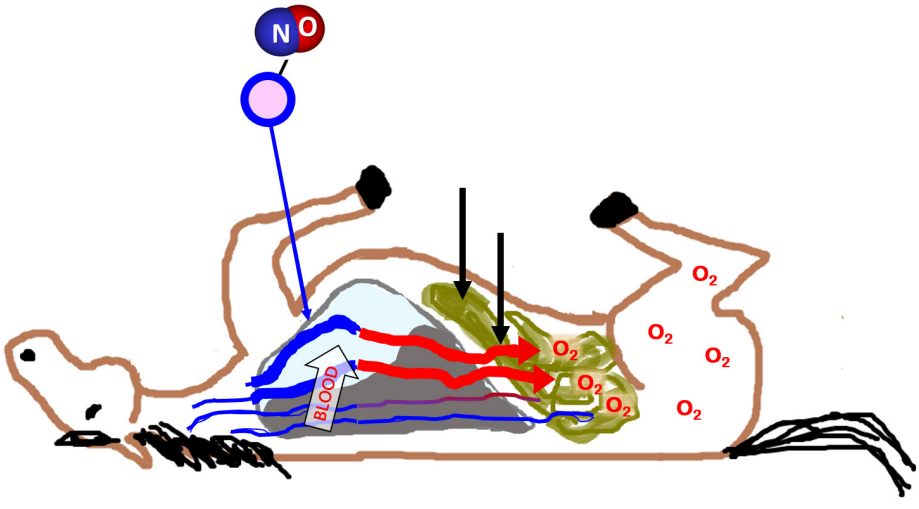
Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:11
Faculty of Veterinary Medicine and Animal Science

Clinical implementation of Pulsed inhaled Nitric Oxide in equine anaesthesia

Maja Wiklund



Clinical implementation of Pulsed inhaled Nitric Oxide in equine anaesthesia

Maja Wiklund
Faculty of Veterinary Medicine and Animal Science
Department of clinical sciences
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Uppsala 2023

Acta Universitatis Agriculturae Sueciae
2023:11

Cover: Illustration by M. Wiklund

ISSN 1652-6880
ISBN (print version) 978-91-8046-074-3
ISBN (electronic version) 978-91-8046-075-0
https://doi.org/10.54612/a.c3epjjvual

Print: SLU Service/Repro, Uppsala 2023

Clinical implementation of Pulsed inhaled Nitric Oxide in equine anaesthesia

Abstract

Hypoxaemia is a common complication during horse anaesthesia that is a challenge to treat. The primary cause of hypoxaemia is the development of a large intra-pulmonary shunt that leads to significant ventilation-perfusion mismatch. Opening and ventilating atelectasis during anaesthesia requires high airway pressures and repeated manoeuvres. Another approach to improve gas exchange in the lung is to redistribute blood flow from atelectatic to well-ventilated lung regions. In experimental studies, it has been shown possible to target the vasodilating gas nitric oxide to open ventilated lung parts by pulsing the gas at the beginning of inspiration.

In the first experimental study of the present thesis, the effect of Pulsed inhaled Nitric Oxide (PiNO) was evaluated during different modes of ventilation in combination with normal or low cardiac output and blood pressure. In the following clinical studies, PiNO was evaluated in colic horses undergoing abdominal surgery and in healthy horses undergoing arthroscopy. In the last study, it was investigated if PiNO-induced improved arterial oxygenation had an impact on the quality of recovery after anaesthesia compared to controls. The results showed that PiNO was effective during mechanical ventilation only if cardiac output blood pressure were adequate, while PiNO was effective under both hypotensive and normotensive conditions during spontaneous breathing. In the clinical studies, PiNO effectively improved arterial oxygenation in both healthy and colic horses, ventilated spontaneously or mechanically. In the last study, horses that received PiNO during anaesthesia had better recovery quality compared to controls. In addition, a positive correlation between arterial oxygenation during anaesthesia and recovery quality was seen.

In summary, PiNO offers a simple and effective option to treat hypoxaemia in anaesthetised horses. In the future, the effect of oxygenation on the quality of recovery needs to be further investigated as well as the long-term effects, such as wound healing in the postoperative period.

Keywords: horse, anaesthesia, hypoxaemia, nitric oxide, recovery, ventilation

Implementering av pulsad inhalerad kväveoxid under hästanestesi i kliniken

Sammanfattning

Hypoxemi är en vanlig komplikation hos sövda hästar som är en utmaning att behandla. Den primära orsaken till hypoxemi är en stor intrapulmonell shunt som leder till betydande obalans mellan ventilation och perfusion. Att öppna upp och ventilera atelektaserna under anestesi kräver höga luftvägstryck och upprepade manövrar. Ett annat tillvägagångssätt för att förbättra gasutbytet i lungan är att omfördela blodflödet från atelektatiska till välventilerade lungdelar. I experimentella studier har det visat sig möjligt att använda den kärlvidgande gasen kväveoxid som en puls i början av varje andetag för att öka blodflödet i de ventilerade lungdelarna.

I den första experimentella studien i avhandlingen utvärderades effekten av Pulserad inhalerad kväveoxid (PiNO) under olika ventilationssätt i kombination med normalt eller låg blodtryck. I följande kliniska studier utvärderades PiNO hos kolikhästar som genomgick bukkirurgi och hos friska hästar som genomgick artroskopi. I den sista studien undersöktes om PiNO-inducerad förbättrad arteriell syresättning hade en inverkan på kvaliteten på uppvaket efter anestesi. Resultaten visade att PiNO var effektivt under mekanisk ventilation endast om hjärtminutvolymen och blodtrycket var normalt, medan PiNO var effektivt under både hypotensiva och normotensiva förhållanden under spontanandning. I de kliniska studierna förbättrade PiNO effektivt arteriell syresättning hos både friska hästar och kolikhästar, ventilerade spontant eller mekaniskt. I den sista studien hade hästar som fick PiNO under anestesi bättre uppvakskvalitet jämfört med kontroller. Dessutom sågs ett positivt samband mellan arteriell syresättning under anestesi och uppvaksskvalitet.

Sammanfattningsvis är PiNO en effektiv alternativ metod för att behandla hypoxemi hos sövda hästar. I framtiden behöver effekten av syresättning på kvaliteten på uppvakningen undersökas ytterligare liksom de långsiktiga effekterna, såsom sårläkning i den postoperativa perioden.

Nyckelord: häst, anestesi, hypoxemi, kväveoxid, uppvak, spontanandning, mekanisk ventilation

Dedication

To my family

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Auckburally A, Grubb TL, **Wiklund M**, Nyman G. (2019) Effects of ventilation mode and blood flow on arterial oxygenation during pulse-delivered inhaled nitric oxide in anesthetized horses. *Am J Vet Res*, 80 (3), 275-283.

II. **Wiklund M**, Granswed I, Nyman G. (2017) Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery. *Vet Anaesth Analg*, 44 (5), 1139-1148.

III. **Wiklund M**, Kellgren M, Wulcan S, Nyman G. (2020) Effects of pulsed inhaled nitric oxide on arterial oxygenation during mechanical ventilation in anaesthetised horses undergoing elective arthroscopy or emergency colic surgery. *EVJ*, 52 (1) 76-82.

IV. **Wiklund M**, Grubb T, Nyman G. (2023) Effect of arterial oxygenation during equine anaesthesia on the quality of recovery. (*manuscript*)

.

Papers I-III are reproduced with the permission of the publishers.

The contribution of Maja Wiklund to the papers included in this thesis was as follows:

I. Study design, data collection, critical revision of the manuscript

II. Study design, data collection, statistical analysis and interpretation, manuscript preparation, critical revision of the manuscript

III. Study design, data collection, statistical analysis and interpretation, manuscript preparation, critical revision of the manuscript

IV. Study design, data collection, statistical analysis and interpretation, manuscript preparation, critical revision of the manuscript

Abbreviations

CaO_2	Arterial oxygen content
cGMP	Cyclic guanosine monophosphate
CI	Cardiac index
CO	Cardiac output
DO_2	Oxygen delivery
$ETCO_2$	End-tidal partial pressure of carbon dioxide
FiO_2	Fraction of inspired oxygen
*f*R	Respiratory frequency
FRC	Functional residual capacity
F-shunt	Oxygen content-based index of Qs/Qt
Hb	Haemoglobin
HR	Heart rate
IM	Intramuscularly
iNO	Inhaled nitric oxide
IV	Intravenously
IQR	Interquartile range
MAP	Mean arterial blood pressure
MV	Mechanical ventilation
NO	Nitric oxide
NO_2	Nitrogen dioxide
OER	Oxygen extraction ratio
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
PAO_2	Alveolar partial pressure of oxygen
$P(A\text{-}a)O_2$	Alveolar-arterial oxygen gradient
PEEP	Positive end expiratory pressure

PiNO	Pulsed inhaled nitric oxide
PIP	Peak inspiratory pressure
ppm	Parts per million
Qs/Qt	Pulmonary shunt fraction
RM	Recruitment manoeuver
SaO_2	Arterial oxygen saturation
SB	Spontaneous breathing
V_A/Q	Ventilation-perfusion ratio
V_D/V_T	Alveolar dead space-to-tidal volume ratio
V_T	Tidal volume

1. Introduction

Horses are extreme athletes; they are prey animals and therefor adapted to run fast. However, they are not suited to be under general anaesthesia, which can be seen from the statistics in comparison to humans, dogs and cats. The anaesthesia-related mortality rate in horses is around 1%, (Johnston et al. 2002; Dugdale et al. 2016), while the mortality rate in humans and small animals is much lower, 0.17% for dogs, 0.24% for cats (Brodbelt et al. 2008) and in humans <0.01% (Lagasse 2002). Interestingly, the mortality rate in these species has declined over the last 20 years whereas this is not the case for horses. Most of the fatalities related to equine anaesthesia occur during the recovery period (Johnston et al. 2002), which is when the horse wakes up from surgery and has to get up into a standing position.

One major complication that can occur during anaesthesia is hypoxaemia, i.e. low oxygen content in the blood. Hypoxaemia during equine anaesthesia has been associated with increased lactate concentrations in the blood, decreased skeletal muscle oxygenation, post anaesthetic cerebral necrosis and sudden cardiac arrest in horses (Steffey et al. 1992; Whitehair et al. 1996; McGoldrick et al. 1998; Taylor 1999; McKay et al. 2002; Portier et al. 2009). Apnoea in horses with hypoxaemia can drastically aggravate the hypoxaemia that causes cardiovascular instability, which in worse case can progress to cardiac arrest (Guedes et al. 2016). There is also one study that showed that intraoperative hypoxaemia increases the risk of postoperative wound infection (Costa-Farré et al. 2014). Reducing the oxygen delivery to the tissues during anaesthesia can also have a negative impact on the recovery after surgery. This thesis explores a new method for improving oxygenation during anaesthesia which might reduce the mortality rate during anaesthesia and complications during recovery in horses.

2. Background

As mentioned in the introduction, the anaesthesia-related mortality rate in healthy horses is relatively high, around 1% (Johnston et al. 2002; Laurenza et al. 2020), however, the mortality for compromised horses, such as horses with colic, is much higher. The higher mortality rate is at least partly because these compromised horses often have impaired circulation and more often are hypoxaemic during anaesthesia. Horses are exposed to anaesthesia for many reasons, both planned elective procedures as well as emergency surgeries like wound repair or abdominal surgery. The compromised circulation in horses with colic can be due to dehydration and endotoxaemia as well as having acid base disturbances. In addition to this, some colic horses have distended intestines, which besides from causing severe pain also impairs the ventilation during anaesthesia. Compared to healthy horses, horses with colic have a negative oxygen balance before they are anaesthetised and they also have an insufficient muscle oxygenation during anaesthesia (Edner et al. 2007). In a retrospective study by Pascoe et al. (1983), 13.1% of the horses with colic that underwent abdominal surgery were hypoxaemic during anaesthesia. The anaesthesia-related mortality rate in horses with colic undergoing abdominal surgery is much higher than for horses undergoing other types of surgery. In the study by Johnston et al. (2002), the mortality rate was 8.0% in colic horses undergoing abdominal surgery. In a later study, the anaesthesia related mortality in colic horses was 3%, however if horses that were euthanized due to an inoperable lesion were included the mortality rate was 33% (Laurenza et al. 2020).

2.1 Hypoxaemia

The incidence of anaesthesia-induced hypoxaemia, commonly defined as an arterial oxygen tension (PaO_2) below 8 kPa, is not fully known but is suggested to be around 12-17% (Pascoe et al. 1983; Day et al. 1995; Whitehair & Willits 1999). There are five causes for hypoxaemia; hypoventilation, low inspired fraction of oxygen (FiO_2), vascular shunt, diffusion impairment and ventilation/perfusion mismatch (V_A/Q) (Auckburally & Nyman 2017).

When a horse is placed in dorsal recumbency during general anaesthesia there is a cardiopulmonary impairment which often leads to hypoxaemia (Nyman & Hedenstierna 1989; Nyman et al. 1990; Wagner 2008a). If no interventions are done the hypoxaemia will persist not only during the whole time that the horse is recumbent but also during the time when the horse is in lateral position during the recovery period (Trim & Wan 1990). In horses, the hypoxaemia is primarily due to atelectasis formation in the dependent areas of the lung (Nyman et al. 1990). The atelectasis leads to an increase in V_A/Q mismatch, which is most prominent when the horse is placed in dorsal recumbency (Dobson et al. 1985; Nyman & Hedenstierna 1989).

During general anaesthesia the respiratory muscles become relaxed and this decreases the functional residual capacity (FRC) (Sorenson & Robinson 1980; Moreno-Martinez et al. 2022). When the FRC decreases bronchioles will collapse and the distal alveoli to theses bronchioles will no longer be ventilated (Moreno-Martinez et al. 2022). Generally horses will receive a gas mixture with high FiO_2, and high FiO_2 can lead to absorption atelectasis (Marntell et al. 2005). Both these factors contribute to an impairment in gas exchange during anaesthesia. Decreasing the FiO_2 in horses during anaesthesia has various results. While lower FiO_2 decreases the alveolar-arterial oxygen gradient ($P(A\text{-}a)O_2$) it has a negative impact on the oxygenation, thus higher FiO_2 in horses during inhalation anaesthesia will result in higher oxygen levels in the blood (Savvas et al. 2021).

As stated in the introduction, there are numerous consequences of hypoxaemia during equine anaesthesia, even though it is not fully understood. The older a horse is the worse its oxygenation during anaesthesia is (Laurenza et al. 2020). A high PaO_2/FiO_2 ratio seems to decrease the risk of respiratory complications, like pulmonary oedema, and also decrease the risk of cardiovascular complications (Laurenza et al. 2020).

2.2 Improvement of oxygenation by controlling the ventilation

Hypoventilation frequently occurs in horses during general anaesthesia. Drugs that are commonly used for premedication and induction of general anaesthesia all cause respiratory depression to some extent. Both romifidine and xylazine have been shown to increase arterial partial pressure of carbon dioxide ($PaCO_2$) in horses (Ringer et al. 2013). However, the main respiratory depressants are the inhalation anaesthetics used for maintenance of anaesthesia (Hubbell & Muir 2015). The respiratory rate decreases with isoflurane and can be as low as two breaths per minute. The tidal volume (V_T) is not usually effected that much unless high doses of isoflurane are used (Steffey 2002).

Since hypoventilation often occurs during general anaesthesia, some form of ventilation strategy is commonly used. Applying mechanical ventilation (MV) is effective for overcoming hypoventilation, and decreasing $PaCO_2$ (Moreno-Martinez et al. 2022). However, when MV is applied there will be a positive intrathoracic pressure that decreases the venous return and there by a decrease in cardiac output (CO), this in turn can worsen the hypoxaemia and oxygen delivery (DO_2) (Cournand & Motley 1948). Mechanical ventilation with recruitment manoeuver (RM) and applied positive end expiratory pressure (PEEP) continuously improved PaO_2 in a clinical study, but gastrointestinal oxygenation was decreased (Hopster et al. 2011, 2017). The use of RM and PEEP requires transiently high inspiratory pressures to open up collapsed alveoli and the elevated mean airway pressure can decrease CO, resulting in decreased oxygen delivery to the tissues (Hopster et al. 2017). The use of selective MV of dependent lung regions with a combination of PEEP decreases V_A/Q mismatch and increases PaO_2 during anaesthesia (Nyman et al. 1987; Moens et al. 1992, 1994). Unfortunately this method requires interventions that are not suitable for a clinical situation (Moens et al. 1992).

Assisted ventilation is a form of controlled mandatory ventilation (Mosing & Senior 2018). During assisted ventilation, the patient's breath triggers the ventilator and a mechanical positive pressure is then applied to increase the V_T (Moens 2013). This allows the patient to contribute to the minute ventilation and offers several advantages over controlled ventilation (Hodgson et al. 1986; Kacmarek et al. 2015), it can decrease the risk of

barotrauma, improve intrapulmonary gas distribution and enhance blood flow (Hodgson et al. 1986).

2.3 Improving oxygenation by controlling the perfusion

A second way to improve the V_A/Q matching is to regulate lung perfusion. In equine anaesthesia one method to improve arterial oxygenation is to give salbutamol as an aerosol. Salbutamol is a β-adrenergic agonist that has a selectivity for β_2-receptors and was first used for treatment of bronchospasm in horses with recurrent airway disease (Derksen et al. 1999). Later on it was studied in horses during general anaesthesia to improve oxygenation (Robertson & Bailey 2002). Salbutamol improves arterial oxygenation, however unwanted cardiovascular effects and profuse sweating is not uncommon (Robertson & Bailey 2002; Casoni et al. 2014). The cardiovascular side effects include tachycardia, hypotension and also a case of ventricular arrhythmia has been reported (Casoni et al. 2014). These unwanted side effects suggest that there is a systemic uptake of salbutamol when delivered as an inhalant. There are also reports on reduction in arterial potassium levels after salbutamol administration (Adami et al. 2020; Loomes 2021), indicating that extra hematologic monitoring may be necessary during and after if salbutamol is chosen for treatment of hypoxaemia.

A way to improve arterial oxygenation without systemic effects is therefore desired and that is where pulsed inhaled nitric oxide (PiNO) comes in.

2.3.1 Nitric oxide

In 1987 nitric oxide (NO) was revealed to be the endothelium-derived relaxing factor that causes vasodilation in the body (Palmer et al. 1987), before this discovery, NO was only considered to be a pollutant for the environment. Nitric oxide is a potent vasodilator that is normally produced endogenously and causes smooth muscle relaxation (Ignarro et al. 1987; Blitzer et al. 1996).

Nitric oxide can be administered as a gas during inhalation; this is called inhaled nitric oxide (iNO). Inhaled nitric oxide has been commonly used in human medicine, e.g. for adults and children with acute hypoxaemic respiratory failure and for neonates with pulmonary hypertension (Rossaint et al. 1993; Dobyns et al. 1999; Clark et al. 2000). When NO is inhaled it

diffuses across the alveolar membrane to the smooth muscles of the pulmonary vessels, where it increases cyclic guanosine monophosphate (cGMP) causing relaxation of the vascular smooth muscle which results in vasodilation (Ichinose et al. 2004; Bhatia et al. 2021).

There are few studies done on iNO during anaesthesia of horses. In neonatal foals with experimentally induced pulmonary hypertension, continuous delivery of iNO did improve arterial oxygenation (Lester et al. 1999). Conversely, continuous delivery of iNO in adult horses showed no positive effect on oxygenation (Young et al. 1999). Heinonen et al. (2000) compared pulsed delivery of iNO (PiNO) during inspiration to constant inspired concentration of NO in anaesthetized pigs and concluded that with PiNO the NO was used more effectively and environmental exhausts were reduced. This method of pulsed delivery of NO was developed in an additional study by the same research group, where PiNO turned out to be an effective way to counteract hypoxaemia in horses during general anaesthesia (Heinonen et al. 2001). Further studies showed that a pulse of iNO during the first part of inspiration (30-45% of the inspiration time) improved arterial oxygenation in healthy horses (Heinonen et al. 2002; Grubb et al. 2008, 2013b; a; Nyman et al. 2012). Additionally, a study using multiple inert gas elimination technique (Wagner 2008b), and scintigraphy demonstrated that pulmonary blood flow was redistributed from dependent atelectatic lung regions to non-dependent ventilated areas during PiNO, resulting in a reduction of right to left vascular shunt (Grubb et al. 2014). The improvement in arterial oxygenation during PiNO has been shown to be sustained throughout 2.5 hours of anaesthesia (Nyman et al. 2012) and no adverse effects, i.e. rebound after PiNO discontinuation, have been observed in the studies done on horses (Grubb et al. 2008, 2013b; a, 2014; Nyman et al. 2012).

2.3.2 Metabolism of nitric oxide

When nitric oxide is given as an inhalant, the excess diffuses to the blood and forms methaemoglobin and nitrate after a reaction with oxyhaemoglobin (Ichinose et al. 2004). In humans around two thirds of the NO that is inhaled is absorbed and then excreted as nitrate via the kidneys within 48 hours after inhalation (Young et al. 1996). If iNO is given in higher doses it can recirculate in the breathing system and then react with oxygen and form nitrogen dioxide (NO_2) (Bhatia et al. 2021). Nitrogen dioxide is a toxic gas

that even in small concentrations causes irritation in the airways (Weinberger et al. 2001). To reduce the risk of NO_2 toxicity and lethal methaemoglobinaemia the recommended maximum iNO dose from the U.S. Food and Drug Administration is 80 parts per million (ppm). For preterm infants the highest recommended dose is 20 ppm for a maximum of 14 days (Witek & Lakhkar 2022).

