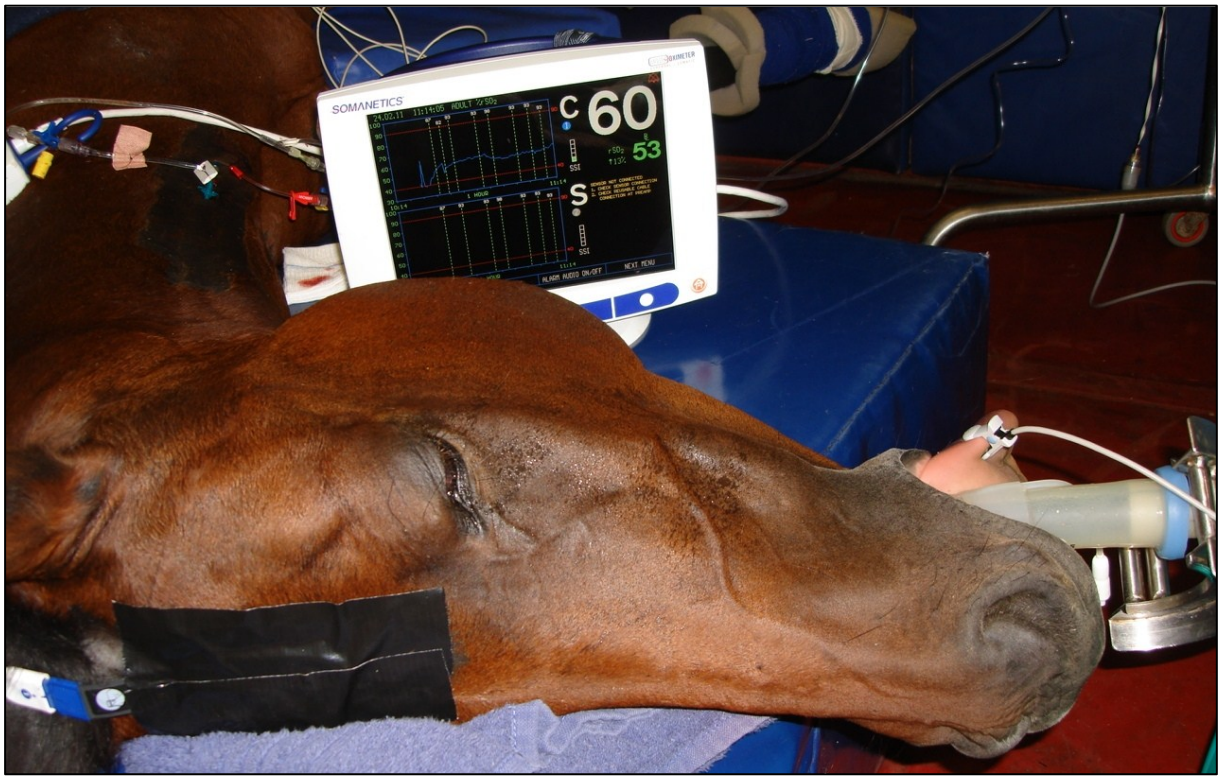


Figure 8: Mean ± s.e. values for regional cerebral oxygen saturation (rSO_2), arterial and venous oxygen tensions (PaO_2 and PvO_2) and arteriovenous oxygen saturations ($SavO_2$). Cerebral rSO_2 and $SavO_2$ values are reported as % (left y-axis), whilst PaO_2 and PvO_2 values are recorded in mmHg (right y-axis).

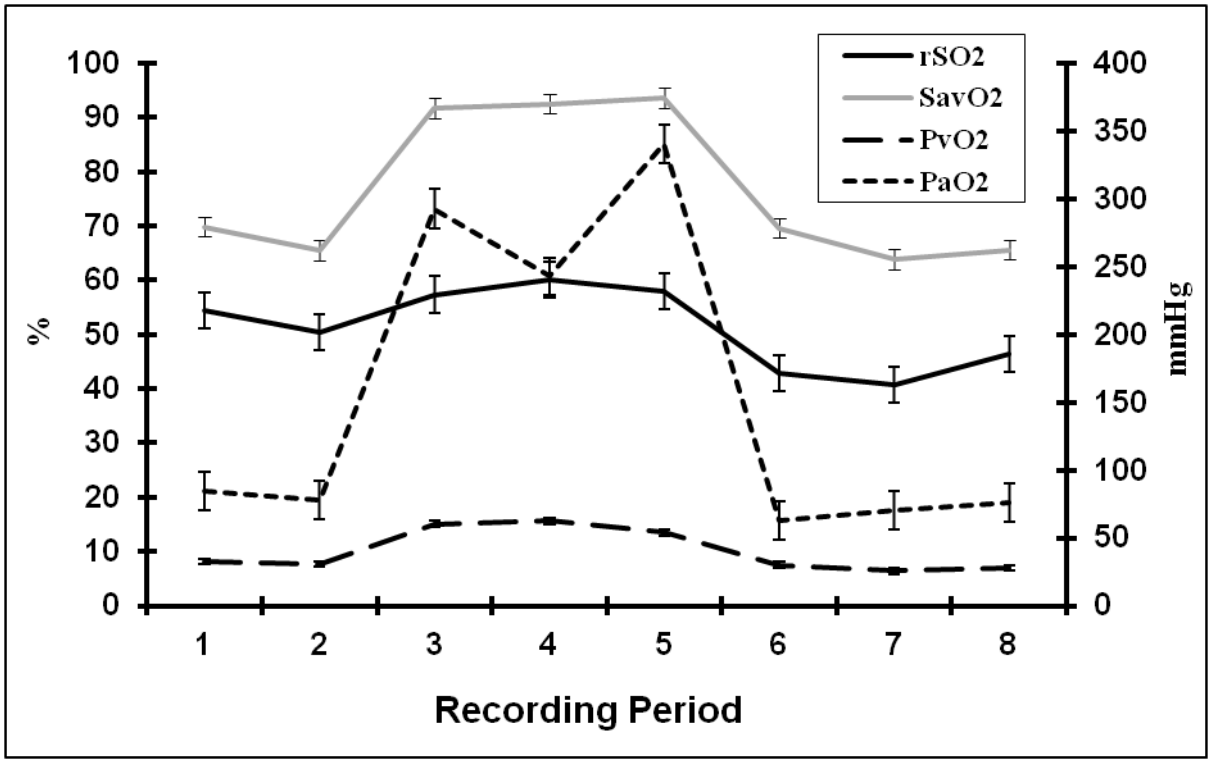


Figure 9: Scatter plot of PaO_2 *versus* rSO_2 values recorded from each of the 6 horse during the 8 recording periods. A significant moderate positive correlation was identified ($r = 0.448$, $P < 0.001$).