2.4 Recovery

The recovery period is the most critical part during the perioperative period in horses (Johnston et al. 2002; Dugdale & Taylor 2016; Laurenza et al. 2020; Gozalo-Marcilla & Ringer 2021). For a horse to recover after anaesthesia it has to stand up, more or less on its own. Complications occur more frequently in horses than in other companion animals, and fatal injuries, like fractures and severe myopathies are not uncommon. Most recovery-related injuries are less severe, such as abrasions and lacerations to the head or extremities.

Several studies have investigated what factors can influence the quality of recovery in horses. Factors proven to affect the quality of the recovery and to potentially lead to mortality during the recovery period are numerous and include, for example, the age and health of the horse as well as the duration of anaesthesia (Dugdale et al. 2016; Laurenza et al. 2020). Although it has not been certain how hypoxaemia affects recovery, there are publications that suggest that hypoxaemia during anaesthesia leads to inferior recoveries in horses (Trim et al. 1989; Rüegg et al. 2016). In one study, looking at ventilation and recruitment of collapsed alveoli, the horses that had a higher PaO_2 during anaesthesia had shorter time to sternal position and fewer attempts to stand compared to horses with lower PaO_2 (Hopster et al. 2011). In another study, a possible correlation between PaO_2 and recovery score was seen and horses that received medetomidine had higher PaO_2 during anaesthesia and a better recovery score compared to horses receiving S-ketamine (Menzies et al. 2016). Hypoxaemia during anaesthesia was found to be a risk factor for lower recovery quality in one study looking at non-assisted versus head and tail rope-assisted recovery (Rüegg et al. 2016).

2.4.1 Scoring of recovery quality

To date, there is no validated method for scoring the quality of recovery in horses after general anaesthesia. Vettorato et al. (2010) examined and compared four different systems for scoring of recovery quality. The recovery quality scoring systems were a visual analogue scale, a composite rating scale, a simple descriptive scale and the Edinburgh scoring system. All four systems were comparable and had a low inter-observer variability. A simple descriptive scale was used in 91 of 124 studies included in a review looking at the influence of anaesthetic protocol on recovery quality (Loomes & Louro 2022), indicating that this scoring system, even though not validated is used extensively. Of three scoring systems, whereas two were included in the study by Vettorato et al. (2010), all systems seemed to be reproducible and repeatable (Suthers et al. 2011). One simple descriptive scale that was included in these studies is from Young & Taylor (1993), and is considered to be easily applied and provides a rapid overview (Suthers et al. 2011) and this system was chosen for the study on recovery in this thesis.

3. Aims of the thesis

The first hypothesis, in the experimental study, was that mechanical ventilation and increased pulmonary blood flow would optimize the effect of PiNO on arterial oxygenation. The second hypothesis was that PiNO during inhalation anaesthesia improves arterial oxygenation during anaesthesia in healthy as well as colic horses undergoing elective and emergency surgery.

The overall aim was to confirm that PiNO can be used to improve arterial oxygenation in anaesthetised horses in a clinical setting.

The specific aims were to:

- Determine the impact of two ventilation modes (mechanical ventilation and spontaneous breathing) and two perfusion conditions (mean arterial blood pressure $<$ or $\geq$ 70 mmHg) on PiNO-mediated oxygenation.
- Evaluate the effect of PiNO on arterial oxygenation and subsequent blood lactate concentration in colic horses breathing spontaneously during abdominal surgery.
- Determine and compare, with a control group, the effect of PiNO on arterial oxygenation in anaesthetised mechanically ventilated horses that were anaesthetised for either elective or emergency surgery.
- Determine if there is a correlation between intraoperative PaO_2 and quality of recovery in healthy horses undergoing arthroscopy.

4. Materials and methods

4.1 Inclusion criteria Study II-IV

Inclusion criteria for the healthy group were determination of clinical health (ASA status I-II) by physical examination and admission to the hospital for elective arthroscopy (Group A in study III) in dorsal recumbency. Inclusion criteria for the compromised group were all adult horses with signs of abdominal pain (colic) referred to the clinic for emergency abdominal surgery (Group C in Study III) in dorsal recumbency. Horses under the age of 6 months were excluded from both groups.

In Study II, every other horse received PiNO and the rest served as controls. In Study II all horses were breathing spontaneously (SB) during anaesthesia. In Study III, the horses which, according to the approved owner consent, could be included in the study were divided so that every other horse received PiNO, labelled A-INO and C-INO, A standing for arthroscopy and C for colic. Control horses, not receiving PiNO, were labelled A-CN and C-CN. All horses were scheduled for mechanical ventilation during anaesthesia.

4.2 Horses

In Study I, which was an experimental study, 27 healthy Standardbred trotters were included. The age ranged from 1 to 25 years and the body weight ranged from 375 to 610 kg.

Study II-IV were clinical studies and informed, written owner consents were obtained. Thirty horses with colic undergoing abdominal surgery were included in study II, 15 received PiNO and 15 served as controls (C). The

study included 14 Warmbloods (six PiNO, eight C), six Standardbred trotters (three PiNO, three C) and 10 various breeds including ponies, Icelandic horses and draft horses (six PiNO, four C). The age in horses receiving PiNO was 10 ± 6 years and 11 ± 6 years in the control group. The body weight ranged from 337 to 688 kg and 250 to 616 kg in the PiNO and control group, respectively. In study III, a total of 80 horses were included, 50 horses were healthy horses undergoing elective arthroscopy (Group A) and 30 horses had signs of colic and underwent acute abdominal surgery (Group C). In both groups every second horse received PiNO and the rest served as controls. In horses undergoing arthroscopy, 23 were Standardbred trotters (12 PiNO, 11 C), 23 Warmbloods (12 PiNO, 11 C) and four were of various breeds (one PiNO, three C). The age ranged from 1 to 12 and from 1 to 15 years in PiNO and control group, the body weight ranged from 330 to 690 and from 340 to 700 kg in PiNO and control group, respectively. Amongst horses undergoing acute abdominal surgery, 20 were Warmbloods (eight PiNO, 12 C) and the rest were of various breeds (seven PiNO, three C). The age ranged from 1 to 19 and from 1 to 18 years in PiNO and control group, the body weight ranged from 360 to 700 and from 390 to 690 kg in PiNO and control group respectively.

In Study IV 26 of the 50 healthy horses undergoing arthroscopy in Study III were included. Twelve of the horses received PiNO and 14 served as controls. The study included 17 Warmbloods, six Standardbred trotters and three of various breeds. The horses were in average 6.5 ± 4 years old and weighed 535 ± 97 kg.

4.3 Anaesthesia

4.3.1 Study I

Food, but not water, was withheld for 12 hours prior to anaesthesia. Acepromazine (0.03 mg kg^{-1}) was administered intramuscular (IM) approximately 30 minutes before induction of anaesthesia. The subcutaneous tissues over the jugular veins were infiltrated with lidocaine before catherisation. The left jugular vein was catheterized with a 14 gauge catheter, and the right jugular vein was catheterized with two 8.5F sheath introducers (one positioned distally in the vein and the other positioned proximally in the vein).

Additional premedication consisted of xylazine hydrochloride (1.1 mg kg^{-1}) intravenously (IV) and butorphanol tartrate (0.025 mg kg^{-1}) IV. When sedation was apparent, anaesthesia was induced by rapid IV administration of a bolus of ketamine hydrochloride (2.2 mg kg^{-1}) and diazepam (0.05 mg kg^{-1}). After the horse was recumbent, the trachea was intubated with a cuffed endotracheal tube (internal diameter, 26 mm). The horse was hoisted onto a padded surgical table, positioned in dorsal recumbency, and connected to a large-animal breathing system and anaesthetic machine (Tafonius, Vetronic Services, Devon, UK). Anaesthesia was maintained with isoflurane vaporized in oxygen. The FiO_2 was maintained at approximately 0.9 by the computer-controlled FiO_2 technology of the anaesthetic machine. Ventilatory mode was SB or continuous mandatory MV. The mean arterial blood pressure (MAP) was deliberately allowed to decrease to < 70 mm Hg or was maintained at ≥ 70 mm Hg by administration of a variable rate infusion of dobutamine.

4.3.2 Study II-IV

After aseptic preparation, a 14 gauge catheter (Milacath®; Mila International Inc., Erlanger, USA) was placed in one of the jugular veins for administration of intravenous (IV) drugs and fluids and collection of blood for venous blood gas analysis. For all horses premedication included 1.1 mg kg^{-1} flunixine meglumine (Flunixin N-vet®; N-vet AB, Sweden) IV, 0.1 mg kg^{-1} romifidine (Sedivet®; Boehringer Ingelheim Vetmedica, Sweden) IV and 0.025 mg kg^{-1} butorphanol (Butador®; Vetoquinol, Sweden) IV. Group A horses also received 0.03 mg kg^{-1} acepromazine (Plegicil®; Pharmaxim, Sweden) IM. For induction of anaesthesia, 0.03 mg kg^{-1} diazepam (Diazepam-ratiopharm®; Ratiopharm, Germany) and 2.2 mg kg^{-1} ketamine (Ketaminol®; Intervet, Sweden) were administered IV. The tracheas of the horses were intubated and anaesthesia was maintained with isoflurane (IsoFlo; Orion Pharma Animal Health, Sweden) in oxygen (fresh gas flow 1 L 100 kg^{-1} $minute^{-1}$). In Study I mechanical ventilation began when the horse was connected to the large animal anaesthetic machine (Tafonius, Vetronic Services, Devon, UK). In Group A, MV was initiated within five minutes after intubation and in Group C after 5-30 minutes. The initial settings for MV were a V_T of 10 mL kg^{-1} and a respiratory frequency (f_R) of six to eight breaths $minute^{-1}$. The settings were changed during anaesthesia in response to blood gas values.

For horses in Study II and III, low arterial blood pressure was treated with intravenous crystalloid fluids (Ringer-acetat; Fresenius Kabi, Sweden), colloid fluids (Voluven; Fresenius Kabi, Sweden) and 0.5-2 μg kg^{-1} $minute^{-1}$ dobutamine (Dobutamin Carino; Carinopharm GmbH, Germany) with the goal to maintain mean arterial blood pressure (MAP) >65 mmHg. Horses in Group C received a 2 mg kg^{-1} lidocaine (Xylocain; AstraZeneca, Sweden) bolus administered over 20 minutes immediately after induction and followed by a lidocaine constant rate infusion (2 mg kg^{-1} $hour^{-1}$) until 20-30 minutes before recovery.

4.4 Delivery of PiNO

Nitric oxide was administered through a device which immediately triggered the delivery of the gas when the horse produced a negative inspiratory pressure during spontaneous breathing (Datex-Ohmeda Research Unit, Helsinki, Finland). In Study II, the length of the pulse was adjusted step wise to effect from onset of inspiration to the first 30, 45 or 60% of the total inspiratory time. The shortest pulse to improve PaO_2 was used throughout the anaesthesia. In Study I and III, the pulse length was 45% of the total inspiratory time. The delivery device was connected with a plastic tube to an adapter located at the proximal end of the endotracheal tube (Figure 1) and once started the horse would get a pulse of NO with every breath. The NO was supplied from a cylinder of 2,000 ppm NO in nitrogen gas (AGA AB, Sweden).

Figure 1. The device that delivers PiNO (NOrse) and the arrow indicates where the line is attached to the patient Y connector.

4.5 Instrumentation

4.5.1 Study I

After a horse was positioned on the surgical table, an 18 gauge catheter was inserted in the transverse facial or mandibular branch of the facial artery for measurement of blood pressure and collection of arterial blood samples for analysis. The catheter was connected via a 3-way stopcock to a pressure-monitoring line and transducer, which was connected to the multiparameter monitor integrated in the anaesthetic machine. A 7.5F Swan-Ganz catheter was inserted via the distal introducer in the right jugular vein and guided into the pulmonary artery by use of pressure guidance from another multiparameter monitor. This catheter was used to measure mean pulmonary arterial pressure as well as CO (determined by thermodilution) and to enable collection of mixed-venous blood samples. By use of a similar technique, a 7.5F pigtail multiple-hole catheter was inserted through the proximal introducer in the right jugular vein and guided into the right atrium. This catheter was used to measure right atrial pressure and to inject ice-cold saline (0.9% NaCl) solution for CO measurement. Both catheters were secured by use of the Luer-lock adaptor on the introducers. The ECG was monitored during placement of the catheters. All pressure transducers were calibrated to zero at the level of the shoulder joint.

Thermodilution was used to measure CO. The Swan-Ganz catheter was connected to the multiparameter monitor, and a 20 mL bolus of ice-cold (0°C) saline solution was manually injected through the pigtail catheter. A minimum of three boluses were injected, and the mean value was calculated and recorded.

4.5.2 Study II-IV

After aseptic preparation, a catheter was placed in the facial artery for blood pressure monitoring and for collection of arterial blood. Monitoring during anaesthesia was performed with a multi-parameter monitor (Tafonius, Vetronic Services, Devon, UK) and included arterial blood pressure, heart rate, electrocardiogram, ventilation parameters (End-tidal partial pressure of carbon dioxide [$ETCO_2$], FiO_2, fR, V_T, peak inspiratory pressure [PIP]) and continuous respiratory gas analysis including measurement of the volatile anaesthetic agent concentration (isoflurane).

Arterial and jugular venous blood samples were obtained for assessment of PaO_2, jugular venous oxygen tension, $PaCO_2$, arterial oxygen saturation (SaO_2), pH, haemoglobin (Hb) and lactate concentrations. A device from Radiometer (ABL 90 flex, Radiometer, Denmark) was used for the blood-gas analysis. Since no mixed venous blood samples were obtained, an oxygen content-based index of Qs/Qt (F-shunt) was calculated. All blood samples were stored on ice if not analysed immediately. Baseline data was collected during anaesthesia, after instrumentation before PiNO delivery to treatment group. Then blood-gas samples were taken every 15 to 30 minutes during anaesthesia, the last sample during anaesthesia was labelled end value.

The respiratory gas and blood-gas analysers were calibrated routinely according to the manufacturers' guidelines.

4.6 Recovery

The recovery was recorded using a camera (Foscam, Foscam Intelligent Technology Co., Ltd, US) that was installed in one corner in the recovery box, visualizing the whole box. All videos were stored on a computer and the persons scoring the quality of the recovery were blinded to physiologic status, including PaO_2, as well as to allocation to treatment or control, during anaesthesia. The quality of recovery was analysed using a simple descriptive scale scoring system developed for recovery of horses after anaesthesia (Young & Taylor 1993). There were three people doing the blinded scoring, one licenced veterinary nurse and two veterinary nurse students. The score is presented as a number from 0 to 5, where 5 is indicating the best recovery, for details see Table 1.

Table 1. Scoring of quality of recovery (Young & Taylor 1993)

Score	Description
5	No ataxia, no struggling, stands up at first attempt as if fully conscious
4	Slight ataxia and staggering, stands up at first or second attempt, no serious instability
3	Some ataxia and staggering, a few unsuccessful attempts to stand, ataxic immediately after standing
2	Excitement, paddling when recumbent, several attempts to stand, severe ataxia once standing, may fall, danger of self-inflicted injury
1	Excitement when recumbent, persistent unsuccessful attempts to stand, severe ataxia and falls once standing, aimless walking, high risk of self-inflicted injury
0	Very violent ("wall of death"), self-inflicted injury, prolonged struggling or unable to stand 2 hours after the end of anaesthesia

4.7 Calculations

Oxygen content of end capillary ($Cc'O_2$), arterial (CaO_2) and mixed venous ($C\bar{v}O_2$) blood were calculated as $Cx O_2 = (1.36 \times [Hb] \times Sx O_2) + (0.003 \times Px O_2)$, where *x* is c', a or $\bar{v}$; alveolar partial pressure of oxygen (PAO_2) was used to calculate $Cc'O_2$.

PAO_2 was calculated using the alveolar gas equation:
$PAO_2 = FIO_2$ (barometric pressure – water vapor pressure) - $PACO_2/RQ$.
Where: $PACO_2$ is substituted with $PaCO_2$ and RQ is the respiratory quotient taken as 0.8 (West 2012).
The alveolar-arterial oxygen partial pressure difference $P(A\text{-}a)O_2 = PAO_2 – PaO_2$.

Oxygen delivery (DO_2) was calculated as $CaO_2 \times \dot{Q}t$.

Venous admixture ($\dot{Q}s/\dot{Q}t$) was calculated using the Berggren shunt formula:
$\dot{Q}s/\dot{Q}t = (Cc'O_2 – CaO_2)/(Cc'O_2 – C\bar{v}O_2)$ (Berggren 1942)

Minute volume (V_E) was calculated as $fR \times V_T$.

The oxygen extraction ratio (OER) was calculated as:
$OER = C(a - \bar{v})O_2 / CaO_2$.

Pulmonary vascular resistance (PVR) was calculated as:
$PVR = MPAP\text{-}PCWP/ \dot{Q}t \times 80$

Cardiac index (CI) was calculated as CI = cardiac output/body weight

Alveolar dead space (V_D/V_T) was estimated as $V_D/V_T = (PaCO_2 - ETCO_2)/PaCO_2$

F-shunt was calculated using:
$F\text{-shunt} = (Cc'O_2 - CaO_2) / ([Cc'O_2 - CaO_2] + 35)$
Where 35 is a fixed value of $C(a\text{-}v)O_2$ in ml L^{-1} (Araos et al. 2012; Briganti et al. 2015)

4.8 Statistics

The raw data collected were entered in Microsoft Excel 2010 (Microsoft Office, Microsoft, WA, USA) and then processed. For statistical calculations in Study I and II, the statistical package GraphPad Prism 5 (GraphPad Software, CA, USA) was used. Data were tested for Gaussian distribution using the Shapiro-Wilk test.

In Study I, Mann-Whitney tests were used to compare differences between SB and MV in hypotensive and normotensive horses, and a Friedman ANOVA and Dunn multiple comparisons post hoc test were used to compare differences over time within each group.

In Study II, Mann-Whitney tests were used for individual changes and unpaired t tests were used to compare the difference between the two groups and Wilcoxon tests and paired t tests to compare differences within the groups.

In Study III, the statistical package Minitab 18 was used for all statistical calculations. Data from Group A and data from Group C were analysed separately. A general linear model, with horse ID as a random effect, was used. Residual plots were used to determine if residual are normally distributed with equal variances. PaO_2 and lactate were not normally distributed and the raw data for these parameters were log transformed before

analysed. The Tukey method with a family error rate of 0.05 (equal to a 95% simultaneous confidence level) was used to compare baseline and end values for each parameter.

In Study IV, the raw data collected were entered in Microsoft Excel 2016 (Microsoft Office, Microsoft, WA, USA) and then processed. For statistical calculations of intra-class correlation (ICC) between the three different people who scored the recovery, Microsoft Excel 2016 was used. In Microsoft Excel 2016 it is not possible to calculate for missing values and there were three missing values so in ICC, the statistics was calculated for 24 scores. The statistical package GraphPad Prism 9 (GraphPad Software, CA, USA) was used for correlation calculations. Correlation was calculated, using Spearman correlation, between the mean recovery score and different parameters. For values that change throughout the anaesthesia an average value during the anaesthesia, baseline, before commencement of PiNO, value excluded, was calculated and used for correlation calculations. Comparisons between groups was done using students t-test.

Data are presented as mean ± standard deviation, mean and range or as median and interquartile range (IQR) for normally or not normally distributed data, respectively. In all studies the difference was considered significant when $p < 0.05$ with a confidence interval of 95%.

5. Results & discussion

Pulsed inhaled nitric oxide improved arterial oxygenation in both compromised and healthy horses during general anaesthesia.

5.1 Arterial oxygenation and delivery of PiNO

In horses with colic that underwent abdominal surgery and were breathing spontaneously (Study II), the effect of PiNO was both immediate and sustained throughout the whole duration of anaesthesia (Figure 2). The PaO_2 increased and the calculated F-shunt decreased compared to baseline and also compared to the control group. As long as the PiNO was delivered it had a positive effect, even when the anaesthesia time was more than 2.5 h, this is in line with previous studies (Nyman et al. 2012; Grubb et al. 2013b). In Study III, the arterial oxygenation in the colic horses that received PiNO improved compared to baseline and also compared to the control group (Figure 3). The PaO_2 increased during administration of PiNO from 7.7 (6.4, 8.5) median and IQR at baseline to 15.5 (9.9, 26.9) kPa at end of anaesthesia.