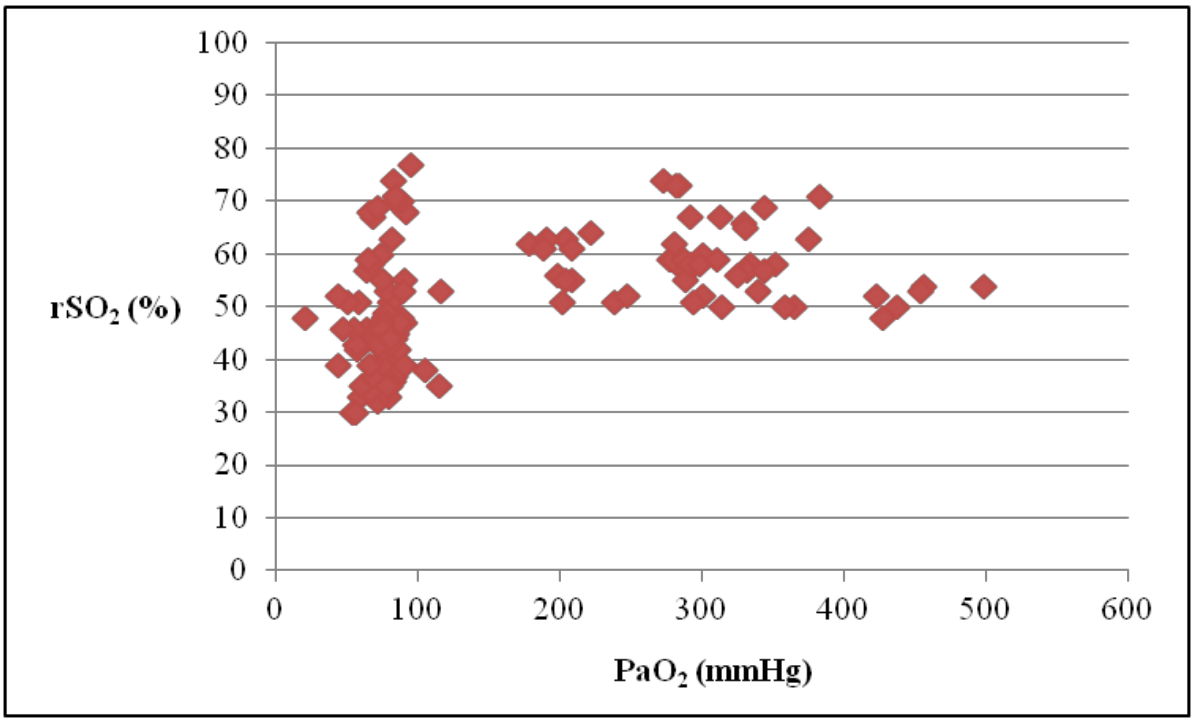


Figure 10: Scatter plot of PvO_2 *versus* rSO_2 values recorded from each of the 6 horses during the 8 recording periods. A significant moderate positive correlation was identified ($r = 0.512$, $P < 0.001$).

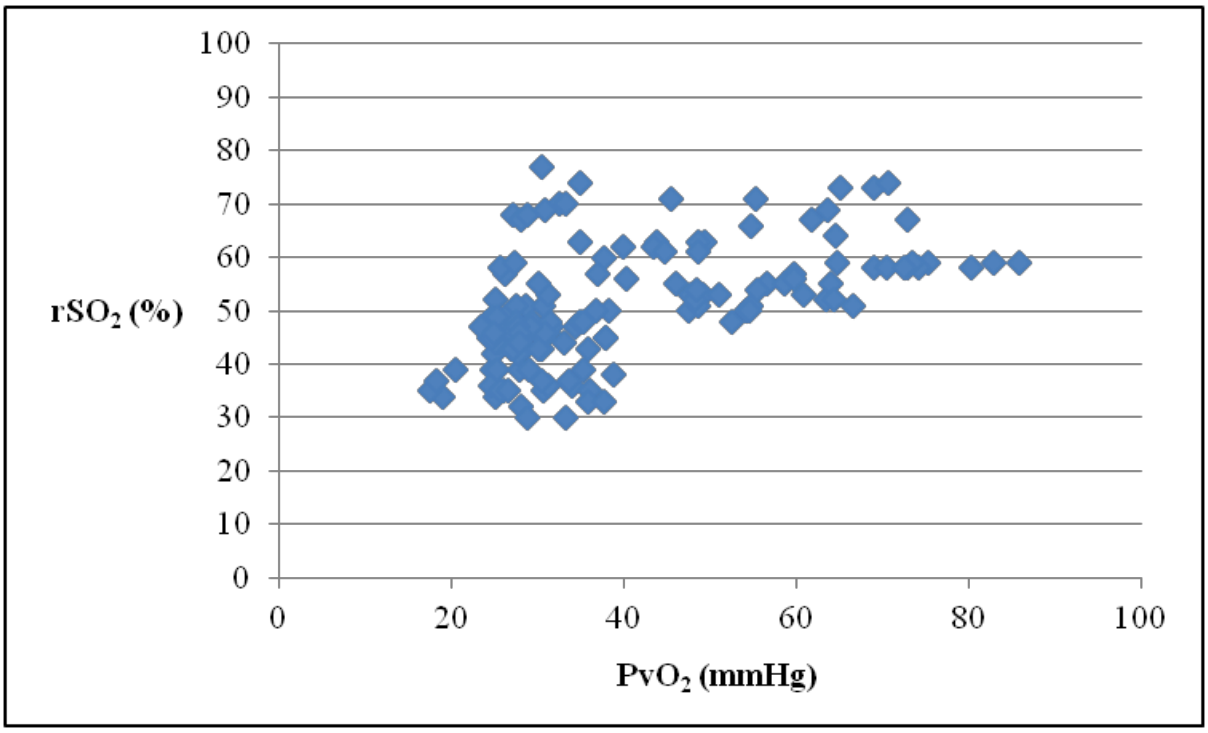


Figure 11: Scatter plot of $SavO_2$ *versus* rSO_2 values recorded from each of the 6 horses during the 8 recording periods. A significant moderate positive correlation was identified ($r = 0.469$, $P < 0.001$).