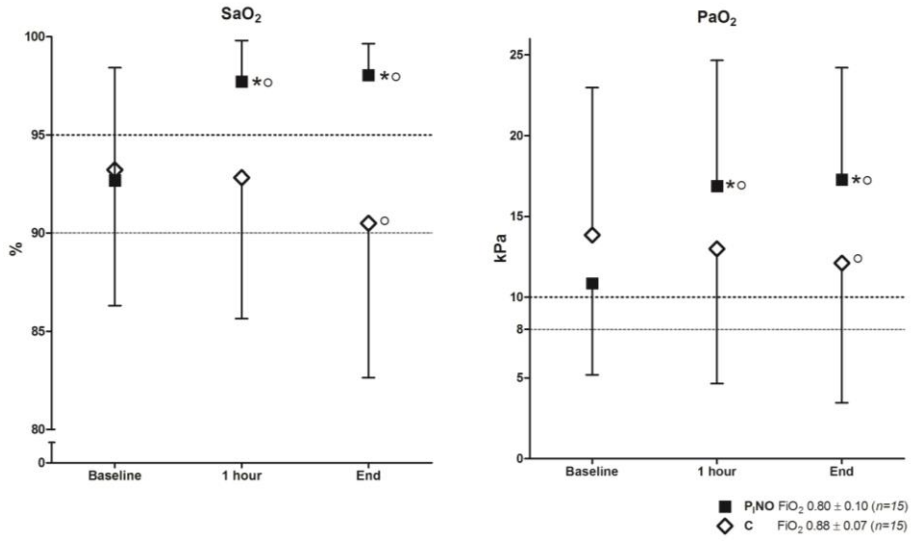


Figure 2. Study II. Arterial oxygen saturation (SaO_2) and arterial oxygen tension (PaO_2) in horses that received pulsed inhaled nitric oxide (PiNO group) and those that did not receive PiNO (C group) during anaesthesia. Data is presented as mean ± SD at baseline (beginning of anaesthesia before PiNO delivery commenced), at 1 hour and at end of anaesthesia in PiNO group and C group, respectively. The dotted lines indicate 90 and 95% SaO_2, and PaO_2 8 and 10 kPa (60 and 75 mm Hg). *Significantly different compared to C; °significantly different from baseline value.

In Study II, the pulse length of PiNO was started at 30% of the total inspiratory time. If there was no clinically significant increase in PaO_2 after 15 minutes the pulse length was adjusted to 45% and if still no significant increase in PaO_2 after 15 minutes it was increased to 60% of the total inspiratory time. Administration of the shortest pulse length, 30%, of PiNO improved PaO_2 in eight of the 15 horses, six horses responded with the 45% pulse length and one horse needed a pulse length of 60% to have a clinically significant increase in PaO_2. The result on pulse length from Study II was then used when it was decided to administer a pulse length of 45% of the total inspiratory time in Study I and III.

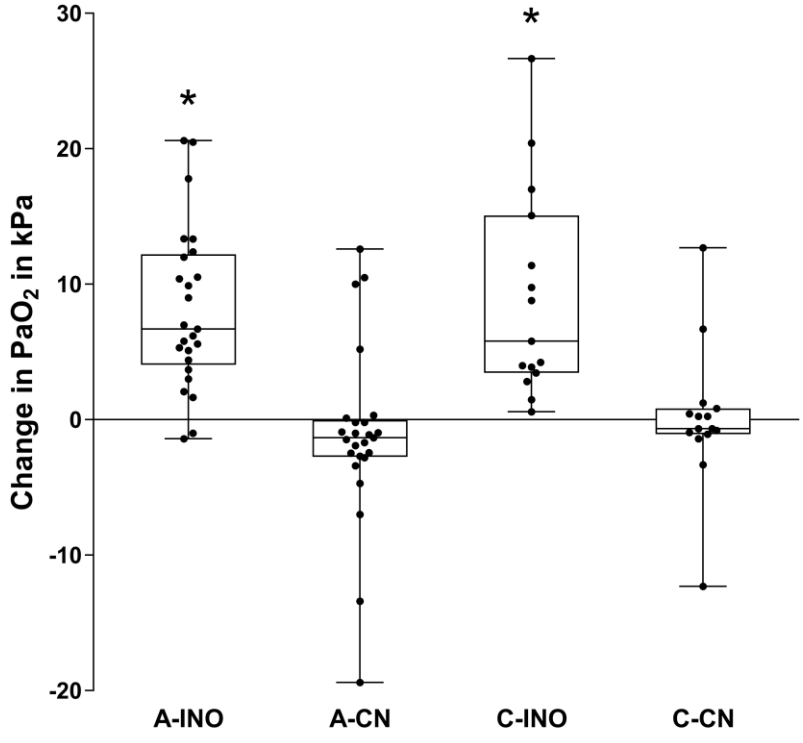


Figure 3. Study III. Boxplot, with individual points and whiskers min to max, of change in arterial oxygen tension (PaO_2) in kPa from baseline (beginning of anaesthesia before PiNO delivery commenced) to end of inhalation anaesthesia in horses that were administered pulsed inhaled nitric oxide (PiNO) and underwent arthroscopy (A-INO) or colic surgery (C-INO) and controls (A-CN, C-CN). *Indicates significant change ($P<0.05$) from baseline to end within respective group.

5.2 Pulmonary shunt

Pulmonary shunt is the amount of blood that flows through the lungs without participating in the gas exchange. It has been shown that treatment with PiNO reduces the pulmonary shunt via altering the blood perfusion and blood flow by redistribution of blood from the atelectasis to the well ventilated areas of the lung (Auckburally et al. 2022). As Study II and III were clinical studies performed on equine patients, the use of a Swan-Ganz catheter was excluded and thus it was not possible to collect mixed venous blood. However, it is described by Briganti et al. (2015) that it is accurate to use the calculated F-shunt for estimation of changes in pulmonary shunt in equine clinical studies, so this calculation was used in Study II and III. In horses

receiving PiNO in Study II, the F-shunt decreased during anaesthesia, from a baseline value of 39 ± 10% to 27 ± 6% at end of anaesthesia. In horses in the control group, the F-shunt went from a baseline value of 40 ± 12% to 44 ± 12% at end of anaesthesia. Amongst the colic horses in Study III, the F-shunt did not change during anaesthesia in the control group. In the colic horses receiving PiNO in that study, the F-shunt decreased significantly, from a baseline value of 46 ± 9% to 35 ± 7% at the end of anaesthesia (Figure 4). In Study I, mixed venous blood was collected; hence, the Berggren formula for pulmonary shunt calculation was used. In Study I, the shunt fraction decreased after PiNO delivery commenced, irrespectively of ventilation mode or blood pressure. Interestingly, the shunt fraction decreased in the MV-L group however, the PaO_2 did not increase significantly in this group. In the MV-L group the PvO_2 was significantly lower compared to the other groups and it did not increase when PiNO was administered, and with a slight, but not significant, increase in PaO_2, the result was a decreased shunt fraction.

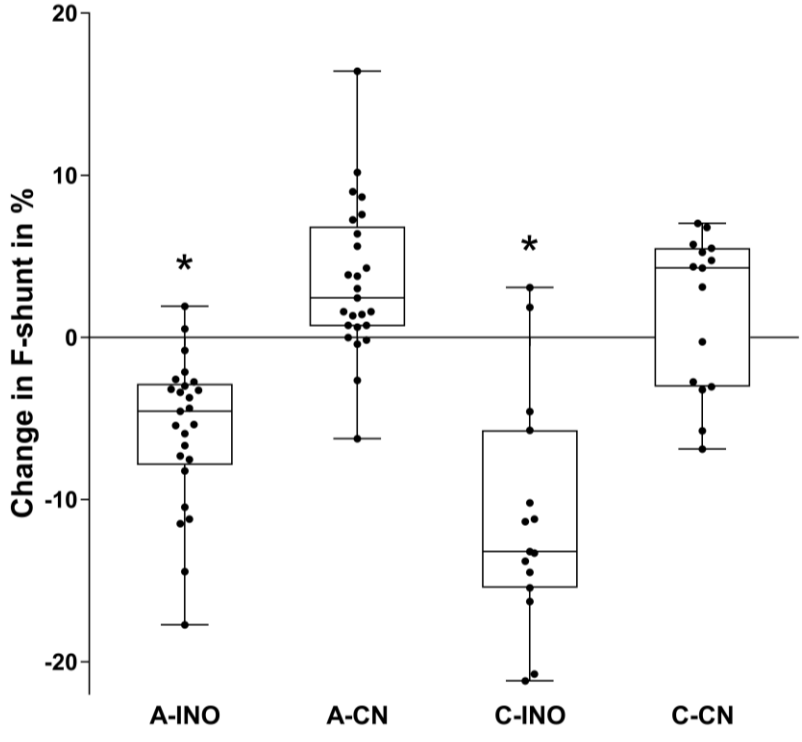


Figure 4. Study III. Boxplot, with individual points and whiskers min to max, of change in F-shunt in % from baseline (beginning of anaesthesia before PiNO delivery commenced) to end of inhalation anaesthesia in horses that were administered pulsed inhaled nitric oxide (PiNO) and underwent arthroscopy (A-INO) or colic surgery (C-INO) and controls (A-CN, C-CN). *Indicates significant change ($P<0.05$) from baseline to end within respectively group.

5.3 Blood pressure and ventilation

To obtain an optimal effect of PiNO the cardiac output and blood pressure also has to been taken into account, especially if the horses are ventilated with positive pressure ventilation. For an optimal gas exchange the ventilation and perfusion has to be matched. The effect of PiNO during different ventilation modes and circulatory status was evaluated in Study I (Figure 5). There was no response to PiNO when the horses with low MAP were ventilated (MV-L). On the contrary, arterial oxygenation improved in hypotensive but spontaneously breathing horses during PiNO administration.

In hypotensive, ventilated horses the V_D/V_T was increased compared to horses in the MV-N group, and the lack of effect of PiNO on gas exchange might be due to profound V_A/Q mismatch with decreased perfusion to well ventilated lung regions. It is well known that positive pressure during mechanical ventilation affects the circulation negatively. Auckburally et al. (2022) recently showed that the perfusion in nondependent lung regions was reduced during mechanical ventilation compared to spontaneous breathing. Mechanical ventilation decreases CO and if DO_2 is lower than the oxygen demand in the tissues, the venous oxygen saturation decreases and as a consequence PaO_2 will be reduced as well (Cournand & Motley 1948; Day et al. 1995).

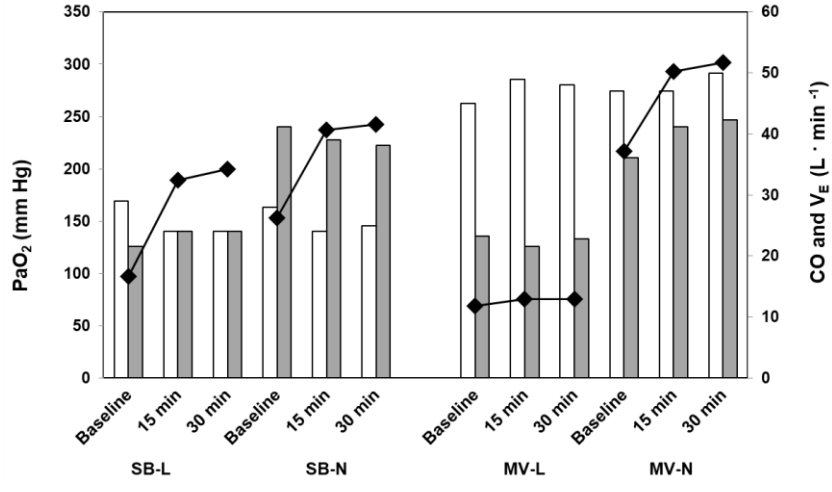


Figure 5. Study I. Mean PaO_2 (diamonds), blood flow (as determined by measurement of CO; gray bars), and minute ventilation (V_E; white bars) for 4 groups of anesthetized horses: SB with low (< 70 mm Hg) MAP (group SB-L; n = 7); SB with physiologically normal (≥ 70 mm Hg) MAP (group SB-N; 8), MV with low MAP (group MV-L; 6), and MV with physiologically normal MAP (group MV-N; 6). Horses were anesthetized and allowed a 60-minute equilibration period. At the end of that period (time 0; baseline), PiNO was delivered for 30 minutes. Variables were determined at baseline and 15 and 30 minutes after the start of PiNO.

The horses in the clinical studies II and III were treated with IV fluids and dobutamine to maintain a MAP above 70 mmHg during anaesthesia. Cardiac output was not measured in the clinical equine patients, but previous studies and the results in Study I show that PiNO itself has no effect on CO. (Heinonen et al. 2000, 2001, 2002; Nyman et al. 2012; Grubb et al. 2013b).

Horses breathing spontaneously in Study I hypoventilated, at end of anaesthesia $PaCO_2$ was 9.1 ± 2.3 and 9.3 ± 1.9 kPa in PiNO and control group, respectively. In Study III, there was no target set regarding $PaCO_2$ during ventilation, it was up to the anaesthetist to adjust the fR and V_T. The horses in this study were slightly hypoventilated, although not to the same extent as the horses in Study II. Since mechanical ventilation has a negative impact on the circulation, the anaesthetists might for this reason have been reluctant to ventilate the horses as much as would have been needed to reach a normal pH and a normal $PaCO_2$. Also an increased $PaCO_2$ has been shown to increase the cardiac index in horses during anaesthesia (Khanna et al. 1995).

The effect of pulsed inhaled nitric oxide on oxygenation has also been tested under ventilation modes other than spontaneous breathing or mechanical ventilation (Wiklund et al. 2018). Under certain circumstances, it can be beneficial to let the horse breathe spontaneously and tailor the ventilator settings to each individual horse's breathing pattern, so-called assisted ventilation. Examination of assisted breathing on nine horses showed that the effect of PiNO on oxygenation also functioned during inhalation anaesthesia (Figure 6). Compared to baseline, PaO_2, $P(A\text{-}a)O_2$ and SaO_2 improved significantly. While Qs/Qt decreased significantly, $PaCO_2$ did not change compared to baseline. Even though the horses in this experimental study were hypoventilating despite assisted ventilation, this mode of ventilation can be favourable compared to mechanical ventilation. Assisted mode of ventilation may reduce the risk of barotrauma, improve pulmonary gas distribution and preserve blood flow (Hodgson et al. 1986).

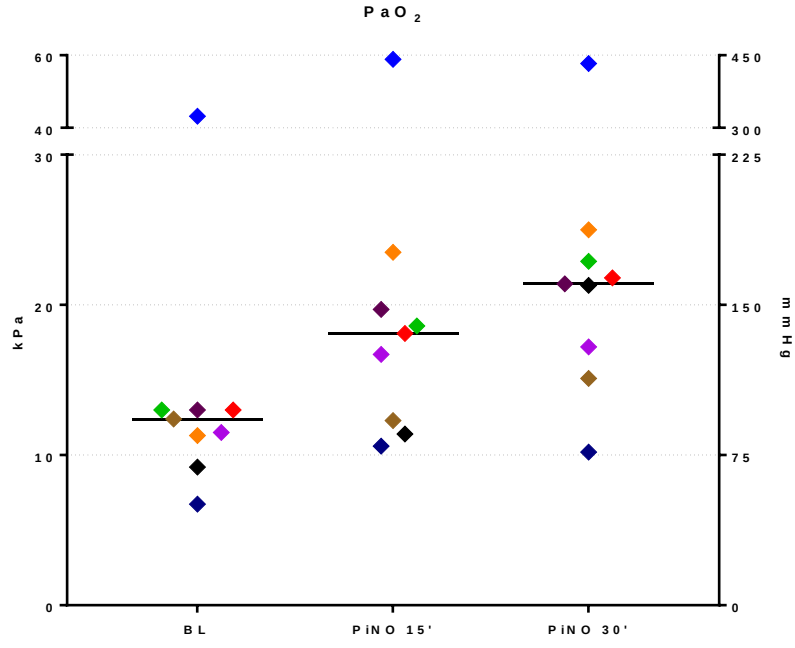


Figure 6. Individual and median arterial oxygen tension (PaO_2), baseline before PiNO delivery (BL) and at 15 minutes and 30 minutes of PiNO delivery in anaesthetised horses during assisted ventilation.

5.4 Lactate

Lactate is produced as a result of anaerobic metabolism and a normal plasma lactate concentration is < 1.5 mmol/L (Radcliffe et al. 2015). Tissue hypoxia due to hypoperfusion is the most common cause for lactic acidosis (Allen & Holm 2008), and hence hypoxaemia can worsen this effect. High lactate concentrations in the blood can affect the anaesthetic outcome. In a study on horses undergoing emergency laparotomy for acute intestinal diseases and healthy horses undergoing elective surgery, an arterial lactate in recovery ≥ 5 mmol/L was associated with a 2.25 times greater relative risk of complications (McCoy et al. 2011).

Measurements of lactate in Study II showed that the blood lactate concentration decreased 17 ± 21% in the PiNO group during anaesthesia, from 3.1 ± 2.6 to 2.3 ± 1.3 mmol L^{-1} ($p = 0.01$). During anaesthesia, the blood lactate concentration decreased in 10 horses in the PiNO group, in two horses the concentration did not change and in three horses the blood lactate

concentration increased (Figure 7). There was no significant change (2 ± 31%) in blood lactate concentration in the control group, the concentration went from 2.4 ± 1.2 to 2.3 ± 1.1 mmol L^{-1} ($p = 0.8$). However, looking at individual horses in the control group, blood lactate concentration decreased in eight horses and an increase was measured in seven horses during anaesthesia. In a study by Edner et al. (2007) blood lactate concentration did not change during a 3-hour anaesthesia in colic horses undergoing abdominal surgery, however, lactate content increased in the gluteus muscle. Interestingly, in Study II a decline in blood lactate concentration during anaesthesia was evident in the PiNO group, whereas amongst controls no changes occurred. The treatment of impaired circulation, based on infusion of crystalloid fluids and dobutamine during anaesthesia, did not differ between the two groups. However, decreasing lactate levels in the PiNO group and increased OER compared to the control group suggests improved peripheral perfusion and oxygenation. Still, many factors may influence lactate levels in individual horses, and the clinical effects of improved arterial oxygenation remains to be investigated. In Study III, no changes in the average lactate concentration were measured between horses treated with PiNO compared with controls, differences were only noted on an individual basis. It has been shown previously that the increase in plasma lactate concentration is not paralleled by similar increases in muscle lactate (Edner et al. 2002). Dissimilarities may be due to individual differences in venous drainage and an accumulation of produced lactate within the muscle during anaesthesia, thus, more studies are needed to investigate if the effect of PiNO can influence muscle metabolism and lactate concentration. In Study I, blood lactate was slightly but significantly higher in the MV-L group compared to MV-N, however, all lactate values in Study I was within normal limits. The reason for the higher lactate in the MV-L group could be the lower arterial oxygenation as well as the lower blood flow causing tissue hypoxia in this group.

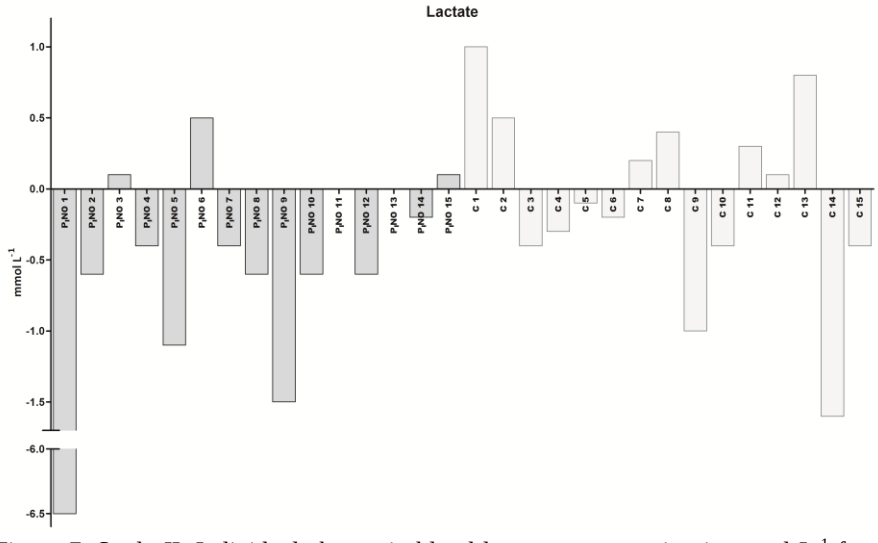


Figure 7. Study II. Individual change in blood lactate concentration in mmol L^{-1} from baseline to end of anaesthesia in horses that received pulsed inhaled nitric oxide (PiNO) and those that did not received PiNO (C). The baseline value is at the beginning of anaesthesia before PiNO delivery commenced.

5.5 Dobutamine

Enhanced blood flow can improve oxygenation by improving cardiac output and pulmonary perfusion which can then increase the uptake of oxygen from the lungs (Kelman et al. 1967). Dobutamine infusions during anaesthesia have been shown to increase CO and systemic and pulmonary pressure in horses (Gasthuys et al. 1991; Mizuno et al. 1994). However, dobutamine can have a negative impact on oxygenation. In healthy awake humans, dobutamine infusion lead to recruitment of intrapulmonary shunt vessels due to the increased CO, which increased the Qs/Qt and $P(A\text{-}a)O_2$ (Bryan et al. 2012). Dobutamine also had a negative effect on PaO_2 in critically ill humans that were mechanically ventilated, by increasing the shunt (Rennotte et al. 1989). However, in horses intrapulmonary shunt vessels have not been described in the lungs, and it is unlikely that any recruitment occurs when the CO is increased, and therefore there is little influence on arterial oxygenation (Manohar & Goetz 2005). In another study on horses dobutamine increased CO during anaesthesia, while $P(A\text{-}a)O_2$, Qs/Qt and V_D/V_T did not change (Swanson & Muir 1986).

In Study I, Qs/Qt and $P(A\text{-}a)O_2$ decreased in both MV and SB horses, likely because of the simultaneous administration of dobutamine during PiNO. Dobutamine administered alone to humans with pulmonary hypertension worsens shunting, but administration of a combination of NO and dobutamine had favourable effects on the pulmonary circulation (Vizza et al. 2001). In that study, selective pulmonary vasodilation due to NO was augmented by an increase in CO, with the deleterious effects of dobutamine on gas exchange being offset by the NO (Vizza et al. 2001). The same mechanism likely contributed to the increase in PaO_2 with PiNO and concurrent dobutamine administration in Study I. However, it cannot be ruled out that this could have been an effect of dobutamine alone because a control group that received dobutamine without PiNO was not included. The greatest decrease in $P(A\text{-}a)O_2$ was seen in the MV-N group most likely because the nondependent portion of the lungs was better ventilated in combination with an increase in pulmonary perfusion as a result of simultaneous administration of PiNO and dobutamine infusion.

5.6 Potential side effects of PiNO

Endothelin-1 is a potent vasoconstrictor and in lambs an increase in ET-1 has been detected after discontinuation of delivery of iNO (Ross et al. 2005). In piglets plasma ET-1 concentrations increased both during and after withdrawal of iNO (Chen et al. 2001) and in humans pulmonary hypertension has been seen after prolonged treatment with iNO after iNO was discontinued (Atz et al. 1996).

In the studies included in this thesis, no side effects were discovered during or after delivery of PiNO. Cardiac output was only measured in Study I, and in that study, the CO did not change after initiation of PiNO delivery. It cannot be ruled out that the CO changed in Study II and III after PiNO delivery commenced, but it is unlikely since no adverse effects of PiNO on CO has been measured in several previous experimental studies (Heinonen et al. 2000, 2001, 2002; Nyman et al. 2012; Grubb et al. 2013b).

Endothelin-1 (ET-1) was not measured in Study I-III, however previously done studies in horses have not revealed increased plasma ET-1 concentration during PiNO or after PiNO delivery has ended (Grubb et al. 2008, 2013a; b). In one of the studies ET-1 was measured during anaesthesia while PiNO was administered and after abrupt cessation of PiNO, the ET-1

concentration was not affected (Grubb et al. 2008). In another study, looking at plasma ET-1 concentrations during the early recovery phase after discontinuation of isoflurane anaesthesia, the control group had higher ET-1 concentrations compared to the group that received PiNO during anaesthesia (Grubb et al. 2013a).

5.7 Effects of oxygenation on the quality of recovery

An interesting aspect of treatment with PiNO under anaesthesia is whether the effect persists during the early recovery period.

When the horses in Study II-III were disconnected from the anaesthesia machine for transportation to the recovery box, they were breathing room air. In the recovery box they got supplementary oxygen into the endotracheal tube with a flow of 10 L per minute, however, this extra oxygen will only result in a FiO_2 around 40% (Wilson et al. 2006), compared up to approximately 90% during anaesthesia. The decrease in FiO_2 will lead to a decrease in arterial oxygenation.

In a previous study by Grubb et al. (2013a) the effect of PiNO on arterial oxygenation was measured and lasted 30 minutes after discontinuation compared to horses that were not treated with PiNO during anaesthesia. Inhaled NO has shown to be a successful treatment for severe hypoxaemia in humans after surgery (Teman et al. 2015; Zhang et al. 2020), and since the horses remain in lateral position for longer than 30 minutes in the recovery box it would be favourable to be able to continue to administer PiNO after the end of inhalation anaesthesia.

Study IV is the first study to investigate if improved PaO_2 through treatment with PiNO and improved arterial oxygenation throughout the entire course of anaesthesia has an effect on the quality of recovery in horses. The results in Study IV show a positive correlation between PaO_2 and recovery score (Figure 8), which is in line with previous studies (Hopster et al. 2011; Menzies et al. 2016; Rüegg et al. 2016). In their studies the PaO_2 during the course of the whole anaesthesia time is not taken into account and a single blood gas during anaesthesia probably does not reflect how well oxygenated the blood and tissues were throughout the anaesthesia. The strength of the present study is that the average PaO_2 for each individual horse during the entire anaesthesia was used for correlation calculations and that the assessors were blinded to the PiNO treatment. The intra-class correlation between the

three observers were 0.79, which is considered to be good. The mean recovery score for all horses were 3.8 ± 0.8, with a range from 1.67 to 5.0. There was a significant ($p = 0.02$) difference in recovery score between horses receiving PiNO and controls. Horses that received PiNO had a mean score of 4.2 ± 0.4, while horses in the control group had a recovery score of 3.5 ± 0.9. Two horses had a recovery score at or below 2, both of them were in the control group.

As far as the authors are aware, the duration of hypoxia necessary to have a negative effect on the quality of recovery is not known. Treatment with PiNO during anaesthesia has previously been shown to have an impact on the oxygenation during recovery (Grubb et al. 2013a). In that research study, horses receiving PiNO during 2.5 hours of inhalation anaesthesia showed higher PaO_2 and SaO_2 up to 30 minutes after discontinuing the treatment compared to a control group (Grubb et al. 2013a).

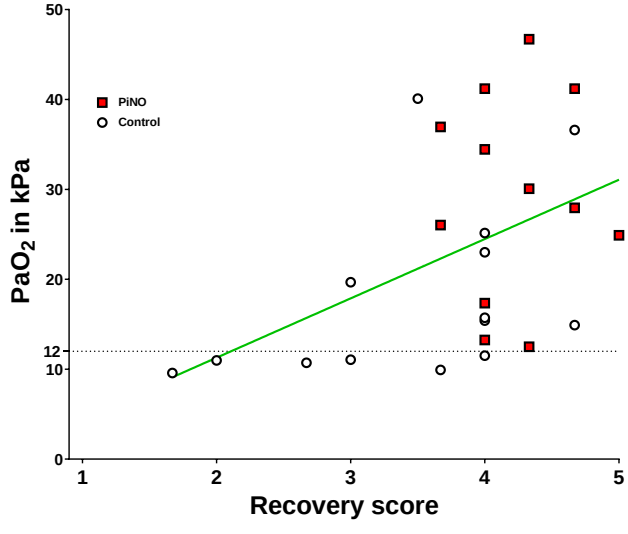


Figure 8. Correlation between arterial oxygen tension (PaO2) and recovery score. Horses that received PiNO during anaesthesia are presented as red squares and controls are presented as unfilled circles.

In human literature, the neurological effects of hypoxaemia have been described. For example it has been shown to cause delirium (Powell et al. 1996; O'reilly et al. 2000). As far as the authors are concerned, it is not known if this could happen to horses as well. If horses develop delirium

because of hypoxaemia during anaesthesia this might be a factor that effects the recovery quality.

6. Conclusions

The main conclusion in this thesis is that pulsed inhaled nitric oxide was an effective method to improve arterial oxygenation in horses during general anaesthesia and surgery in a clinical setting.

In the experimental Study (I), the degree of pulmonary perfusion affected PiNO efficacy during MV but not during SB. Use of PiNO failed to increase oxygenation in the MV-L group, likely because of profound ventilation-perfusion mismatching induced the impact on both ventilation and perfusion by positive pressure ventilation.

During SB, PiNO improved oxygenation irrespective of the magnitude of blood flow, but hypoventilation and hypercapnia persisted. Use of PiNO was most effective in horses with adequate perfusion.

In the clinical studies (II-IV) the results showed that it is possible to effectively reduce the F-shunt and improve arterial oxygenation in both healthy and compromised horses, ventilated spontaneously or mechanically, by continuous delivery of PiNO.

Results in this thesis suggest that improved oxygenation during anaesthesia can have a positive effect on the recovery quality in horses. Since PiNO improves oxygenation, it can benefit the recovery postoperatively. In the future, the effect of oxygenation on recovery quality needs to be further investigated as well as the long-time effects, such as wound healing.

References

Adami, C., Westwood-Hearn, H., Bolt, D.M. & Monticelli, P. (2020). Prevalence of Electrolyte Disturbances and Perianesthetic Death Risk Factors in 120 Horses Undergoing Colic Surgery. *Journal of Equine Veterinary Science*, 84, 102843. https://doi.org/10.1016/j.jevs.2019.102843

Allen, S.E. & Holm, J.L. (2008). Lactate: physiology and clinical utility. *Journal of Veterinary Emergency and Critical Care*, 18 (2), 123–132. https://doi.org/10.1111/j.1476-4431.2008.00286.x

Araos, J.D., Larenza, M.P., Boston, R.C., De Monte, V., De Marzo, C., Grasso, S., Haskins, S.C., Crovace, A. & Staffieri, F. (2012). Use of the oxygen content-based index, Fshunt, as an indicator of pulmonary venous admixture at various inspired oxygen fractions in anesthetized sheep. *American Journal of Veterinary Research*, 73 (12), 2013–2020. https://doi.org/10.2460/ajvr.73.12.2013

Atz, A.M., Adatia, I. & Wessel, D.L. (1996). Rebound pulmonary hypertension after inhalation of nitric oxide. *The Annals of Thoracic Surgery*, 62 (6), 1759–1764. https://doi.org/10.1016/s0003-4975(96)00542-5

Auckburally, A. & Nyman, G. (2017). Review of hypoxaemia in anaesthetized horses: predisposing factors, consequences and management. *Veterinary Anaesthesia and Analgesia*, 44 (3), 397–408. https://doi.org/10.1016/j.vaa.2016.06.001

Auckburally, A., Wiklund, M.K., Lord, P.F., Hedenstierna, G. & Nyman, G. (2022). Effects of pulsed inhaled nitric oxide delivery on the distribution of pulmonary perfusion in spontaneously breathing and mechanically ventilated anesthetized ponies. *American Journal of Veterinary Research*, 1–9. https://doi.org/10.2460/ajvr.21.03.0036

Berggren, S.M. (1942). *The oxygen deficit of arterial blood caused by non-ventilating parts of the lung*. Erscheinungsort nicht ermittelbar: Verlag nicht ermittelbar.

Bhatia, V., Elnagary, L. & Dakshinamurti, S. (2021). Tracing the path of inhaled nitric oxide: Biological consequences of protein nitrosylation. *Pediatric Pulmonology*, 56 (2), 525–538. https://doi.org/10.1002/ppul.25201

Blitzer, M.L., Loh, E., Roddy, M.-A., Stamler, J.S. & Creager, M.A. (1996). Endothelium-derived nitric oxide regulates systemic and pulmonary vascular resistance during acute hypoxia in humans. *Journal of the American College of Cardiology*, 28 (3), 591–596. https://doi.org/10.1016/0735-1097(96)00218-5

Briganti, A., Portela, D.A., Grasso, S., Sgorbini, M., Tayari, H., Bassini, J.R.F., Vitale, V., Romano, M.S., Crovace, A., Breghi, G. & Staffieri, F. (2015). Accuracy of different oxygenation indices in estimating intrapulmonary shunting at increasing infusion rates of dobutamine in horses under general anaesthesia. *The Veterinary Journal*, 204 (3), 351–356. https://doi.org/10.1016/j.tvjl.2015.04.002

Brodbelt, D.C., Blissitt, K.J., Hammond, R.A., Neath, P.J., Young, L.E., Pfeiffer, D.U. & Wood, J.L.N. (2008). The risk of death: the Confidential Enquiry into Perioperative Small Animal Fatalities. *Veterinary Anaesthesia and Analgesia*, 35 (5), 365–373. https://doi.org/10.1111/j.1467-2995.2008.00397.x

Bryan, T.L., van Diepen, S., Bhutani, M., Shanks, M., Welsh, R.C. & Stickland, M.K. (2012). The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *Journal of Applied Physiology*, 113 (4), 541–548. https://doi.org/10.1152/japplphysiol.00404.2012

Casoni, D., Spadavecchia, C. & Adami, C. (2014). Cardiovascular changes after administration of aerosolized salbutamol in horses: five cases. *Acta Veterinaria Scandinavica*, 56 (1), 49. https://doi.org/10.1186/s13028-014-0049-z

Chen, L., He, H., Fernandez Mondejar, E., Fredén, F., Wiklund, P., Alving, K. & Hedenstierna, G. (2001). Endothelin-1 and nitric oxide synthase in short rebound reaction to short exposure to inhaled nitric oxide. *American Journal of Physiology. Heart and Circulatory Physiology*, 281 (1), H124-131. https://doi.org/10.1152/ajpheart.2001.281.1.H124

Clark, R.H., Kueser, T.J., Walker, M.W., Southgate, W.M., Huckaby, J.L., Perez, J.A., Roy, B.J., Keszler, M. & Kinsella, J.P. (2000). Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. *New England Journal of Medicine*, 342 (7), 469–474

Costa-Farré, C., Prades, M., Ribera, T., Valero, O. & Taurà, P. (2014). Does intraoperative low arterial partial pressure of oxygen increase the risk of surgical site infection following emergency exploratory laparotomy in horses? *The Veterinary Journal*, 200 (1), 175–180. https://doi.org/10.1016/j.tvjl.2014.01.029

Cournand, A. & Motley, H.L. (1948). Physiological studies of the effects of intermittent positive pressure breathing on cardiac output in man. *The American Journal of Physiology*, 152 (1), 162–174. https://doi.org/10.1152/ajplegacy.1947.152.1.162

Day, T.K., Gaynor, J.S., Muir, W.W., Bednarski, R.M. & Mason, D.E. (1995). Blood gas values during intermittent positive pressure ventilation and spontaneous ventilation in 160 anesthetized horses positioned in lateral or dorsal recumbency. *Veterinary surgery: VS*, 24 (3), 266–276

Derksen, F., Olszewski, M., Robinson, N., Berney, C.E., Hakala, J., Matson, C. & Ruth, D. (1999). Aerolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. *American journal of veterinary research*, 60, 689–93

Dobson, A., Gleed, R.D., Meyer, R.E. & Stewart, B.J. (1985). Changes in blood flow distribution in equine lungs induced by anaesthesia. *Quarterly Journal of Experimental Physiology*, 70 (2), 283–297

Dobyns, E.L., Cornfield, D.N., Anas, N.G., Fortenberry, J.D., Tasker, R.C., Lynch, A., Liu, P., Eells, P.L., Griebel, J., Baier, M., & others (1999). Multicenter randomized controlled trial of the effects of inhaled nitric oxide therapy on gas exchange in children with acute hypoxemic respiratory failure. *The Journal of pediatrics*, 134 (4), 406–412

Dugdale, A.H., Obhrai, J. & Cripps, P.J. (2016). Twenty years later: a single-centre, repeat retrospective analysis of equine perioperative mortality and investigation of recovery quality. *Veterinary Anaesthesia and Analgesia*, 43 (2), 171–178. https://doi.org/10.1111/vaa.12285

Dugdale, A.H. & Taylor, P.M. (2016). Equine anaesthesia-associated mortality: where are we now? *Veterinary Anaesthesia and Analgesia*, 43 (3), 242–255. https://doi.org/10.1111/vaa.12372

Edner, A., Nyman, G. & Essén-Gustavsson, B. (2002). The relationship of muscle perfusion and metabolism with cardiovascular variables before and after detomidine injection during propofol–ketamine anaesthesia in horses. *Veterinary Anaesthesia and Analgesia*, 29 (4), 182–199. https://doi.org/10.1046/j.1467-2995.2002.00101.x

Edner, A.H., Nyman, G.C. & Essén-Gustavsson, B. (2007). Metabolism before, during and after anaesthesia in colic and healthy horses. *Acta Veterinaria Scandinavica*, 49, 34. https://doi.org/10.1186/1751-0147-49-34

Gasthuys, F., de Moor, A. & Parmentier, D. (1991). Influence of Dopamine and Dobutamine on the Cardiovascular Depression During a

Standard Halothane Anaesthesia in Dorsally Recumbent, Ventilated Ponies. *Journal of Veterinary Medicine Series A*, 38 (1–10), 494–500. https://doi.org/10.1111/j.1439-0442.1991.tb01040.x

Gozalo-Marcilla, M. & Ringer, S.K. (2021). Recovery after General Anaesthesia in Adult Horses: A Structured Summary of the Literature. *Animals : an Open Access Journal from MDPI*, 11 (6), 1777. https://doi.org/10.3390/ani11061777

Grubb, T., Edner, A., Frendin, J.H., Funkquist, P., Rydén, A. & Nyman, G. (2013a). Oxygenation and plasma endothelin-1 concentrations in healthy horses recovering from isoflurane anaesthesia administered with or without pulse-delivered inhaled nitric oxide. *Veterinary Anaesthesia and Analgesia*, 40 (6), e9–e18. https://doi.org/10.1111/j.1467-2995.2012.00735.x

Grubb, T., Frendin, J.H.M., Edner, A., Funkquist, P., Hedenstierna, G. & Nyman, G. (2013b). The effects of pulse-delivered inhaled nitric oxide on arterial oxygenation, ventilation-perfusion distribution and plasma endothelin-1 concentration in laterally recumbent isoflurane-anaesthetized horses. *Veterinary Anaesthesia and Analgesia*, 40 (6), e19-30. https://doi.org/10.1111/vaa.12037

Grubb, T.L., Högman, M., Edner, A., Frendin, J.H.M., Heinonen, E., Malavasi, L.M., Frostell, C.G., Ryden, A., Alving, K. & Nyman, G.C. (2008). Physiologic responses and plasma endothelin-1 concentrations associated with abrupt cessation of nitric oxide inhalation in isoflurane-anesthetized horses. *American Journal of Veterinary Research*, 69 (3), 423–430. https://doi.org/10.2460/ajvr.69.3.423

Grubb, T.L., Lord, P.F., Berger, M., Larsson, C., Rydén, A., Frendin, J., Funkquist, P., Edner, A. & Nyman, G. (2014). Effects of pulse-delivered inhaled nitric oxide administration on pulmonary perfusion and arterial oxygenation in dorsally recumbent isoflurane-anesthetized horses. *American Journal of Veterinary Research*, 75 (11), 949–955. https://doi.org/10.2460/ajvr.75.11.949

Guedes, A., Aleman, M., Davis, E. & Tearney, C. (2016). Cardiovascular, respiratory and metabolic responses to apnea induced by atlanto-occipital intrathecal lidocaine injection in anesthetized horses. *Veterinary Anaesthesia and Analgesia*, 43 (6), 590–598. https://doi.org/10.1111/vaa.12344

Heinonen, E., Hedenstierna, G., Meriläinen, P., Högman, M. & Nyman, G. (2001). Pulsed delivery of nitric oxide counteracts hypoxaemia in the anaesthetized horse. *Veterinary Anaesthesia and Analgesia*, 28 (1), 3–11

Heinonen, E., Högman, M. & Meriläinen, P. (2000). Theoretical and experimental comparison of constant inspired concentration and pulsed delivery in NO therapy. *Intensive care medicine*, 26 (8), 1116–1123

Heinonen, E., Nyman, G., Meriläinen, P. & Högman, M. (2002). Effect of different pulses of nitric oxide on venous admixture in the anaesthetized horse. *British Journal of Anaesthesia*, 88 (3), 394–398

Hodgson, D.S., Steffey, E.P., Grandy, J.L. & Woliner, M.J. (1986). Effects of spontaneous, assisted, and controlled ventilatory modes in halothane-anesthetized geldings. *American Journal of Veterinary Research*, 47 (5), 992–996

Hopster, K., Kästner, S.B., Rohn, K. & Ohnesorge, B. (2011). Intermittent positive pressure ventilation with constant positive end-expiratory pressure and alveolar recruitment manoeuvre during inhalation anaesthesia in horses undergoing surgery for colic, and its influence on the early recovery period. *Veterinary Anaesthesia and Analgesia*, 38 (3), 169–177. https://doi.org/10.1111/j.1467-2995.2011.00606.x

Hopster, K., Wogatzki, A., Geburek, F., Conze, P. & Kästner, S.B.R. (2017). Effects of positive end-expiratory pressure titration on intestinal oxygenation and perfusion in isoflurane anaesthetised horses. *Equine Veterinary Journal*, 49 (2), 250–256. https://doi.org/10.1111/evj.12555

Hubbell, J.A.E. & Muir, W.W. (2015). Oxygenation, oxygen delivery and anaesthesia in the horse: Oxygenation, oxygen delivery and anaesthesia. *Equine Veterinary Journal*, 47 (1), 25–35. https://doi.org/10.1111/evj.12258

Ichinose, F., Roberts, J.D. & Zapol, W.M. (2004). Inhaled Nitric Oxide. *Circulation*, 109 (25), 3106–3111. https://doi.org/10.1161/01.CIR.0000134595.80170.62

Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E. & Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences*, 84 (24), 9265–9269

Johnston, G., Eastment, J., Wood, J. & Taylor, P. (2002). The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of Phases 1 and 2. *Veterinary Anaesthesia and Analgesia*, 29 (4), 159–170. https://doi.org/10.1046/j.1467-2995.2002.00106.x

Kacmarek, R.M., Pirrone, M. & Berra, L. (2015). Assisted mechanical ventilation: the future is now! *BMC Anesthesiology*, 15 (1). https://doi.org/10.1186/s12871-015-0092-y

Kelman, G.R., Nunn, J.F., Prys-Roberts, C. & Greenbaum, R. (1967). The influence of cardiac output on arterial oxygenation: a theoretical study. *British Journal of Anaesthesia*, 39 (6), 450–458. https://doi.org/10.1093/bja/39.6.450

Khanna, A.K., McDonell, W.N., Dyson, D.H. & Taylor, P.M. (1995). Cardiopulmonary effects of hypercapnia during controlled intermittent positive pressure ventilation in the horse. *Canadian Journal of Veterinary Research*, 59 (3), 213–221