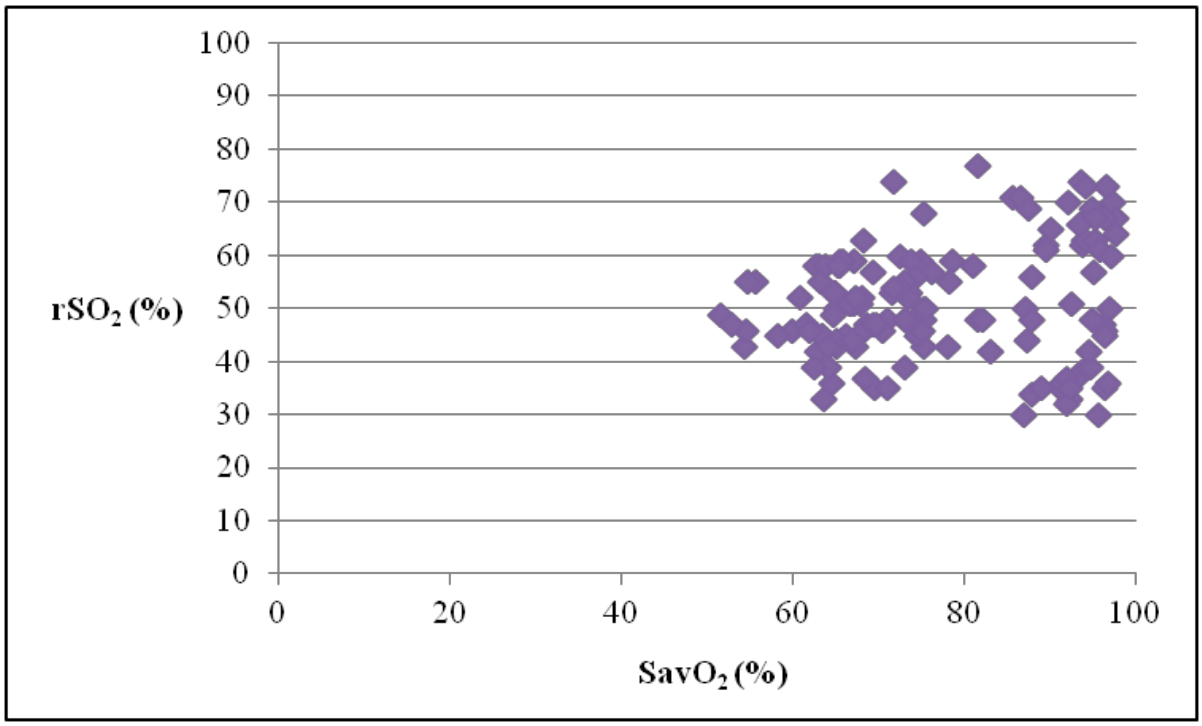


Figure 12: Scatter plot of $PaCO_2$ *versus* rSO_2 values recorded from each of the 6 horses during the 8 recording periods. A significant weak positive correlation was identified ($r = 0.198$, $P < 0.05$).

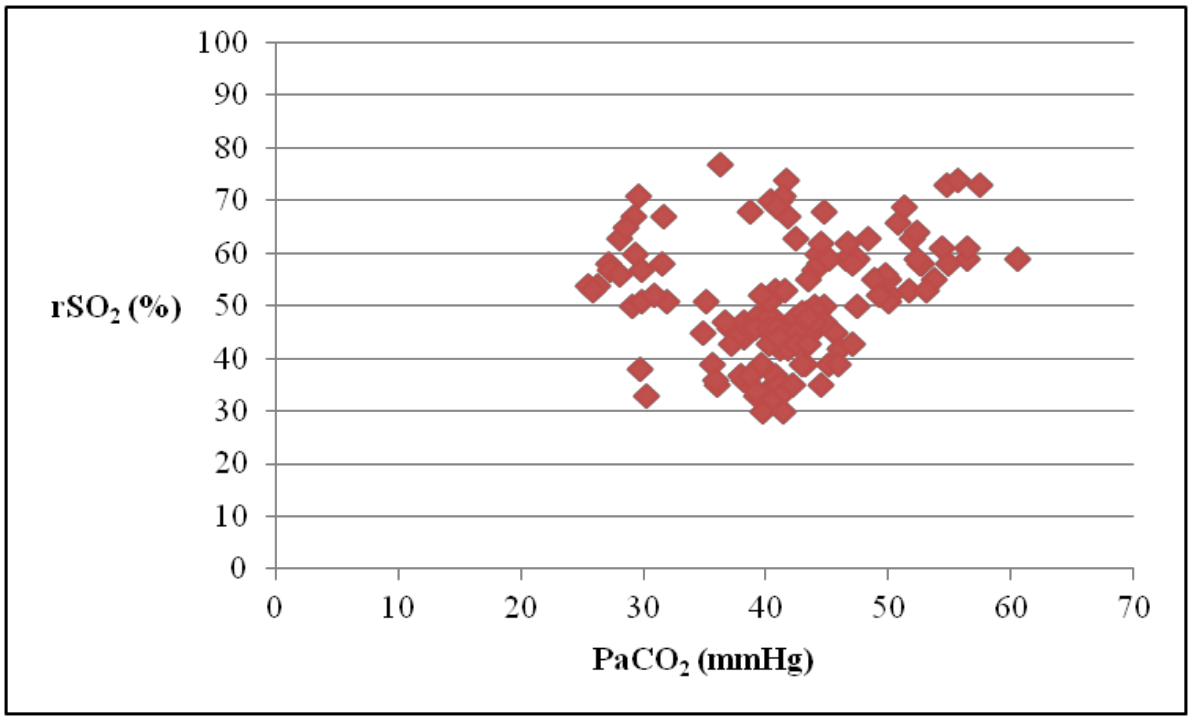


Figure 13: Scatter plot of $PvCO_2$ *versus* rSO_2 values recorded from each of the 6 horses during the 8 recording periods. No significant correlation was identified (r = 0.056, P = 0.51).

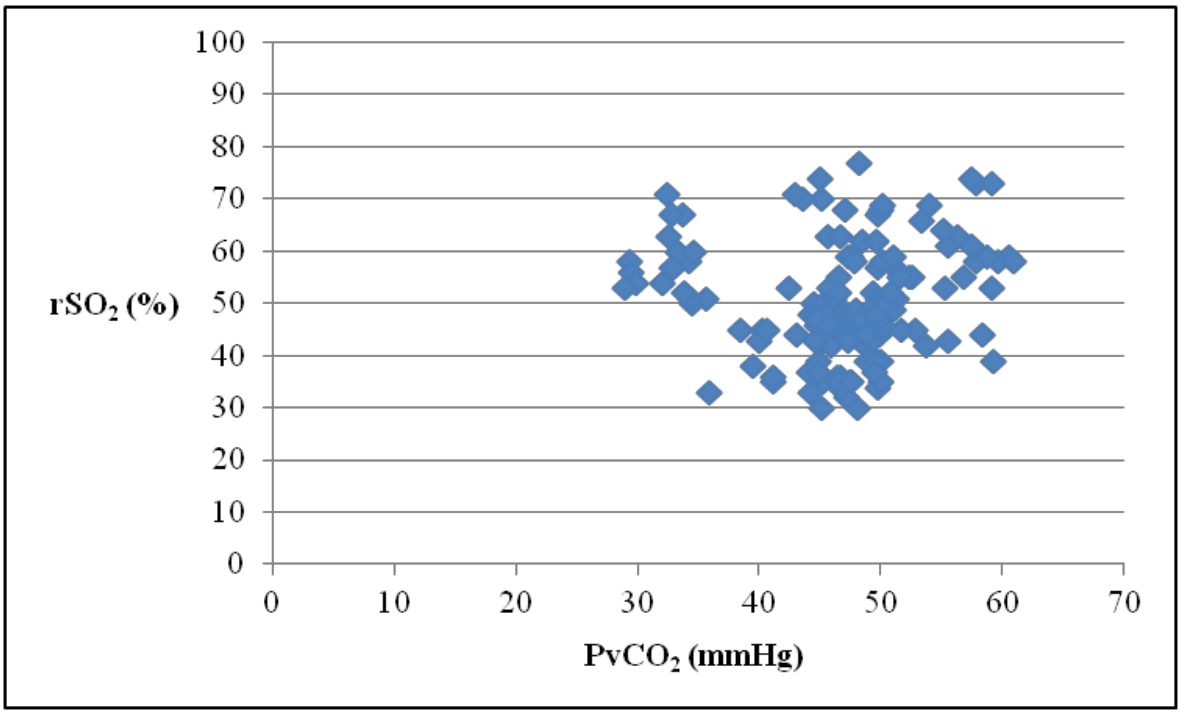


Figure 14: Scatter plot of [LAC] *versus* rSO_2 values recorded from each of the 6 horses during the 8 recording periods. No significant correlation was identified (r = -0.257, P = 0.08).