Lagasse, R.S. (2002). Anesthesia Safety: Model or Myth?: A Review of the Published Literature and Analysis of Current Original Data. *Anesthesiology*, 97 (6), 1609–1617. https://doi.org/10.1097/00000542-200212000-00038

Laurenza, C., Ansart, L. & Portier, K. (2020). Risk Factors of Anesthesia-Related Mortality and Morbidity in One Equine Hospital: A Retrospective Study on 1,161 Cases Undergoing Elective or Emergency Surgeries. *Frontiers in Veterinary Science*, 6, 514. https://doi.org/10.3389/fvets.2019.00514

Lester, G.D., DeMarco, V.G. & Norman, W.M. (1999). Effect of inhaled nitric oxide on experimentally induced pulmonary hypertension in neonatal foals. *American Journal of Veterinary Research*, 60 (10), 1207–1212

Loomes, K. (2021). Effect of Aerosolised Salbutamol Administration on Arterial Potassium Concentration in Anaesthetised Horses. *Journal of Equine Veterinary Science*, 103, 103667. https://doi.org/10.1016/j.jevs.2021.103667

Loomes, K. & Louro, L.F. (2022). Recovery of horses from general anaesthesia: A systematic review (2000-2020) of risk factors and influence of interventions during the recovery period. *Equine Veterinary Journal*, 54 (2), 201–218. https://doi.org/10.1111/evj.13517

Manohar, M. & Goetz, T.E. (2005). Intrapulmonary arteriovenous shunts of >15 μm in diameter probably do not contribute to arterial hypoxemia in maximally exercising Thoroughbred horses. *Journal of Applied Physiology*, 99 (1), 224–229. https://doi.org/10.1152/japplphysiol.01230.2004

Marntell, S., Nyman, G. & Hedenstierna, G. (2005). High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses. *Veterinary Anaesthesia and Analgesia*, 32 (6), 338–347. https://doi.org/10.1111/j.1467-2995.2005.00199.x

McCoy, A.M., Hackett, E.S., Wagner, A.E., Mama, K.R. & Hendrickson, D.A. (2011). Pulmonary Gas Exchange and Plasma Lactate in

Horses with Gastrointestinal Disease Undergoing Emergency Exploratory Laparotomy: A Comparison with an Elective Surgery Horse Population. *Veterinary Surgery*, 40 (5), 601–609. https://doi.org/10.1111/j.1532-950X.2011.00840.x

McGoldrick, T.M., Bowen, I.M. & Clarke, K.W. (1998). Sudden cardiac arrest in an anaesthetised horse associated with low venous oxygen tensions. *The Veterinary Record*, 142 (22), 610–611

McKay, J.S., Forest, T.W., Senior, M., Kelly, D.F., Jones, R.S., de, L.A. & Summers, B.A. (2002). Postanaesthetic cerebral necrosis in five horses. *The Veterinary Record*, 150 (3), 70–74

Menzies, M.P.L., Ringer, S.K., Conrot, A., Theurillat, R., Kluge, K., Kutter, A.P., Jackson, M., Thormann, W. & Bettschart-Wolfensberger, R. (2016). Cardiopulmonary effects and anaesthesia recovery quality in horses anaesthetized with isoflurane and low-dose S-ketamine or medetomidine infusions. *Veterinary Anaesthesia and Analgesia*, 43 (6), 623–634. https://doi.org/10.1111/vaa.12359

Mizuno, Y., Aida, H. & Fujinaga, T. (1994). Effects of Dobutamine Infusion in Dorsally Recumbent Isoflurane-Anesthetized Horses. *Journal of Equine Science*, 5 (3), 87–94. https://doi.org/10.1294/jes.5.87

Moens, Y. (2013). Mechanical Ventilation and Respiratory Mechanics During Equine Anesthesia. *Veterinary Clinics of North America: Equine Practice*, 29 (1), 51–67. https://doi.org/10.1016/j.cveq.2012.12.002

Moens, Y., Gootjes, P. & Lagerweij, E. (1992). A tracheal tube-in-tube technique for functional separation of the lungs in the horse. *Equine veterinary journal*, 24 (2), 103–106

Moens, Y., Lagerweij, E., Gootjes, P. & Poortman, J. (1994). Differential artificial ventilation in anesthetized horses positioned in lateral recumbency. *American Journal of Veterinary Research*, 55 (9), 1319–1326

Moreno-Martinez, F., Mosing, M. & Senior, M. (2022). Controlled mechanical ventilation in equine anaesthesia: Physiological background and basic considerations (Part 1). *Equine Veterinary Education*, 34 (6), 320–329. https://doi.org/10.1111/eve.13476

Mosing, M. & Senior, J.M. (2018). Maintenance of equine anaesthesia over the last 50 years: Controlled inhalation of volatile anaesthetics and pulmonary ventilation. *Equine Veterinary Journal*, 50 (3), 282–291. https://doi.org/10.1111/evj.12793

Nyman, G., Frostell, C., Hedenstierna, G., Funkquist, B., Kvart, C. & Blomqvist, H. (1987). Selective mechanical ventilation of dependent

lung regions in the anaesthetized horse in dorsal recumbency. *British Journal of Anaesthesia*, 59 (8), 1027–1034

Nyman, G., Funkquist, B., Kvart, C., Frostell, C., Tokics, L., Strandberg, A., Lundquist, H., Lundh, B., Brismar, B. & Hedenstierna, G. (1990). Atelectasis causes gas exchange impairment in the anaesthetised horse. *Equine Veterinary Journal*, 22 (5), 317–324

Nyman, G., Grubb, T.L., Heinonen, E., Frendin, J., Edner, A., Malavasi, L.M., Frostell, C. & Högman, M. (2012). Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses. *Veterinary Anaesthesia and Analgesia*, 39 (5), 480–487. https://doi.org/10.1111/j.1467-2995.2012.00740.x

Nyman, G. & Hedenstierna, G. (1989). Ventilation-perfusion relationships in the anaesthetised horse. *Equine Veterinary Journal*, 21 (4), 274–281

O'reilly, D., Williams, M. & County, B. (2000). Adult Hypoxaemia in the Perioperative Period: A Review of the Literature. *British Journal of Perioperative Nursing (United Kingdom)*, 10 (4), 204–212. https://doi.org/10.1177/175045890001000405

Palmer, R.M.J., Ferrige, A.G. & Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, 327 (6122), 524–526. https://doi.org/10.1038/327524a0

Pascoe, P.J., McDonell, W.N., Trim, C.M. & Van Gorder, J. (1983). Mortality rates and associated factors in equine colic operations—a retrospective study of 341 operations. *The Canadian Veterinary Journal*, 24 (3), 76

Portier, K., Crouzier, D., Guichardant, M., Prost, M., Debouzy, J.-C., Kirschvink, N., Fellmann, N., Lekeux, P. & Coudert, J. (2009). Effects of high and low inspired fractions of oxygen on horse erythrocyte membrane properties, blood viscosity and muscle oxygenation during anaesthesia. *Veterinary Anaesthesia and Analgesia*, 36 (4), 287–298. https://doi.org/10.1111/j.1467-2995.2009.00459.x

Powell, J.F., Menon, D.K. & Jones, J.G. (1996). The effects of hypoxaemia and recommendations for postoperative oxygen therapy. *Anaesthesia*, 51 (8), 769–772. https://doi.org/10.1111/j.1365-2044.1996.tb07893.x

Radcliffe, R.M., Buchanan, B.R., Cook, V.L. & Divers, T.J. (2015). The clinical value of whole blood point-of-care biomarkers in large animal emergency and critical care medicine: Biomarkers in large

animal emergency critical care. *Journal of Veterinary Emergency and Critical Care*, 25 (1), 138–151. https://doi.org/10.1111/vec.12276

Rennotte, M.T., Reynaert, M., Clerbaux, Th., Willems, E., Roeseleer, J., Verlter, C., Rodenstein, D. & Frans, A. (1989). Effects of two inotropic drugs, dopamine and dobutamine, on pulmonary gas exchange in artificially ventilated patients. *Intensive Care Medicine*, 15 (3), 160–165. https://doi.org/10.1007/BF01058567

Ringer, S.K., Schwarzwald, C.C., Portier, K.G., Ritter, A. & Bettschart-Wolfensberger, R. (2013). Effects on cardiopulmonary function and oxygen delivery of doses of romifidine and xylazine followed by constant rate infusions in standing horses. *The Veterinary Journal*, 195 (2), 228–234. https://doi.org/10.1016/j.tvjl.2012.06.036

Robertson, S.A. & Bailey, J.E. (2002). Aerosolized salbutamol (albuterol) improves PaO2 in hypoxaemic anaesthetized horses - a prospective clinical trial in 81 horses. *Veterinary Anaesthesia and Analgesia*, 29 (4), 212–218. https://doi.org/10.1046/j.1467-2995.2002.00100.x

Ross, G.A., Oishi, P., Azakie, A., Fratz, S., Fitzgerald, R.K., Johengen, M.J., Harmon, C., Hendricks-Munoz, K., Xu, J., Black, S.M. & Fineman, J.R. (2005). Endothelial alterations during inhaled NO in lambs with pulmonary hypertension: implications for rebound hypertension. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, 288 (1), L27-35. https://doi.org/10.1152/ajplung.00144.2004

Rossaint, R., Falke, K.J., Lopez, F., Slama, K., Pison, U. & Zapol, W.M. (1993). Inhaled Nitric Oxide for the Adult Respiratory Distress Syndrome. *New England Journal of Medicine*, 328 (6), 399–405. https://doi.org/10.1056/NEJM199302113280605

Rüegg, M., Bettschart-Wolfensberger, R., Hartnack, S., Junge, K.H., Theiss, F. & Ringer, S.K. (2016). Comparison of non-assisted versus head and tail rope-assisted recovery after emergency abdominal surgery in horses: *Pferdeheilkunde Equine Medicine*, 32 (5), 469–478. https://doi.org/10.21836/PEM20160508

Savvas, I., Pavlidou, K., Braun, C., Schauvliege, S., Staffieri, F. & Moens, Y. (2021). Evaluation of the Effect of the Inspired Oxygen Fraction on Blood Oxygenation during Inhalant Anaesthesia in Horses: A Systematic Review with Meta-Analysis. *Animals : an Open Access Journal from MDPI*, 11 (8), 2245. https://doi.org/10.3390/ani11082245

Sorenson, P.R. & Robinson, N.E. (1980). Postural effects on lung volumes and asynchronous ventilation in anesthetized horses. *Journal of*

Applied Physiology: Respiratory, Environmental and Exercise Physiology, 48 (1), 97–103. https://doi.org/10.1152/jappl.1980.48.1.97

Steffey, E.P. (2002). Recent advances in inhalation anesthesia. *Veterinary Clinics of North America: Equine Practice*, 18 (1), 159–168. https://doi.org/10.1016/S0749-0739(01)00007-4

Steffey, E.P., Willits, N. & Woliner, M. (1992). Hemodynamic and respiratory responses to variable arterial partial pressure of oxygen in halothane-anesthetized horses during spontaneous and controlled ventilation. *American Journal of Veterinary Research*, 53 (10), 1850–1858

Suthers, J.M., Christley, R.M. & Clutton, R.E. (2011). Quantitative and qualitative comparison of three scoring systems for assessing recovery quality after general anaesthesia in horses. *Veterinary Anaesthesia and Analgesia*, 38 (4), 352–362. https://doi.org/10.1111/j.1467-2995.2011.00629.x

Swanson, C.R. & Muir, W.W. (1986). Dobutamine-induced augmentation of cardiac output does not enhance respiratory gas exchange in anesthetized recumbent healthy horses. *American Journal of Veterinary Research*, 47 (7), 1573–1576

Taylor, P.M. (1999). Effects of hypoxia on endocrine and metabolic responses to anaesthesia in ponies. *Research in veterinary Science*, 66 (1), 39–44

Teman, N.R., Thomas, J., Bryner, B.S., Haas, C.F., Haft, J.W., Park, P.K., Lowell, M.J. & Napolitano, L.M. (2015). Inhaled Nitric Oxide to Improve Oxygenation for Safe Critical Care Transport of Adults With Severe Hypoxemia. *American Journal of Critical Care*, 24 (2), 110–117. https://doi.org/10.4037/ajcc2015570

Trim, C.M., Adams, J.G., Cowgill, L.M. & Ward, S.L. (1989). A retrospective survey of anaesthesia in horses with colic. *Equine Veterinary Journal*, 21 (S7), 84–90. https://doi.org/10.1111/j.2042-3306.1989.tb05663.x

Trim, C.M. & Wan, P.Y. (1990). Hypoxaemia during anaesthesia in seven horses with colic. *Veterinary Anaesthesia and Analgesia*, 17 (1), 45–49

Vettorato, E., Chase-Topping, M.E. & Clutton, R.E. (2010). A comparison of four systems for scoring recovery quality after general anaesthesia in horses. *Equine Veterinary Journal*, 42 (5), 400–406. https://doi.org/10.1111/j.2042-3306.2010.00093.x

Vizza, C.D., Della Rocca, G., Roma, D.A., Iacoboni, C., Pierconti, F., Venuta, F., Rendina, E., Schmid, G., Pietropaoli, P. & Fedele, F.

(2001). Acute hemodynamic effects of inhaled nitric oxide, dobutamine and a combination of the two in patients with mild to moderate secondary pulmonary hypertension. *Critical Care*, 5 (6), 355. https://doi.org/10.1186/cc1069

Wagner, A.E. (2008a). Complications in Equine Anesthesia. *Veterinary Clinics of North America: Equine Practice*, 24 (3), 735–752. https://doi.org/10.1016/j.cveq.2008.10.002

Wagner, P.D. (2008b). The multiple inert gas elimination technique (MIGET). *Intensive Care Medicine*, 34 (6), 994–1001. https://doi.org/10.1007/s00134-008-1108-6

Weinberger, B., Laskin, D.L., Heck, D.E. & Laskin, J.D. (2001). The toxicology of inhaled nitric oxide. *Toxicological Sciences*, 59 (1), 5–16

West, J.B. (2012). *Respiratory Physiology: The Essentials*. Lippincott Williams & Wilkins.

Whitehair, K.J., Steffey, E.P., Woliner, M.J. & Willits, N.H. (1996). Effects of inhalation anesthetic agents on response of horses to three hours of hypoxemia. *American Journal of Veterinary Research*, 57 (3), 351–360

Whitehair, K.J. & Willits, N.H. (1999). Predictors of arterial oxygen tension in anesthetized horses: 1,610 cases (1992-1994). *Journal of the American Veterinary Medical Association*, 215 (7), 978–981

Wiklund, M., Auckburally, A. & Nyman, G. (2018). Effects of Pulsed inhaled Nitric Oxide on oxygenation during assisted ventilation. *Proceedings of WCVA Venice*, 2018.

Wilson, D.V., Schott, H.C., Robinson, N.E., Berney, C.E. & Eberhart, S.W. (2006). Response to nasopharyngeal oxygen administration in horses with lung disease. *Equine Veterinary Journal*, 38 (3), 219–223. https://doi.org/10.2746/042516406776866345

Witek, J. & Lakhkar, A.D. (2022). Nitric Oxide. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK554485/ [2022-11-07]

Young, J.D., Sear, J.W. & Valvini, E.M. (1996). Kinetics of methaemoglobin and serum nitrogen oxide production during inhalation of nitric oxide in volunteers. *British Journal of Anaesthesia*, 76 (5), 652–656. https://doi.org/10.1093/bja/76.5.652

Young, L.E., Marlin, D.J., McMurphy, R.M., Walsh, K. & Dixon, P.M. (1999). Effects of inhaled nitric oxide 10 ppm in spontaneously breathing horses anaesthetized with halothane. *British Journal of Anaesthesia*, 83 (2), 321–324

Young, S.S. & Taylor, P.M. (1993). Factors influencing the outcome of equine anaesthesia: a review of 1,314 cases. *Equine Veterinary Journal*, 25 (2), 147–151. https://doi.org/10.1111/j.2042-3306.1993.tb02926.x

Zhang, H., Liu, Y., Meng, X., Yang, D., Shi, S., Liu, J., Yuan, Z., Gu, T., Han, L., Lu, F., Xu, Z., Liu, Y. & Yu, M. (2020). Effects of inhaled nitric oxide for postoperative hypoxemia in acute type A aortic dissection: a retrospective observational study. *Journal of Cardiothoracic Surgery*, 15 (1), 25. https://doi.org/10.1186/s13019-020-1069-6

Popular science summary

PiNO – A method during anaesthesia that can save horses' lives

Did you know that 1 out of 100 horses that are anaesthetised die? That is a staggeringly high mortality rate of 1% compared to the mortality rate 0.04% for the world's most dangerous sport, BASE jumping. By using PiNO, a method that I have introduced to horse patients through my PhD thesis, more horses might be saved from an early death, in Sweden and worldwide.

Horses are among the most popular domestic animals in Sweden, from competition at high levels to trail riding, and surgeries that require anaesthesia are common. Every year, thousands of horses undergo anaesthesia for surgery in Sweden alone. Horses might undergo anaesthesia for planned surgeries, for example castration, but also for emergency surgeries like abdominal surgery due to colic, a painful condition where the intestines need surgical correction.

A horse under anaesthesia has a relatively high risk of not surviving. This can be because often when a horse is in surgery it has to be positioned on its back, in a special padded bed shaped to fit a horse. In a horse the intestines are very heavy and take up 34% of the body weight – compare that to dogs where the intestines only take up 13% of the body weight. The unnatural position causes the horse's heavy intestines to compress the lungs, which can lead to complications.

One common complication during horse anaesthesia when the horse is on its back is low oxygen in the blood (hypoxaemia) which is why my PhD project is focused on a new way to treat hypoxaemia. The method, called PiNO

(Pulsed inhaled Nitric Oxide) delivery involves giving a small pulse of the gas nitric oxide in the beginning of each breath during anaesthesia. The molecule nitric oxide changes how the blood flows throughout the horse's lungs and greatly improves oxygen levels in the horse's blood.

In my research, I divided the horses into two groups, one group that was treated with PiNO and one control group. When treated with PiNO, both healthy horses, undergoing scheduled surgeries and horses that were sick and undergoing emergency surgeries, improved their oxygen level in the blood during anaesthesia. With PiNO, horses that had better oxygen level in the blood during anaesthesia also had better recovery from anaesthesia, for example the horses needed fewer attempts to stand and were more stable once standing.

PiNO is currently being spread throughout the world of horse anaesthesia, in the hope that it will have a positive impact and make it possible for more horses to survive anaesthesia and have better recovery.

Populärvetenskaplig sammanfattning

PiNO – en metod under anestesin som kan rädda hästars liv

Visste du att 1 av 100 hästar som sövs inte överlever operationen? Det är en hög dödlighet på 1 %, jämfört en med dödlighet på 0,04 % för världens farligaste sport, BASE-hoppning. Med metoden PiNO under narkos kan man rädda fler hästar från en tidig död, i Sverige och världen över.

Hästar är bland de populäraste husdjuren i Sverige, från tävlingar på hög nivå till hobbyridning, och det är vanligt med operationer som kräver anestesi. Enbart i Sverige genomgår varje år flera tusen hästar operationer där de behöver anestesi. Hästar kan sövas för planerade operationer, till exempel för kastration, men även för akuta kirurgier som buköppning på grund av kolik, ett mycket smärtsamt tillstånd där hästens tarmar behöver rättas till vid en operation.

En häst som sövs har relativt stor risk att inte klara sig. Det beror bland annat på att när hästen opereras måste den ofta ligga på rygg, på ett speciellt bord med vaddering som är formad för att passa en häst. Tarmarna hos en häst är mycket tunga och tar upp 34 % av kroppsvikten, jämfört med hundar där de bara tar upp 13 %. När hästen ligger på rygg pressar tarmarna ihop lungorna, vilket leder till komplikationer.

En vanlig komplikation vid anestesi av hästar när de ligger på rygg är syrebrist i blodet (hypoxemi). Min avhandling handlar om ett nytt sätt att behandla hypoxemi. Metoden, som kallas PiNO (Pulsed inhaled Nitric Oxide, pulsad inhalerad kväveoxid), innebär att man ger en liten puls av gasen kväveoxid i början av varje andetag under anestesin. Kväveoxid-

molekylerna förändrar hur blodet strömmar genom hästens lungor och förbättrar avsevärt syrehalten i hästens blod.

I min forskning delade jag in hästarna i två grupper, de som fick PiNO och en kontrollgrupp. PiNO gör att både friska hästar som genomgår planerade operationer och sjuka hästar som genomgår akuta operationer förbättrar sin syrehalt i blodet under anestesin. Med PiNO får hästarna också bättre återhämtning efteråt, till exempel behöver hästen färre försök att stå upp och är mer stabil när den väl står upp.

PiNO förmedlas för närvarande över hela världen i hopp om att det kan ha en positiv inverkan och göra det möjligt för fler hästar att överleva narkosen och få bättre uppvakning efteråt.

Acknowledgements

The work included in this thesis was carried out at the Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science at the Swedish University of Agricultural Sciences, Uppsala, Sweden. The Swedish-Norwegian Foundation for Equine Research generously supported the project.

There is one person that I would specifically like to thank and without him, this thesis would never have been completed. You have supported me throughout the years, lifted me up when things have been rough, and pushed me so I could finish on time. **Andreas Ramsell**, you are the love of my life and I cannot state how much I appreciate you and how much you mean to me.

I would also like to thank my family; **Inga-Britt**, **Dan**, **Hanna** and **Erik**, even though I do not get to see you as often as I would like, I know you are always there for me.

Thank you to all my friends for believing in me and supported me over the years. No one mentioned, no one forgotten :)

I would like to express my gratitude to my supervisor group:

My main supervisor, **Görel Nyman**, thank you for showing me the world of anaesthesia. Also thanks to my co-supervisors **Tamara Grubb**, **Katarina Nostell** and **Ove Wattle**, for your support.

I would also like to express my gratitude to:

All the staff at the University Equine Clinic for helping me out throughout the years, special thanks to **Cia Eriksson**, **Jennie Ginyard**, **Malin Persson**, **Moa Alkarp**, **Ulrika Holm**, **Yvette Åberg**, **Anna-Stina Persson, Andrea Colombo** and **Lisa Wilteus**. Also thanks to Mälaren Equine Practice for letting me conduct part of my research and collect cases there.

The former "kontorsråttorna", **Emma Wahlqvist** and **Jonna Nilsson**, thank you for your support and all the laughs in the office. Thanks to the GT-team; **Elina Åhlander**, **Elina Henriksson** and also **Emma Wahlqvist** and **Ellen Gottberg** (even though you do not drink GT).

Adam Auckburally, thank you for everything!

Lena Olsen, thank you for all the support and encouragement.

Ylva Sjunnesson, for your support and for helping with what seemed to be endless paperwork during all these years.

Ewa Olszowy, one of the strongest and nicest persons I know. Thank you for all the support you have given me!

Dylan Gorvy, thank you for all the interesting discussions regarding anaesthesia and surgery.

Marianne Grauers, without your support I may not have been able to finish this thesis.

Erica Gumpert Herlofson, **Vanessa Bettembourg**, **Hafiz Bakri**, **Anneli Rydén** and **Eva-Maria Hedin** – old and new colleges that have enrichened my life.

And last but not least, **Dior**, the cute little monster that made sure I did not get stuck by the computer for too many hours at the same time.

I

Effects of ventilation mode and blood flow on arterial oxygenation during pulse-delivered inhaled nitric oxide in anesthetized horses

Adam Auckburally BVSc
Tamara L. Grubb DVM, PhD
Maja Wiklund DVM
Görel Nyman DVM, PhD

Received May 15, 2018.
Accepted July 30, 2018.

From the Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden (Auckburally, Wiklund, Nyman); and the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 (Grubb).

Address correspondence to Dr. Auckburally (Adam.Auckburally@scvetspecialists.co.uk).

OBJECTIVE
To determine the impact of mechanical ventilation (MV) and perfusion conditions on the efficacy of pulse-delivered inhaled nitric oxide (PiNO) in anesthetized horses.

ANIMALS
27 healthy adult horses.

PROCEDURES
Anesthetized horses were allocated into 4 groups: spontaneous breathing (SB) with low (< 70 mm Hg) mean arterial blood pressure (MAP; group SB-L; n = 7), SB with physiologically normal (≥ 70 mm Hg) MAP (group SB-N; 8), MV with low MAP (group MV-L; 6), and MV with physiologically normal MAP (group MV-N; 6). Dobutamine was used to maintain MAP > 70 mm Hg. Data were collected after a 60-minute equilibration period and at 15 and 30 minutes during PiNO administration. Variables included Pa_{O_2}, arterial oxygen saturation and content, oxygen delivery, and physiologic dead space-to-tidal volume ratio. Data were analyzed with Shapiro-Wilk, Mann-Whitney *U*, and Friedman ANOVA tests.

RESULTS
Pa_{O_2}, arterial oxygen saturation, arterial oxygen content, and oxygen delivery increased significantly with PiNO in the SB-L, SB-N, and MV-N groups; were significantly lower in group MV-L than in group MV-N; and were lower in MV-N than in both SB groups during PiNO. Physiologic dead space-to-tidal volume ratio was highest in the MV-L group.

CONCLUSIONS AND CLINICAL RELEVANCE
Pulmonary perfusion impacted PiNO efficacy during MV but not during SB. Use of PiNO failed to increase oxygenation in the MV-L group, likely because of profound ventilation-perfusion mismatching. During SB, PiNO improved oxygenation irrespective of the magnitude of blood flow, but hypoventilation and hypercarbia persisted. Use of PiNO was most effective in horses with adequate perfusion. (*Am J Vet Res* 2019;80:275–283)

ABBREVIATIONS

Ca_{O_2}	Arterial oxygen content
CI	Cardiac index
CO	Cardiac output
D_{O_2}	Oxygen delivery
F_{IO_2}	Fraction of inspired oxygen
HR	Heart rate
MAP	Mean arterial blood pressure
MV	Mechanical ventilation
NO	Nitric oxide
OER	Oxygen extraction ratio
P_{AO_2}	Alveolar partial pressure of oxygen
P_{AO_2} - Pa_{O_2}	Alveolar-arterial difference in partial pressure of oxygen
P_{ETCO_2}	End-tidal partial pressure of carbon dioxide
PiNO	Pulsed-delivered inhaled nitric oxide
Qs/Qt	Pulmonary shunt fraction
RR	Respiratory rate
Sa_{O_2}	Arterial oxygen saturation
SB	Spontaneous breathing
$S\bar{v}_{O_2}$	Mixed-venous oxygen saturation
V_D/V_T	Physiologic dead space-to-tidal volume ratio
$\dot{V}/Q$	Ventilation-perfusion ratio
V_T	Tidal volume

Mortality rates associated with general anesthesia of horses are unacceptably high (approx 0.9% to 1.1% in healthy horses).[1,2] A deficiency in tissue D_{O_2} may be associated with morbidity and fatalities.[3] The D_{O_2}, which is dependent on both perfusion and ventilation, is commonly decreased in anesthetized horses as a direct result of the cardiovascular and respiratory depressant effects of volatile anesthetics and the physical effect of recumbency.[4]

Decreased tissue perfusion, which may be a consequence of reduced CO (a determinant of blood flow), is identified clinically as hypotension (defined as an MAP < 70 mm Hg in horses[5]). In anesthetized horses, hypotension is often treated with an infusion of dobutamine[6] (a nonselective β-adrenoceptor agonist that has positive inotropic effects) to increase ventricular output.[7] Although blood pressure attributable to increased blood flow is increased with dobutamine, an in-

fusion of dobutamine in horses does not ensure improved arterial oxygenation.[8,9]

Hypoventilation, identified clinically by the presence of hypercarbia, is commonly treated by initiating MV. Although this is effective for treating hypercarbia, hypoxemia and Do_2 can be worsened by MV[10] as a result of numerous factors, including a positive intrathoracic pressure–mediated decrease in CO via prevention of efficient venous return[11] and an increase in lung volume that impedes cardiac filling owing to chamber and pericardial compression effects.[12,13]

Administration of PiNO improves arterial oxygenation in SB anesthetized horses.[14–19] This effect does not occur through changes in ventilation or CO; instead, it is a result of redistribution of pulmonary blood from atelectatic to aerated areas of the lungs.[17] Spontaneously breathing horses anesthetized for abdominal exploratory surgery that receive PiNO require dobutamine infusion for maintenance of normotension.[19] Hypercarbia with subsequent respiratory acidosis commonly develops in horses receiving PiNO that do not receive ventilatory support.[16–19] In healthy research horses, PiNO delivered by MV improved oxygenation; however, PiNO was delivered for only 5 minutes.[14] To fully support anesthetized horses, a combination of PiNO, MV, and dobutamine administration may be necessary, but the combined impact of MV and PiNO during clinically relevant durations of anesthesia is unknown.

The objective of the study reported here was to determine the impact of 2 ventilation modes (MV and SB) and 2 perfusion conditions (MAP < or ≥ 70 mm Hg) on PiNO-mediated oxygenation in anesthetized horses. Our hypothesis was that MV and increased blood flow would optimize the effect of PiNO on arterial oxygenation.

Materials and Methods

Animals

Twenty-seven healthy Standardbreds were used in the study. Age ranged from 1 to 25 years, and body weight ranged from 375 to 610 kg. Horses were randomly (computer-generated numbers) assigned to 4 groups: SB with low (< 70 mm Hg) MAP (group SB-L; n = 7), SB with physiologically normal (≥ 70 mm Hg) MAP (group SB-N; 8), MV with low MAP (group MV-L; 6), and MV with physiologically normal MAP (group MV-N; 6). The experiment was approved by the local Ethical Committee on Animal Experiments in Uppsala, Sweden (C 201/14).

Anesthesia

Food, but not water, was withheld for 12 hours prior to anesthesia. Acepromazine (0.03 mg•kg^{-1}) was administered IM approximately 30 minutes before induction of anesthesia. The subcutaneous tissues over the jugular veins were infiltrated with lidocaine. The left jugular vein was catheterized with a 14-gauge catheter, and the right jugular vein was catheterized with two 8.5F sheath introducers (one positioned distally in the vein and the other positioned proximally in the vein).

Additional premedication consisted of xylazine hydrochloride (1.1 mg•kg^{-1}, IV) and butorphanol tartrate (0.025 mg•kg^{-1}, IV). When sedation was apparent, anesthesia was induced by rapid IV administration of a bolus of ketamine hydrochloride (2.2 mg•kg^{-1}) and diazepam (0.05 mg•kg^{-1}). After each horse was recumbent, the trachea was intubated with a cuffed endotracheal tube (internal diameter, 26 mm). The horse was hoisted onto a padded surgical table, positioned in dorsal recumbency, and connected to a large-animal breathing system and anesthetic machine.[a] Anesthesia was maintained with isoflurane vaporized in oxygen. The F_{IO_2} was maintained at approximately 0.9 by the computer-controlled F_{IO_2} technology of the anesthetic machine. Ventilatory mode was SB or continuous mandatory MV. The MAP was allowed to decrease to < 70 mm Hg or was maintained at ≥ 70 mm Hg by administration of a variable rate infusion of dobutamine.

Instrumentation

After a horse was positioned on the surgical table, an 18-gauge catheter was inserted in the transverse facial or mandibular branch of the facial artery for measurement of blood pressure and collection of arterial blood samples for analysis. The catheter was connected via a 3-way stopcock to a pressure-monitoring line and transducer, which was connected to the multiparameter monitor integrated in the anesthetic machine. A 7.5F Swan-Ganz catheter was inserted via the distal introducer in the right jugular vein and guided into the pulmonary artery by use of pressure guidance from another multiparameter monitor.[b] This catheter was used to measure mean pulmonary arterial pressure as well as CO (determined by thermodilution) and to enable collection of mixed-venous blood samples. By use of a similar technique, a 7.5F pigtail multiple-hole catheter was inserted through the proximal introducer in the right jugular vein and guided into the right atrium. This catheter was used to measure right atrial pressure and to inject ice-cold saline (0.9% NaCl) solution for CO measurement. Both catheters were secured by use of the Luer-lock adaptor on the introducers. The ECG was monitored during placement of the catheters. All pressure transducers were calibrated to zero at the level of the shoulder joint.

Hemodynamic analysis

Thermodilution was used to measure CO. The Swan-Ganz catheter was connected to the multiparameter monitor,[b] and a 20-mL bolus of ice-cold (0°C) saline solution was manually injected through the pigtail catheter. A minimum of 3 boluses were injected, and the mean value was calculated and recorded.

Systolic arterial blood pressure, diastolic arterial blood pressure, and MAP were recorded from the arterial catheter; HR, RR, minute volume, peak inspired pressure, P_{ETCO_2}, end-tidal isoflurane concentration, and F_{IO_2} were recorded from the calibrated multiparameter monitor[a] integrated with the anesthetic machine.

Arterial and mixed-venous blood samples were simultaneously aspirated over a period of 3 breaths from the arterial and pulmonary artery catheters, respectively. Samples were analyzed immediately (arterial pH, mixed-venous pH, Pa_{O_2}, mixed-venous partial pressure of oxygen, Sa_{O_2}, $S\bar{v}_{O_2}$, Pa_{CO_2}, mixed-venous partial pressure of carbon dioxide, and hemoglobin concentration) by use of a standard electrode technique.[c]

Calculated variables

The D_{O_2} was calculated as Ca_{O_2} X CO. Venous admixture was calculated by use of the Berggren shunt formula[20] as follows:

$$Qs/Qt = (C\acute{c}_{O_2} - Ca_{O_2})/(C\acute{c}_{O_2} - C\bar{v}_{O_2})$$

where $C\acute{c}_{O_2}$ is the pulmonary end-capillary oxygen content and $C\bar{v}_{O_2}$ is the mixed-venous oxygen content. The CI was calculated as CO/body weight. Minute ventilation was calculated as RR X V_T. Values for V_D/V_T were calculated as $(Pa_{CO_2} - P_{ETCO_2})/Pa_{CO_2}$. The P_{AO_2} was calculated by use of the alveolar gas equation[21] as follows:

$$P_{AO_2} = (F_{IO_2} \bullet [\text{barometric pressure} - \text{water vapor pressure}]) - (P_{ACO_2}/\text{respiratory quotient})$$

where P_{ACO_2} is the alveolar partial pressure of carbon dioxide (the value for Pa_{CO_2} was used for P_{ACO_2}) and the respiratory quotient is 0.8. The P_{AO_2} - Pa_{O_2} was calculated as P_{AO_2} minus Pa_{O_2}. The OER was calculated as the arterial–mixed-venous of oxygen content divided by Ca_{O_2}.

PiNO

Anesthetized horses were allowed a 60-minute equilibration period. At the end of that period (time 0;

Table 1—Mean ± SD values for blood flow variables at various time points for anesthetized horses with SB with low (< 70 mm Hg) MAP (group SB-L; n = 7), SB with physiologically normal (≥ 70 mm Hg) MAP (group SB-N; 8), MV with low MAP (group MV-L; 6), and MV with physiologically normal MAP (group MV-N; 6).

Variable	Group	Baseline	15 minutes	30 minutes	*P* value*
HR (beats•min^{-1})	SB-L	38 ± 7	36 ± 5	35 ± 6†	NS
	SB-N	48 ± 15	45 ± 14	42 ± 12	NS
	MV-L	35 ± 3†	33 ± 3†	32 ± 3†‡	0.002
	MV-N	45 ± 10	49 ± 11	50 ± 12	NS
MAP (mm Hg)	SB-L	53 ± 10†	66 ± 7†	68 ± 6†‡	0.004
	SB-N	72 ± 5	80 ± 7	80 ± 8	0.018
	MV-L	53 ± 9†	55 ± 8†§	56 ± 9†§	NS
	MV-N	77 ± 11	82 ± 4	81 ± 3	NS
MPAP (mm Hg)	SB-L	13 ± 4	13 ± 3	14 ± 3	NS
	SB-N	16 ± 4	15 ± 4	14 ± 4	NS
	MV-L	15 ± 3	14 ± 3	13 ± 3	NS
	MV-N	12 ± 3	12 ± 3	11 ± 3	NS
CI (mL•kg^{-1}•min^{-1})	SB-L	45 ± 9†	50 ± 10†	50 ± 11†	0.027
	SB-N	84 ± 30	81 ± 23	79 ± 22	NS
	MV-L	45 ± 6†	43 ± 7†	46 ± 5†	NS
	MV-N	72 ± 10	80 ± 8	81 ± 10	NS
Qs/Qt	SB-L	0.35 ± 0.10	0.29 ± 0.12	0.28 ± 0.12‡	< 0.001
	SB-N	0.38 ± 0.10	0.28 ± 0.09‡	0.28 ± 0.11	0.005
	MV-L	0.46 ± 0.07	0.37 ± 0.09	0.36 ± 0.09‡	< 0.001
	MV-N	0.39 ± 0.10	0.36 ± 0.10	0.32 ± 0.07‡	< 0.001
Hemoglobin (g•L^{-1})	SB-L	101 ± 6	96 ± 40†	97 ± 4†	0.004
	SB-N	113 ± 17	110 ± 17	108 ± 17	NS
	MV-L	105 ± 4†	105 ± 3†§	106 ± 3†§	NS
	MV-N	117 ± 9	122 ± 12	123 ± 9	NS
Lactate (mmol•L^{-1})	SB-L‖	0.7 ± 0.3	0.7 ± 0.2	0.8 ± 0.2	NS
	SB-N⁋	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	NS
	MV-L	0.9 ± 0.3	1.0 ± 0.3†	1.1 ± 0.4	NS
	MV-N	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.2	NS

Horses were anesthetized and allowed a 60-minute equilibration period. At the end of that period (time 0; baseline), PiNO was delivered for 30 minutes. Variables were determined at baseline and 15 and 30 minutes after the start of PiNO.

*Within a group, values differ significantly ($P < 0.05$; Friedman ANOVA with the Dunn multiple comparison test) among time points. †Within a time point within a ventilation mode (SB or MV), value differs significantly ($P < 0.05$) from the value for SB-N or MV-N. ‡Within a group, value differs significantly ($P < 0.05$) from the baseline value. §Within a time point, MV value differs significantly ($P < 0.05$) from the corresponding value for SB-L or SB-N. ‖Represents results for only 3 horses. ⁋Represents results for only 4 horses.

MPAP = Mean pulmonary arterial pressure. NS = Not significant ($P \geq 0.05$).

baseline), PiNO was delivered for 30 minutes. Nitric oxide was delivered via the endotracheal tube by use of a specially designed device.[d] The device delivered a pulse of NO at the beginning of inspiration, and the flow sensor was triggered by the negative pressure generated by SB or the positive pressure generated by the ventilator during MV. The device was connected to a cylinder containing 2,000 ppm of NO in nitrogen.[e] The NO was delivered during the first 45% of inspiration (controlled by the device). The most effective timing and duration of delivery have been determined in previous studies.[14,16,17]

Data collection

Arterial and mixed-venous blood gas samples were collected at 0, 15, and 30 minutes (ie, before and 15 and 30 minutes after the start of PiNO) and analyzed to determine Pa_{O_2}, Sa_{O_2}, mixed-venous partial pressure of oxygen, Sv_{O_2}, Pa_{CO_2}, pH, hemoglobin concentration, and venous lactate concentration. At the same time points, HR, RR, systolic and diastolic arterial blood pressures, MAP, mean pulmonary arterial pressure, right atrial pressure, P_{ETCO_2}, end-tidal isoflurane concentration, F_{IO_2}, oxygen saturation measured by use of pulse oximetry, CO, and V_T were recorded, and CI, P_{AO_2}, Ca_{O_2}, mixed-venous oxygen content, D_{O_2}, Qs/Qt, minute ventilation, V_D/V_T, and OER were calculated. At the end of the procedure, horses were moved to a padded recovery stall and allowed to recover from anesthesia or were euthanized (IV administration of a barbiturate overdose), as determined by requirements of concurrent projects.

Statistical analysis

Raw data were entered into a spreadsheet program[f] for processing. For all statistical calculations, a statistical package[g] was used. Data were tested for a Gaussian distribution by use of the Shapiro-Wilk normality test. Mann-Whitney tests were used to compare differences between SB and MV in hypotensive and normotensive horses, and a Friedman ANOVA and Dunn multiple comparisons post hoc test were used to compare differences over time within each group. Differences were considered significant at $P < 0.05$. Data were reported as mean ± SD.

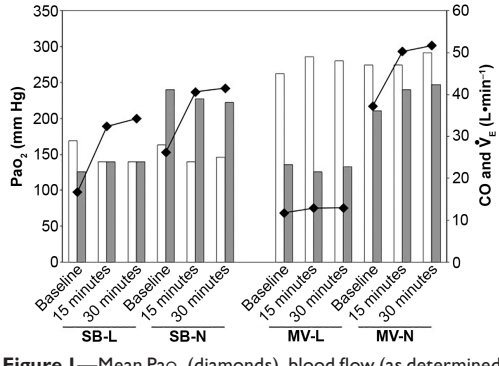


Figure 1—Mean Pa_{O_2} (diamonds), blood flow (as determined by measurement of CO; gray bars), and minute ventilation (V_E; white bars) for 4 groups of anesthetized horses: SB with low (< 70 mm Hg) MAP (group SB-L; n = 7); SB with physiologically normal (≥ 70 mm Hg) MAP (group SB-N; 8), MV with low MAP (group MV-L; 6), and MV with physiologically normal MAP (group MV-N; 6). Horses were anesthetized and allowed a 60-minute equilibration period. At the end of that period (time 0; baseline), PiNO was delivered for 30 minutes. Variables were determined at baseline and 15 and 30 minutes after the start of PiNO.

Results

Animals

Age and body weight did not differ significantly among the groups.

Blood flow

Heart rate was higher in horses with physiologically normal MAP than in horses with low MAP at all time points for MV horses and at 30 minutes for SB horses **(Table 1)**. The MAP was significantly higher in horses with physiologically normal MAP than in horses with

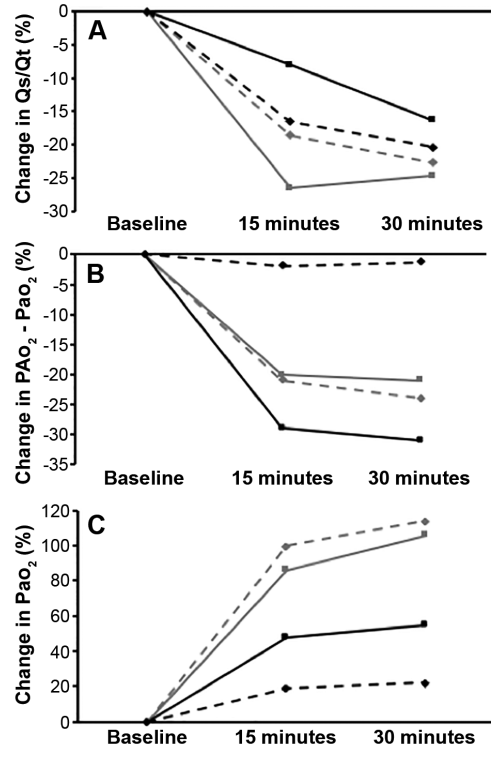


Figure 2—Percentage change in Qs/Qt (A), P_{AO_2} - Pa_{O_2} (B), and Pa_{O_2} (C) in anesthetized horses before (baseline) and 15 and 30 minutes after the start of PiNO for 7 horses in group SB-L (gray diamonds and dashed line), 8 horses in group SB-N (gray squares and solid line), 6 horses in group MV-L (black diamonds and dashed line), and 6 horses in group MV-N (black squares and solid line). ***See*** Figure 1 for remainder of key.