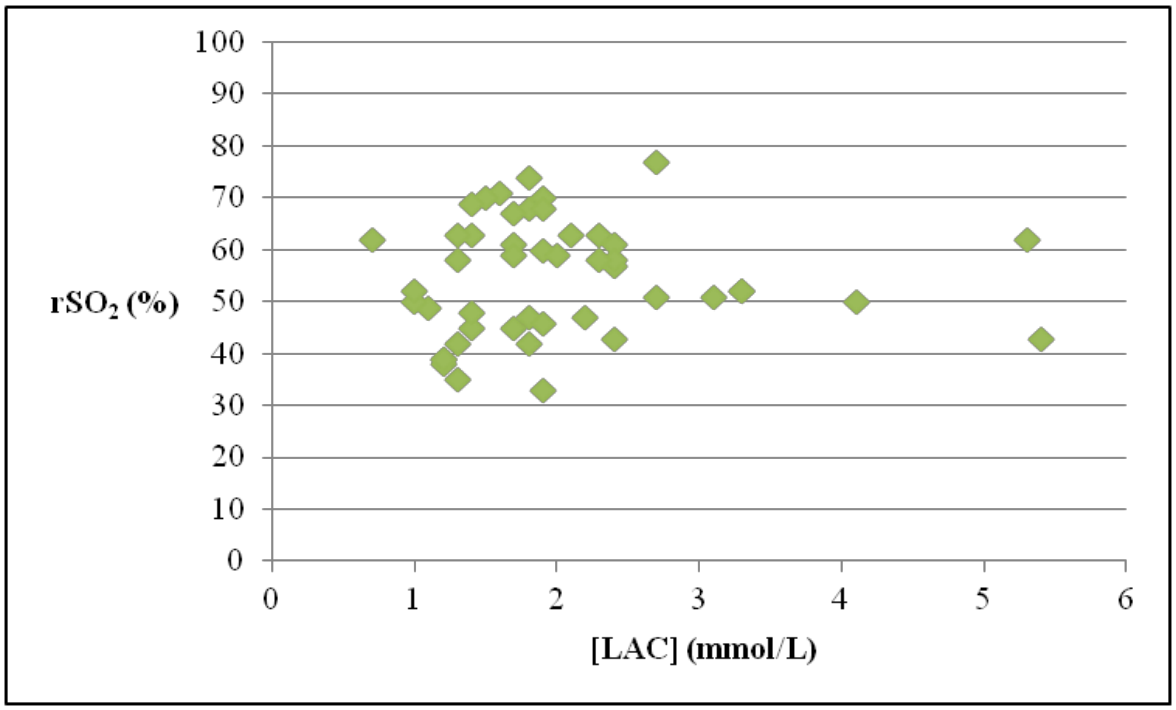


Table 1: Description of each recording period (RP) conducted.

RP	Description
1	Normal standing horse, 60 min following sedation with xylazine[a]
2	Standing sedated horse, 5 min following sedation with romifidine[j]
3	General anaesthesia: normocapnoea, following 5 min stabilisation at $PaCO_2$ = 40-50 mmHg
4	General anaesthesia: hypercapnoea, following 5 min stabilisation at $PaCO_2$ = 50-60 mmHg
5	General anaesthesia: hypocapnoea, following 5 min stabilisation at $PaCO_2$ = 25-35 mmHg
6	Following disconnection from isoflurane/O_2 and sedation with romifidine[j], breathing a combination of 100% O_2 and room air
7	5 min post-recovery
8	2 h post-recovery

$PaCO_2$ = arterial carbon dioxide tension

Table 2: Mean ± s.d. values for regional cerebral oxygen saturation (%), recorded from each of the 6 horses during each recording period (RP).

Horse	RP1	RP2	RP3	RP4	RP5	RP6	RP7	RP8
1	70.5 ± 7.24	61.3 ± 14.3	58.3 ± 0.58	58.6 ± 0.53	57.7 ± 0.58	46.3 ± 3.21*	50.7 ± 7.96*	47.1 ± 1.6*
2	43.9 ± 2.34	35.3 ± 0.99	51.0 ± 1.73	53.2 ± 1.4	60.4 ± 10.8	35.3 ± 1.8	34.1 ± 0.42*	37.7 ± 0.12
3	47.5 ± 0.76	39.3 ± 0.42	62.5 ± 0.42	61.1 ± 1.21	57.9 ± 1.7	34.7 ± 9.47*	35.6 ± 0.87*	44.8 ± 3.61
4	70.3 ± 0.7	68.1 ± 1.75	66.5 ± 2.5	73.6 ± 0.2	66.2 ± 1.06	50.3 ± 1.45*	42.4 ± 1.06*	58.1 ± 0.76
5	48.1 ± 0.42	52.7 ± 1.7	55.2 ± 0.35	55.4 ± 3.98	51.4 ± 0.4	49.2 ± 3.65	36.1 ± 0.81*	48 ± 1.91
6	46.3 ± 0.42	46.4 ± 0.4	50.5 ± 2.1	58.3 ± 0.7	53.7 ± 0.5	40.9 ± 1.1	45.4 ± 0.35	42.5 ± 0.42

RP1-8 as described in Table 1; * denotes ≥ 20% decrease compared to baseline rSO_2 values recorded during RP1.