Table 2—Mean ± SD values for ventilation variables at various time points for 4 groups of anesthetized horses (7 horses in group SB-L, 8 horses in group SB-N, 6 horses in group MV-L, and 6 horses in group MV-N).

Variable	Group	Baseline	15 minutes	30 minutes	*P* value*
Minute ventilation ($L \cdot min^{-1}$)	SB-L	29.5 ± 12.5	24.1 ± 8.6	23.6 ± 2.6	NS
	SB-N	28.5 ± 10.5	24.3 ± 14.1	25.5 ± 9.1	NS
	MV-L	44.8 ± 4.7§	48.9 ± 5.8§	47.6 ± 4.7§	NS
	MV-N	47.2 ± 11.7§	47.1 ± 7.7§	49.6 ± 10.1§	NS
RR ($breaths \cdot min^{-1}$)	SB-L	4.9 ± 2.0	3.6 ± 1.5	3.6 ± 1.3	NS
	SB-N	4.2 ± 2.1	3.6 ± 1.7	3.3 ± 1.6	NS
	MV-L	7.7 ± 0.8§	8.0 ± 0.6§	8.0 ± 0.6§	NS
	MV-N	8.2 ± 1.0§	7.8 ± 0.4§	8.0 ± 0.6§	NS
PIP (cm H_2O)	SB-L	NA	NA	NA	NA
	SB-N	NA	NA	NA	NA
	MV-L	27.2 ± 3.4†	26.7 ± 2.2	27.7 ± 3.9	NS
	MV-N	20.8 ± 3.8	23.0 ± 3.6‡	23.3 ± 3.6‡	0.008
$Paco_2$ (mm Hg)	SB-L	63.0 ± 7.5	69.8 ± 11.3‡	69.0 ± 8.3‡	0.001
	SB-N	76.5 ± 18	74.3 ± 10.5	76.5 ± 13.5	NS
	MV-L	63.0 ± 7.5	55.5 ± 4.5§	55.5 ± 3.8§	NS
	MV-N	60.0 ± 9.8	60.8 ± 11.3§	58.5 ± 8.3§	NS
V_D/V_T	SB-L	0.29 ± 0.07	0.25 ± 0.10	0.24 ± 0.13	NS
	SB-N	0.30 ± 0.06	0.24 ± 0.12	0.22 ± 0.10	NS
	MV-L	0.29 ± 0.08	0.28 ± 0.08†	0.29 ± 0.07	NS
	MV-N	0.22 ± 0.04§	0.18 ± 0.04	0.18 ± 0.06	NS

NA = Not applicable. PIP = Peak inspiratory pressure.
See Table 1 for remainder of key.

low MAP for both MV groups and both SB groups and significantly higher in the SB-L group than the MV-L group at 15 and 30 minutes. There were no significant changes in mean pulmonary arterial pressure. The CO (measured as CI) was significantly higher in the SB-N group and MV-N group than the SB-L group and MV-L group, respectively, at all time points, but there were no differences between the MV-N and SB-N groups or between the MV-L and SB-L groups **(Figure 1)**. The Qs/Qt decreased during PiNO in all groups, but there were no differences among groups **(Figure 2)**.

Minor clinically unimportant changes were evident at various time points. Hemoglobin concentration was higher in horses with physiologically normal MAP than in horses with low MAP. Lactate concentration was higher in the MV-L group than the MV-N group, but no changes in lactate concentration were detected between the SB groups.

Ventilation

Minute ventilation and RR were higher in the MV groups than in the SB groups. However, there were no differences between the SB-L and SB-N groups or between the MV-L and MV-N groups and no change over time in the MV or SB groups **(Table 2)**. The $Paco_2$ was lower in the MV groups than the SB groups at 15 and 30 minutes. There were no differences in $Paco_2$ between the SB-N and SB-L groups or between the MV-N and MV-L groups, and there were no changes over time except for the SB-L group, in which $Paco_2$ was higher at 15 and 30 minutes than at baseline. The V_D/V_T was higher in the MV-L group than the MV-N group at 15 and 30 minutes.

Oxygenation

The P_{AO_2} - Pao_2 decreased during PiNO administration in all groups, except for the MV-L group (**Table 3**; Figure 2). The Pao_2, Sao_2, and Cao_2 increased significantly during PiNO in the SB-L, SB-N, and MV-N groups, compared with respective baseline values (Figure 1). There were no differences between the SB groups, but Pao_2, Sao_2, and Cao_2 were significantly lower in the MV-L group than the MV-N group at all time points. The Sao_2 was significantly lower in the MV-L group than the SB-L group at all time points. The Pao_2 changed over time. The Do_2 increased significantly during PiNO in all groups, except for the MV-L group. The Do_2 was significantly higher at all time points for the SB-N group and MV-N group than for the SB-L group and MV-L group, respectively **(Figure 3)**. There were no differences in $S\bar{v}o_2$ between SB groups. The $S\bar{v}o_2$ was significantly lower in the MV-L group than the MV-N group at all time points. The OER was significantly higher in the MV-L group than the MV-N group, but there were no other differences among groups.

Discussion

Results of the present study supported the hypothesis that MV in combination with adequate blood flow promoted optimal gas exchange during PiNO by improving arterial oxygenation and managing hypercarbia. During MV, the degree of PiNO-mediat-

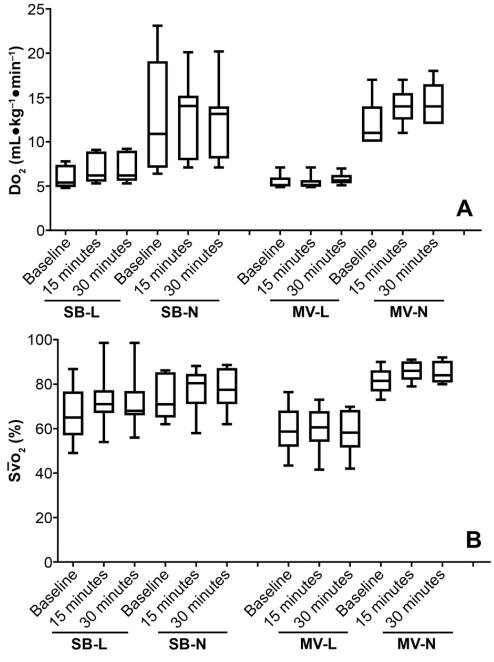


Figure 3—Box-and-whisker plots of D_{O_2} (A) and $S\bar{v}O_2$ (B) at baseline and 15 and 30 minutes after the start of PiNO for 7 horses in group SB-L, 8 horses in group SB-N, 6 horses in group MV-L, and 6 horses in group MV-N. The lower and upper boundaries of each box represent the 25th and 75th percentiles of the data, respectively; the horizontal line in each box represents the median; and the whiskers represent the minimum and maximum values. **See** Figure 1 for remainder of key.

ed improvement in oxygenation, D_{O_2}, and Qs/Qt was related to an increase in blood flow as measured by an increase in CI. It was obvious that MV had a negative impact on the degree of improvement in oxygenation in horses with low blood flow. Interestingly, during SB, arterial oxygenation increased with PiNO regardless of the magnitude of blood flow. However, hypoventilation commonly developed in SB horses, which led to hypercarbia.

The lack of impact of PiNO on oxygenation for the MV-L group was unexpected because PiNO reportedly has a positive effect on oxygenation during short-term use.[14] On the basis of the increase in V_D/V_T for the MV-L group, compared with results for the MV-N group, we speculate that this lack of effect was attributable to profound $\dot{V}/\dot{Q}$ mismatching and a lack of perfusion to some ventilated lung regions. There is optimal gas exchange when ventilation and perfusion are equally matched, but MV can have deleterious effects on $\dot{V}/\dot{Q}$ matching in a number of ways. First, CO is reduced during MV because of a reduction in cardiac filling pressure.[11] If D_{O_2} decreases below tissue oxygen demand, then $S\bar{v}O_2$ decreases and Pa_{O_2} is reduced.[22] In the present study, OER was higher and $S\bar{v}O_2$ was lower in the MV-L group. Thus, a larger fraction of oxygen was extracted from the blood when capillary beds were perfused. This indicated that when hemoglobin saturation with oxygen was adequate, blood flow was proportionately more important than oxygen content for tissue oxygen supply, which agrees with results of other studies.[23,24] In those studies,[23,24] oxygen uptake did not differ between SB and MV, but D_{O_2} decreased during controlled ventilation, which is a reflection of lower blood flow. Second, the $\dot{V}/\dot{Q}$ matching was disturbed because the areas of lung normally ventilated became overventilated. This situation, compounded by the decrease in CO, increases alveolar dead space by reducing perfusion of the overventilated alveoli.[12,22] More importantly, during periods of positive intrathoracic pressure, blood is forced from nondependent areas of the lungs (ie, those that are normally ventilated) and driven toward dependent areas of the lungs, a large proportion of which are atelectatic.[25,26] Conventional MV will not recruit dependent atelectatic areas of the lungs because the alveolar pressure produced is insufficient. Additionally, the compressive effect on the pulmonary capillaries increases pulmonary vascular resistance and thereby further reduces perfusion. Overall, these effects will worsen $\dot{V}/\dot{Q}$ mismatching, increase Qs/Qt and V_D/V_T, and reduce Pa_{O_2}. These negative effects of MV were not detected in horses with sufficient CO during PiNO, presumably because of concomitant improvements of perfusion and vasodilation in well-ventilated pulmonary regions. Analysis of results of the study reported here suggested that the deterioration in $\dot{C}O$ induced by MV affected blood pressure as well as $\dot{V}/\dot{Q}$ matching and gas exchange in the lungs.

In the present study, SB did not have a negative impact on oxygenation, and relative increases in oxygenation and a reduction in Qs/Qt were greatest in these horses. The respiratory mechanics during SB favors the distribution of gas in the lungs and improves ventilation of dependent juxtadiaphragmatic lung regions.[27,28] The negative intrathoracic pressure during inspiration also promotes alveolar recruitment and counteracts cyclic alveolar collapse in dependent lung regions.[28] Furthermore, arterial blood pressure is better preserved during SB through better cardiac preload and higher systemic vascular resistance attributable to acidemia associated with hypoventilation and subsequent hypercarbia.[29] These combined effects likely contributed to improved $\dot{V}/\dot{Q}$ matching in the study reported here. The similar effects of PiNO on arterial oxygenation for both the SB-L and SB-N groups suggested that the mode of ventilation played an integral role in the distribution of pulmonary perfusion, rather than being reliant on total blood flow.

Increased oxygenation with increased blood flow is attributable to an increase in pulmonary perfusion with subsequent uptake of oxygen from the lungs.[30] Infusions of dobutamine increase CO and systemic and pulmonary pressure in anesthetized horses.[31–33] However, dobutamine can also have a negative impact on oxygenation. In healthy awake humans,

Table 3—Mean ± SD values for oxygenation variables at various time points for 4 groups of anesthetized horses (7 horses in group SB-L, 8 horses in group SB-N, 6 horses in group MV-L, and 6 horses in group MV-N).

Variable	Group	Baseline	15 minutes	30 minutes	*P* value*
F_{IO_2}	SB-L	0.90 ± 0.07	0.92 ± 0.03	0.91 ± 0.03	NS
	SB-N	0.90 ± 0.02	0.90 ± 0.03	0.90 ± 0.02	NS
	MV-L	0.85 ± 0.05§	0.84 ± 0.05§	0.85 ± 0.05§	NS
	MV-N	0.90 ± 0.01	0.88 ± 0.02	0.88 ± 0.02	NS
P_{AO_2} - Pa_{O_2} (mm Hg)	SB-L	495.0 ± 42.8	390.8 ± 69.8‡	377.3 ± 60.8‡	0.003
	SB-N	402.0 ± 129.8	318.8 ± 112.5	306.0 ± 99.8‡	0.038
	MV-L	474.0 ± 45.8	465.0 ± 48.8†	466.5 ± 45.8†§	NS
	MV-N	349.5 ± 142.5	264.0 ± 139.5	253.5 ± 133.5‡	0.006
Pa_{O_2} (mm Hg)	SB-L	97.5 ± 28.5†	189 ± 57.8‡	199.5 ± 59.3‡	< 0.001
	SB-N	153.0 ± 120.8	237.0 ± 122.3‡	250.5 ± 97.5‡	< 0.001
	MV-L	69.0 ± 55.5†	75.8 ± 46.5†§	75.8 ± 40.5†§	NS
	MV-N	217.5 ± 145.5	293.3 ± 151.5‡	301.5 ± 142.5‡	0.002
Sa_{O_2} (%)	SB-L	95.7 ± 2.2	98.6 ± 1.3	98.8 ± 1.2‡	< 0.001
	SB-N	94.7 ± 2.7	97.9 ± 1.1‡	97.8 ± 1.7‡	< 0.001
	MV-L	85.0 ± 7.4†§	89.3 ± 6.2†§	89.8 ± 7.1†§	NS
	MV-N	95.5 ± 1.2	96.3 ± 0.7	96.7 ± 0.4‡	< 0.001
Ca_{O_2} (mL•L^{-1})	SB-L	130.6 ± 6.3	136.9 ± 4.8‡	137.5 ± 4.9‡	< 0.001
	SB-N	146.3 ± 24.5	153.2 ± 21.4‡	153.5 ± 20.0‡	< 0.001
	MV-L	120.6 ± 14.9†	126.8 ± 15.4†	127.0 ± 16.7†	NS
	MV-N	169.4 ± 15.6	176.2 ± 16.3‡	178.8 ± 20.9‡	0.002
D_{O_2} (mL•kg^{-1}•min^{-1})	SB-L	6.0 ± 1.2†	6.9 ± 1.5†‡	7.0 ± 1.6†‡	< 0.001
	SB-N	12.8 ± 6.4	12.6 ± 4.9‡	12.3 ± 4.3	NS
	MV-L	5.5 ± 0.8†	5.2 ± 1.0†	5.6 ± 1.0†	NS
	MV-N	12.2 ± 2.8	14.5 ± 2.1	15.1 ± 3.9‡	0.008
$P\bar{v}_{O_2}$ (mm Hg)	SB-L	39.8 ± 7.5	41.3 ± 5.3	42.8 ± 5.3†	NS
	SB-N	51.8 ± 12.0	52.5 ± 9.8	54.0 ± 12.8	NS
	MV-L	31.5 ± 3.8†§	31.5 ± 3.8†§	30.8 ± 3.8†§	NS
	MV-N	51.0 ± 9.0	57.0 ± 7.5‡	57.0 ± 10.5	0.012
$S\bar{v}_{O_2}$ (%)	SB-L	67 ± 12	73 ± 14‡	73 ± 13	0.004
	SB-N	74 ± 10	77 ± 11	78 ± 9	NS
	MV-L	60 ± 11†	60 ± 11†	58 ± 10†	NS
	MV-N	82 ± 6	86 ± 4	85 ± 5	0.029
Ca_{O_2} - $C\bar{v}_{O_2}$ (mL•L^{-1})	SB-L	40.3 ± 15.2	38.2 ± 16.7†	38.8 ± 16.5†	0.004
	SB-N	32.4 ± 9.8	35.5 ± 13.6	34.5 ± 12.8	NS
	MV-L	36.4 ± 3.6†	41.9 ± 4.9†§	44.7 ± 6.5†§	NS
	MV-N	28.3 ± 6.1	25.2 ± 4.2	27.3 ± 4.3	NS
OER (%)	SB-L	31 ± 12	32 ± 8	29 ± 13	NS
	SB-N	23 ± 9	24 ± 11	23 ± 10	NS
	MV-L	31 ± 7†	34 ± 7†	36 ± 7†	NS
	MV-N	17 ± 5	15 ± 4	16 ± 4	NS

Ca_{O_2} - $C\bar{v}_{O_2}$ = Arterial–mixed-venous difference in oxygen content. $P\bar{v}_{O_2}$ = Mixed-venous partial pressure of oxygen.
See Table 1 for remainder of key.

dobutamine infusion results in the recruitment of intrapulmonary shunt vessels owing to increasing CO, with a subsequent increase in Qs/Qt and P_{AO_2} - Pa_{O_2}.[34] Furthermore, in critically ill mechanically ventilated humans, dobutamine adversely affects Pa_{O_2} by increasing shunting.[35] However, intrapulmonary shunt vessels have not been described in the lungs of horses, and there is unlikely to be recruitment in situations of increased CO; consequently, there is little impact on arterial oxygenation.[36] In the present study, Qs/Qt and P_{AO_2} - Pa_{O_2} decreased in both MV and SB horses, likely because of the simultaneous administration of dobutamine during PiNO. Dobutamine administered alone to humans with pulmonary hypertension worsens shunting, but administration of a combination of NO and dobutamine has complementary effects on the pulmonary circulation.[37] In the present study, selective pulmonary vasodilation attributable to NO was augmented by an increase in CO, with the deleterious effects of dobutamine on gas exchange being offset by the NO.[37,38] The same mechanism likely contributed to the increase in Pa_{O_2} with PiNO and concurrent dobutamine administration. However, it is possible that this could have been an effect of dobutamine alone because a control group that received dobutamine without PiNO was not included. The greatest decrease in P_{AO_2} - Pa_{O_2} was in the MV-N group because the nondependent por-

tion of the lungs was better ventilated in combination with an increase in pulmonary perfusion as a result of dobutamine infusion and PiNO.

Other results of the present study can be explained by the interventions intended to support ventilation and perfusion. The use of MV led to significant differences in respiratory variables (RR and $Paco_2$), and the administration of dobutamine resulted in significant differences in some cardiovascular variables (HR, MAP, hemoglobin concentration, and CI).

The present study revealed that the magnitude of blood flow had an impact on the efficacy of PiNO during MV. The use of PiNO failed to increase indices of oxygenation when blood flow was low, likely because of decreased perfusion of ventilated lung regions. Administration of dobutamine during PiNO resulted in dramatic improvements in arterial oxygenation and Do_2 during MV. In SB horses, PiNO improved arterial oxygenation irrespective of the magnitude of blood flow, but hypoventilation and hypercarbia persisted.

Acknowledgments

This manuscript represents a portion of a thesis submitted by Dr. Auckburally to the Swedish University of Agricultural Sciences as partial fulfilment of the requirements for a Doctor of Philosophy degree.

Supported by a grant from the Swedish-Norwegian Foundation for Equine Research. Funding sources were not involved in the study design, data analysis and interpretation, or writing and publication of the manuscript.

The authors declare that there were no conflicts of interest.

Presented in abstract form at the Association of Veterinary Anaesthetists Spring Meeting, Grenada, West Indies, March 2018.

Footnotes

a. Tafonius, Vetronic Services, Devon, England.
b. Datex-Ohmeda AS/3-AM, Datex-Ohmeda, Helsinki, Finland.
c. ABL 90 flex, Radiometer, Brønshøj, Denmark.
d. NOrse, Datex-Ohmeda, Helsinki, Finland.
e. AGA AB, Lidingö, Sweden.
f. Microsoft Excel, version 2010, Microsoft, Redmond, Wash.
g. GraphPad Prism, version 5, GraphPad Software Inc, La Jolla, Calif.