Table 3: Mean ± s.d. values for regional cerebral oxygen saturation (rSO_2) (%), arterial and venous oxygen and carbon dioxide tensions (PaO_2, PvO_2, $PaCO_2$ and $PvCO_2$) (mmHg), arteriovenous oxygen saturations ($SavO_2$) (%) and mean arterial pressure (MAP) (mmHg) recorded during each recording period (RP). Mean arterial pressures were not recorded during RP6-8.

RP	rSO_2	PaO_2	PvO_2	$PaCO_2$	$PvCO_2$	$SavO_2$	MAP
1	54.44 ± 12.0^{ac}	84.41 ± 5.22^{a}	32.44 ± 5.08^{a}	40.27 ± 2.66^{ae}	44.66 ± 2.75^{a}	69.87 ± 8.24^{a}	110.50 ± 15.8^{a}
2	50.52 ± 12.9^{ad}	78.01 ± 6.34^{a}	30.59 ± 3.85^{ac}	41.05 ± 2.63^{ae}	47.56 ± 2.14^{ab}	65.56 ± 6.42^{ac}	106.67 ± 12.8^{a}
3	57.35 ± 6.13^{ae}	295.53 ± 83.3^{b}	58.23 ± 12.3^{bd}	47.40 ± 3.04^{b}	49.46 ± 3.23^{b}	91.44 ± 4.30^{b}	72.33 ± 6.13^{b}
4	60.03 ± 6.92^{ce}	242.78 ± 75.8^{c}	62.41 ± 12.6^{b}	53.78 ± 2.86^{c}	56.82 ± 3.20^{c}	92.55 ± 3.79^{b}	76.17 ± 8.89^{b}
5	57.88 ± 6.17^{ce}	340.79 ± 76.0^{d}	53.52 ± 11.02^{d}	28.87 ± 1.95^{d}	32.58 ± 2.06^{d}	93.44 ± 3.51^{b}	70.29 ± 7.11^{b}
6	42.76 ± 7.46^{b}	62.25 ± 22.2^{a}	30.02 ± 4.95^{ac}	39.11 ± 4.65^{a}	47.44 ± 6.11^{ab}	69.51 ± 7.83^{a}	-
7	40.73 ± 6.77^{b}	70.12 ± 8.07^{a}	25.39 ± 3.82^{c}	41.49 ± 2.32^{ae}	46.84 ± 4.01^{ab}	63.78 ± 7.25^{c}	-
8	46.36 ± 6.62^{bd}	76.21 ± 5.85^{a}	27.87 ± 2.83^{ac}	42.40 ± 2.22^{e}	47.91 ± 3.55^{ab}	65.61 ± 5.10^{ac}	-

RP1-8 as described in Table 1; differing superscript letters denote significant difference between pairs within each category ($P < 0.05$).

Table 4: Temperature-corrected and non temperature-corrected mean ± s.d. values for arterial and venous oxygen and carbon dioxide tensions (PaO_2, PvO_2, $PaCO_2$ and $PvCO_2$) (mmHg) recorded during each recording period (RP).

RP	$_{tc}PaO_2$	PaO_2	$_{tc}PvO_2$	PvO_2	$_{tc}PaCO_2$	$PaCO_2$	$_{tc}PvCO_2$	$PvCO_2$
1	84.41 ± 5.22	81.43 ± 4.44	32.44 ± 5.08	31.23 ± 5.15	40.27 ± 2.66	39.33 ± 2.58	44.66 ± 2.75	43.67 ± 2.34
2	78.01 ± 6.34	74.12 ± 6.08	30.59 ± 3.85	29.22 ± 2.98	41.05 ± 2.63	41.03 ± 3.87	47.56 ± 2.14	45.99 ± 2.29
3	295.53 ± 83.3	293.05 ± 83.0	58.23 ± 12.3	58.62 ± 13.6	47.40 ± 3.04	48.01 ± 2.89	49.46 ± 3.23	50.13 ± 3.20
4	242.78 ± 75.8	246.48 ± 74.0	62.41 ± 12.6	64.71 ± 12.0	53.78 ± 2.86	55.52 ± 3.27	56.82 ± 3.20	56.83 ± 4.29
5	340.79 ± 76.0	344.80 ± 76.2	53.52 ± 11.0	58.59 ± 12.8	28.87 ± 1.95	30.58 ± 2.57	32.58 ± 2.06	33.79 ± 2.07
6	62.25 ± 22.2	68.70 ± 19.0	30.02 ± 4.95	33.51 ± 6.95	39.11 ± 4.65	40.88 ± 5.08	47.44 ± 6.11	49.83 ± 6.77
7	70.12 ± 8.07	75.80 ± 9.32	25.39 ± 3.82	27.67 ± 3.64	41.49 ± 2.32	43.67 ± 2.45	46.84 ± 4.01	49.36 ± 3.14
8	76.21 ± 5.85	76.54 ± 5.47	27.87 ± 2.83	27.97 ± 2.40	42.40 ± 2.22	42.56 ± 2.73	47.91 ± 3.55	48.42 ± 3.56

RP1-8 as described in Table 1; $_{tc}$ = temperature-corrected

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Appendices

Appendix 1: Recording form used for data collection

University of Pretoria
Department of Companion Animal Clinical Studies

Horse number: _____ Drug Administration: _____

Date: _____ Dose: _____

Recording period: _____ Time: _____

Time (min)	1	2	3	4	5	6	7	8	9	10
rSO_2										
MAP										
VBG										
ABG										
Lactate										
HR										
Pulse										
CRT										
Mm										
RR										
T										

Appendix 2: Range of rectal temperatures (°C) recorded for each of the 6 horses during each recording period (RP).

RP	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6
1	38.0 - 38.1	37.2 - 37.6	37.4 - 37.6	37.5 - 37.8	37.2 - 37.4	37.6 - 37.7
2	37.8 - 38.1	38.0	37.5 - 37.7	37.8	37.3 - 37.7	37.6 - 37.7
3	37.1 - 37.5	36.1 - 37.2	35.8 - 35.9	37.0 - 37.5	36.7 - 37.3	36.7 - 36.8
4	36.3 - 36.6	36.9 - 37.1	35.7 - 35.9	36.6 - 36.8	35.7 - 36.0	36.0 - 36.1
5	35.9 - 36.0	36.6 - 36.8	36.0 - 36.4	36.3 - 36.4	35.9 - 36.5	36.0 - 36.3
6	35.7 - 36.0	36.2 - 36.6	35.8	36.2	35.2 - 35.4	35.2 - 35.9
7	35.5 - 35.9	36.1 - 36.3	35.1 - 35.6	36.0 - 36.2	35.1 - 35.4	36.4 - 36.6
8	37.1 - 37.2	36.9 - 37.7	36.7	36.5 - 36.6	36.5 - 36.6	37.3 - 37.4

Appendix 3: Manufacturers addresses

[a] Bayer Health Care, Isando, SA

[b] Arrow International, Reading, Philadelphia, USA

[c] Mila International Inc, Erlanger, Kentucky, USA

[d] Radiometer South Africa (Pty) Ltd, Midrand, Gauteng, SA

[e] Siemens Healthcare Diagnostics, Deerfield, Illinois, USA

[f] Covidien, Midrand, Gauteng, SA

[g] Ingelheim Pharmaceuticals, Randburg, Gauteng, SA

[h] Fresenius Kabi, Port Elizabeth, SA

[i] Micro Health Care Pty Ltd, Bethlehem, Free State, SA

[j]Abbott Laboraties Pty Ltd, Johannesburg, Gauteng, SA

[k] Surgivet DHV 1000 Large Animal Ventilator, Waukesha, Wisconsin, USA

[l] GE Healthcare, Little Chalfont, Buckinghamshire, UK

[m] Adcock Ingrim Critical Care Ltd, Johannesburg, Gauteng, SA

[n] SPSS version 17.0 for Windows, SPSS Inc, Chicago, Illinois, USA

[o] MINITAB Statistical Software, Release 13.32, Minitab Inc, Pennsylvania, USA

Scientific Proceedings Associated with this Dissertation

MCCONNELL, E., SAULEZ, M., BESTER, L., FOSGATE, G., SANZ, M., RIOJA, E., RAATH, R. Use of near-infrared spectroscopy to identify trends in regional cerebral oxygen saturation in horses. *Journal of Veterinary Emergency and Critical Care* 2011; 21:S16.

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