References

1. Johnston GM, Eastment JK, Wood JLN, et al. The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of phases 1 and 2. *Vet Anaesth Analg* 2002;29:159–170.
2. Dugdale AH, Obhrai J, Cripps PJ. Twenty years later: a single-centre, repeat retrospective analysis of equine perioperative mortality and investigation of recovery quality. *Vet Anaesth Analg* 2016;43:171–178.
3. Whitehair KJ, Willits NH. Predictors of arterial oxygen tension in anesthetized horses: 1,610 cases (1992–1994). *J Am Vet Med Assoc* 1999;215:978–981.
4. Hubbell JAE, Muir WW. Oxygenation, oxygen delivery and anaesthesia in the horse. *Equine Vet J* 2015;47:25–35.
5. Schauvliege S, Gasthuys F. Drugs for cardiovascular support in anesthetized horses. *Vet Clin North Am Equine Pract* 2013;29:19–49.
6. de Vries A, Brearly JC, Taylor PM. Effects of dobutamine on cardiac index and arterial blood pressure in isoflurane-anaesthetized horses under clinical conditions. *J Vet Pharmacol Ther* 2009;32:353–358.
7. Swanson CR, Muir WW III, Bednarski RM, et al. Hemodynamic responses in halothane-anesthetized horses given infusions of dopamine or dobutamine. *Am J Vet Res* 1985;46:365–370.
8. Swanson CR, Muir WW III. Dobutamine-induced augmentation of cardiac output does not enhance respiratory gas exchange in anesthetized recumbent healthy horses. *Am J Vet Res* 1986;47:1573–1576.
9. Briganti A, Portela DA, Grasso S, et al. Accuracy of different oxygenation indices in estimating intrapulmonary shunting at increasing infusion rates of dobutamine in horses under general anaesthesia. *Vet J* 2015;204:351–356.
10. Hopster K, Wogatzki A, Geburek F, et al. Effects of positive end-expiratory pressure titration on intestinal oxygenation and perfusion in isoflurane anaesthetised horses. *Equine Vet J* 2017;49:250–256.
11. Cournand A, Motley HL, Werko L, et al. Physiologic studies on the effects of intermittent positive pressure breathing on cardiac output in man. *Am J Physiol* 1948;152:162–174.
12. Tyler DC. Positive end-expiratory pressure: a review. *Crit Care Med* 1983;11:300–308.
13. Shekerdemian L, Bohn D. Cardiovascular effects of mechanical ventilation. *Arch Dis Child* 1999;80:475–480.
14. Heinonen E, Hedenstierna G, Meriläinen P, et al. Pulsed delivery of nitric oxide counteracts hypoxaemia in the anaesthetised horse. *Vet Anaesth Analg* 2001;28:3–11.
15. Heinonen E, Nyman G, Meriläinen P, et al. Effect of different pulses of nitric oxide on venous admixture in the anaesthetized horse. *Br J Anaesth* 2002;88:394–398.
16. Nyman G, Grubb TL, Heinonen E. Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses. *Vet Anaesth Analg* 2012;39:480–487.
17. Grubb T, Frendin JHM, Edner A, et al. The effects of pulse-delivered inhaled nitric oxide on arterial oxygenation, ventilation-perfusion distribution and plasma endothelin-1 concentration in laterally recumbent isoflurane-anaesthetized horses. *Vet Anaesth Analg* 2013;40:e19–e30.
18. Grubb TL, Lord PF, Berger M, et al. Effects of pulse-delivered inhaled nitric oxide administration on pulmonary perfusion and arterial oxygenation in dorsally recumbent isoflurane-anesthetized horses. *Am J Vet Res* 2014;75:949–955.
19. Wiklund M, Granswed I, Nyman G. Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery. *Vet Anaesth Analg* 2017;44:1139–1148.
20. Berggren SM. Oxygen deficit of arterial blood by non ventilating parts of the lung. *Acta Physiol Scand* 1942;4(suppl II):7–92.
21. West JB. Ventilation-perfusion relationships. In: *Respiratory physiology: the essentials*. 9th ed. Philadelphia: Lippincott Williams & Wilkins, 2012;56–76.
22. Day TK, Gaynor JS, Muir WW III, et al. Blood gas values during intermittent positive pressure ventilation and spontaneous ventilation in 160 anesthetized horses positioned in lateral or dorsal recumbency. *Vet Surg* 1995;24:266–276.
23. Hodgson DS, Steffey EP, Grandy JL, et al. Effects of spontaneous, assisted, and controlled ventilatory modes in halothane-anesthetized geldings. *Am J Vet Res* 1986;47:992–996.
24. Edner A, Nyman G, Essén-Gustavsson B. The effects of spontaneous and mechanical ventilation on central cardiovascular function and peripheral perfusion during isoflurane anaesthesia in horses. *Vet Anaesth Analg* 2005;32:136–146.
25. Nyman G, Hedenstierna G. Ventilation-perfusion relationships in the anaesthetised horse. *Equine Vet J* 1989;21:274–281.
26. Cairo JM. Effects of positive-pressure ventilation on the pulmonary system. In: Pilbeam SP, ed. *Pilbeam's mechanical ventilation—physiological concepts and clinical applications*. 5th ed. St Louis: Elsevier, 2012;327–352.
27. Neumann P, Wrigge H, Zinserling J, et al. Spontaneous breathing affects the spatial ventilation and perfusion distribution during mechanical ventilatory support. *Crit Care Med* 2005;33:1090–1095.
28. Wrigge H, Zinserling J, Neumann P, et al. Spontaneous breathing with airway pressure release ventilation favors

ventilation in dependent lung regions and counters cyclic alveolar collapse in oleic-acid-induced lung injury: a randomized controlled computed tomography trial. *Crit Care* 2005;9:R780–R789.
29. Mosing M, Senior JM. Maintenance of equine anaesthesia over the last 50 years: controlled inhalation of volatile anaesthetics and pulmonary ventilation. *Equine Vet J* 2018;50:282–291.
30. Kelman GR, Nunn JF, Prys-Roberts C, et al. The influence of cardiac output on arterial oxygenation: a theoretical study. *Br J Anaesth* 1967;39:450–458.
31. Gasthuys F, de Moor A, Parmentier D. Influence of dopamine and dobutamine on the cardiovascular depression during a standard halothane anaesthesia in dorsally recumbent ventilated ponies. *Zentralbl Veterinarmed A* 1991;38:494–500.
32. Mizuno Y, Aida H, Fujinaga T. Effects of dobutamine infusion in dorsally recumbent isoflurane-anesthetized horses. *J Equine Sci* 1994;5:87–94.
33. Young LE, Blissitt KJ, Clutton RE, et al. Temporal effects of an infusion of dobutamine hydrochloride in horses anesthetized with halothane. *Am J Vet Res* 1998;59:1027–1032.
34. Bryan TL, van Diepen S, Bhutani M, et al. The effects of dobutamine and dopamine on intrapulmonary shunt and gas exchange in healthy humans. *J Appl Physiol* 2012;113:541–548.
35. Rennotte MT, Reynaert M, Clerbaux T, et al. Effects of two inotropic drugs, dopamine and dobutamine, on pulmonary gas exchange in artificially ventilated patients. *Intensive Care Med* 1989;15:160–165.
36. Manohar M, Goetz TE. Intrapulmonary arteriovenous shunts of > 15μm in diameter probably do not contribute to arterial hypoxaemia in maximally exercising Thoroughbred horses. *J Appl Physiol* 2005;99:224–229.
37. Vizza CD, Della Rocca G, Di Roma A, et al. Acute hemodynamic effects of inhaled nitric oxide, dobutamine and a combination of the two in patients with mild to moderate secondary pulmonary hypertension. *Crit Care* 2001;5:355–361.
38. Bradford KK, Bhaskar D, Pearl RG. Combination therapy with inhaled nitric oxide and intravenous dobutamine during pulmonary hypertension in the rabbit. *J Cardiovasc Pharmacol* 2000;36:146–151.

II

RESEARCH PAPER

Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery

Maja Wiklund, Izabella Granswed & Görel Nyman
Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Husbandry, Swedish University of Agricultural Sciences, Uppsala, Sweden

Correspondence: Maja Wiklund, Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Husbandry, Swedish University of Agricultural Sciences, Box 7054, SE 750 07 Uppsala, Sweden. E-mail: maja.wiklund@uds.slu.se.

Abstract

Objective To evaluate the effect of pulsed inhaled nitric oxide (INO) on arterial oxygenation in horses during abdominal surgery.

Study design Prospective, randomized, clinical trial.

Animals Thirty horses that underwent abdominal surgery at the University Animal Hospital in Uppsala, Sweden.

Methods Anaesthesia was induced according to a standard protocol – romifidine, butorphanol, diazepam and ketamine and maintained with isoflurane in oxygen. Fifteen horses were administered pulsed INO and 15 served as controls. After baseline data collection, pulsed INO delivery commenced. Arterial and venous blood were collected and analysed. Cardiorespiratory parameters were measured, and oxygen content and F-shunt were calculated.

Results Arterial oxygen tension (PaO_2) and arterial oxygen saturation (SaO_2) increased from 10.9 ± 5.7 kPa (82 ± 43 mmHg) and $93 \pm 6\%$ to 17.3 ± 6.9 kPa (134 ± 52 mmHg) ($p < 0.0001$) and $98 \pm 2\%$ ($p < 0.0001$), respectively, in horses administered pulsed INO. In the control group, PaO_2 and SaO_2 decreased from 13.9 ± 9.1 kPa (104 ± 68 mmHg) and $93 \pm 7\%$ to 12.1 ± 8.6 kPa (91 ± 65 mmHg) ($p = 0.0413$) and $91 \pm 8\%$ ($p = 0.0256$), respectively. At the end of anaesthesia, the oxygen content was significantly higher in horses administered pulsed INO compared to controls ($p = 0.0126$). The calculated F-shunt decreased from $39 \pm 10\%$ to $27 \pm 6\%$ ($p < 0.0001$) in horses administered pulsed INO, and remained unchanged in controls, $40 \pm 12\%$ to $44 \pm 12\%$. Blood lactate concentration decreased ($-17 \pm 21\%$) in horses administered pulsed INO ($p = 0.0119$), whereas no difference was measured in controls ($2 \pm 31\%$).

Conclusions and clinical relevance The present study showed that it is possible to effectively reduce the F-shunt and improve arterial oxygenation in horses during abdominal surgery by continuous delivery of pulsed INO.

Keywords anaesthesia, colic, horse, hypoxaemia, nitric oxide.

Introduction

General anaesthesia in horses causes cardiopulmonary impairment, which frequently results in hypoxaemia (Nyman & Hedenstierna 1989; Nyman et al. 1990; Wagner 2008). During general anaesthesia, hypoxaemia is mainly resulting from the development of atelectasis in the dependent lung, and ventilation/perfusion ($\dot{V}_A/\dot{Q}$) mismatch is most noticeable when the horse is positioned in dorsal recumbency (Dobson et al. 1985; Nyman & Hedenstierna 1989; Nyman et al. 1990).

There are two hypothetical ways to improve the matching of ventilation and perfusion during anaesthesia: either the ventilation can be directed to lung regions that are well perfused or the perfusion can be redistributed to the well-ventilated lung areas. Most research in the anaesthetized horse has focused on the first way, i.e. improving the ventilation in the perfused lung regions. Nyman & Hedenstierna (1989) showed that neither mechanical ventilation (MV) nor positive end-expiratory pressure (PEEP) had

a positive effect on $\dot{V}_A/\dot{Q}$ ratios. Selective MV of dependent lung regions with PEEP does decrease $\dot{V}_A/\dot{Q}$ mismatch and increase arterial oxygen tension (PaO_2) during general anaesthesia (Nyman et al. 1987; Moens et al. 1994). However, this method requires technical interventions which are difficult to apply in clinical conditions (Moens et al. 1992). More recently, it has been shown in clinical studies that MV with recruitment manoeuver (RM) and constant PEEP improved PaO_2 but simultaneously decreased arterial gastrointestinal oxygenation (Hopster et al. 2011, 2016). This method entails transiently high inspiratory pressures and opens up previously collapsed alveoli. Although the RM was considered to successfully open the lungs based on the increased PaO_2, the manoeuver had to be continually repeated to keep the lungs open. Additionally, the elevated mean airway pressure generated by PEEP decreased cardiac output (CO) and consequently decreased oxygen delivery to the tissues (Hopster et al. 2016).

The second way to improve the $\dot{V}_A/\dot{Q}$ matching is to regulate the lung perfusion. Inhaled nitric oxide (INO) is widely used in human medicine, e.g. for children and adults with acute hypoxaemic respiratory failure and neonates with pulmonary hypertension (Rossaint et al. 1993; Dobyns et al. 1999; Clark et al. 2000). INO works as a selective pulmonary vasodilator and does not cause systemic effects in the rest of the body (Frostell et al. 1991). However, few studies have been done on INO and horses. In one study by Young et al. (1999), continuous delivery of INO in horses showed no positive effect on oxygenation. Conversely, in another study done on neonatal foals with experimentally induced pulmonary hypertension, continuous delivery of INO did improve arterial oxygenation (Lester et al. 1999).

Heinonen et al. (2000) compared pulsed delivery of INO during inspiration to constant inspired concentration of NO in anaesthetized pigs and concluded that the NO gas effect was used more effectively, and environmental exhausts was reduced with pulsed delivery. This method of pulsed delivery of NO was developed in a study by Heinonen et al. (2001), in which pulsed INO turned out to be an effective way to counteract hypoxaemia in horses during general anaesthesia. Further studies showed that a pulse of INO during the first part of inspiration (30–45% of the inspiration time) improved arterial oxygenation in healthy horses (Heinonen et al. 2002; Grubb et al. 2008; Nyman et al. 2012; Grubb et al. 2013a,b). Additionally, Grubb et al. (2014) used multiple inert gas elimination technique and scintigraphy to demonstrate that blood flow was redistributed from dependent atelectatic lung regions to non-dependent ventilated areas during pulsed INO, resulting in a reduction of right to left vascular shunt. The improvement in arterial oxygenation during pulsed delivery of INO has been shown to be sustained throughout 2.5 hours of anaesthesia (Nyman et al. 2012) and no adverse effects, i.e. rebound, have been observed in the studies done on horses (Grubb et al. 2008; Nyman et al. 2012; Grubb et al. 2013a,b, 2014).

The present study is a progression from experimental conditions to a clinical setting. The aim was to evaluate the effect of pulsed INO on arterial oxygenation and subsequent blood lactate concentration in colic horses during abdominal surgery.

Materials and methods

The study was designed as a prospective randomized clinical trial, and informed owner consents were obtained. A sample size calculation indicated that the number of horses should be 11 in each group, based on the results of previous studies (Heinonen et al. 2001, 2002; Nyman et al. 2012; Grubb et al. 2013b, 2014) and with consideration that this study did not include healthy horses. In the end, a total of 30 horses were included in the study, which increased the power from 80% to 90%. The study was approved by the local ethics committee for animal experiments, Uppsala, Sweden (approval number C 201/14).

Horses

Inclusion criteria for the study called for horses to show signs of colic and undergo acute abdominal surgery at the Equine Clinic at the University Animal Hospital in Uppsala, Sweden, between May 2012 and December 2013. Horses with an approved owner consent were randomized so that every second horse was enrolled to the pulsed INO group. Horses younger than 6 months were excluded from the study.

Anaesthesia

The horses were anaesthetized following the standard protocol used at the Equine Clinic at the University Animal Hospital. Premedication included 1.1 mg kg^{-1} flunixin meglumine (Flunixin N-vet; N-vet AB, Sweden), 0.03 mg kg^{-1} acepromazine (Plegicil; Pharmaxim, Sweden), 0.1 mg kg^{-1} romifidine (Sedivet; Boehringer Ingelheim Vetmedica, Sweden) and 0.025 mg kg^{-1} butorphanol (Butador;

Vetoquinol, Sweden). For induction, 0.03 mg kg^{-1} diazepam (Diazepam-ratiopharm; Ratiopharm, Germany) and 2.2 mg kg^{-1} ketamine (Ketaminol; Intervet, Sweden) were administered. The horses' tracheas were intubated and anaesthesia was maintained with isoflurane (IsoFlo; Orion Pharma Animal Health, Sweden) in oxygen (fresh gas flow 1 L 100 kg^{-1} $minute^{-1}$). The horses were left to breath spontaneously. Low blood pressure was treated with intravenous crystalloid fluids (Ringer-acetat; Fresenius Kabi, Sweden) and 0.5–2 μg kg^{-1} $minute^{-1}$ dobutamine (Dobutamin Carino; Carinopharm GmbH, Germany) as required. A 2 mg kg^{-1} lidocaine (Xylocain; AstraZeneca, Sweden) bolus administered over approximately 20 minutes followed by a constant rate infusion (2 mg kg^{-1} $hour^{-1}$) was administered in the event of more complicated surgery.

Delivery of pulsed INO

The NO was administered through a device that immediately triggered the delivery of the gas when the horse produced a negative inspiratory pressure during spontaneous breathing (Datex-Ohmeda Research Unit, Finland). The length of the pulse was adjusted stepwise, to effect, from onset of inspiration to the first 30%, 45% or 60% of the total inspiratory time. To minimize exhalation of NO, the shortest pulse that improved PaO_2 was used throughout the anaesthesia. The delivery device was connected with a plastic tube to an adapter located at the proximal end of the endotracheal tube, and once started the horse would get a pulse of NO with every breath. The NO was supplied from a cylinder of 2000 p.p.m. NO in nitrogen gas (AGA AB, Sweden).

Instrumentation

All horses were instrumented with electrocardiogram electrodes placed for lead II analysis and measurement of heart rate. After aseptic preparation, the horses were instrumented with a catheter in the facial artery and in the jugular vein for collection of arterial and venous blood. The arterial catheter was also used for blood pressure monitoring.

Blood-gas analysis

Arterial and venous blood samples were obtained for assessment of PaO_2, venous oxygen tension (PvO_2), arterial carbon dioxide tension ($PaCO_2$), arterial oxygen saturation (SaO_2), pH, blood glucose and blood lactate concentrations. Devices from Radiometer (ABL 500 and ABL 90 flex), calibrated routinely according to the manufacturer, were used for the blood-gas analysis. Venous blood samples were also collected in ethylenediamine tetra-acetic acid (EDTA) tubes for analysis of haemoglobin (Hb). All blood samples were stored on ice. Hb was analysed with the Advia 2120 Hematology System (Siemens Healthineers, Germany) at the Hematology and Chemistry Laboratory at the University Animal Hospital in Uppsala.

Collection of data

Once completely instrumented, data were collected and used as anaesthesia baseline data. After baseline data collection, pulsed INO delivery commenced for horses in the pulsed INO group. Pulse rate was counted by manual palpation of the pulse on the facial artery and respiratory rate was counted by watching thoracic movements. Continuous respiratory gas analysis (fractions of O_2 and CO_2) including measurement of the volatile anaesthetic agent concentration (isoflurane) was performed with a dedicated multi-parameter anaesthesia monitor (Mindray, BeneView T5; Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China) using side-stream gas sampling at the level of the connection of the endotracheal tube to the Y piece of the circuit.

Calculated data

F-shunt was calculated as follows:

$$\text{F-shunt} = (CcO_2 - CaO_2)/([CcO_2 - CaO_2] + 35),$$

where 35 is a fixed value of $C(a\text{-}v)O_2$ in mL L^{-1} (Araos et al. 2012; Briganti et al. 2015).

Oxygen extraction ratio (O_2ER) was calculated as:

$$C(a\text{-}v)O_2/CaO_2$$

CcO_2, CaO_2, and CvO_2 are oxygen content in mL L^{-1} of capillary, arterial and venous blood, respectively. These values were calculated as: $CxO_2 = (Hb \times 1.36 \times SxO_2) + (0.227 \times PxO_2)$. The value CcO_2 was calculated as: $CcO_2 = (Hb \times 1.36 \times 1) + (0.227 \times PaO_2)$.

Statistics

The raw data collected were entered in Microsoft Excel 2010 (Microsoft Office, Microsoft, WA, USA) and then processed. For all statistical calculations, the statistical package GraphPad Prism 5 (GraphPad

Software, CA, USA) was used. Data were tested for Gaussian distribution using the Shapiro–Wilk test. For individual changes, Mann–Whitney tests and unpaired *t* tests were used to compare the difference between the two groups and Wilcoxon tests and paired *t* tests to compare differences within the groups. The difference was considered significant when $p < 0.05$ with a confidence interval of 95%. Data are presented as mean ± standard deviation.

Results

Fifteen horses were administered pulsed INO (pulsed INO group) and 15 served as controls (C group).

Horses included in the study were on average 10–11 years old (pulsed INO: 10 ± 6 years; C: 11 ± 6 years). The weight in the pulsed INO and C group was 543 ± 114 (337–688) kg and 508 ± 105 (250–616) kg, respectively. Fourteen warmbloods (six pulsed INO, eight C), six Standardbred trotters (three pulsed INO, three C) and 10 various breeds including ponies, Icelandic horses and draft horses (six pulsed INO, four C) were included in the study. During anaesthesia, crystalloid fluids were administered continuously, 12.4 ± 5.5 L to horses in the pulsed INO group and 13.0 ± 3.5 L to horses in the C group. Twenty-two horses (11 pulsed INO, 11 C) were administered a lidocaine infusion during surgery.

The time between induction of anaesthesia and the baseline recordings was approximately 30 minutes. Data were collected at 30-minute intervals until the end of anaesthesia. This clinical study has several missing values; Hb was not analysed in two horses (one pulsed INO, one C) and venous blood samples in three horses (one pulsed INO, two C). Physiological data and calculations are presented in Table 1. Administration of the shortest pulse length of pulsed INO improved PaO_2 in eight of the 15 horses; six horses responded with the 45% pulse length, and one horse needed a longer pulse.

Arterial oxygen tension and saturation

All horses in the pulsed INO group ($n = 15$) presented a higher PaO_2 and SaO_2 at the end of anaesthesia compared to baseline values (Fig. 1 and Table 2). Five horses in the pulsed INO group had a low baseline

Table 1 Physiological data and calculations during anaesthesia in horses that were administered pulsed inhaled nitric oxide (pulsed INO) and those that were not (C).

Parameter	Group	Baseline	*n*	End	*n*	*p* value
HR (beats minute^{-1})	Pulsed INO	40 ± 6	15	40 ± 6	15	ns
	C	44 ± 22	15	40 ± 8	15	
ABP (mmHg)	Pulsed INO	78 ± 21	15	82 ± 22	15	ns
	C	73 ± 17	15	79 ± 9	15	
f_R (breaths minute^{-1})	Pulsed INO	6 ± 3	15	6 ± 3	15	ns
	C	6 ± 4	15	7 ± 3	15	
FIO_2	Pulsed INO	0.80 ± 0.10*	15	0.80 ± 0.10*	15	0.0091*
	C	0.88 ± 0.07	15	00.88 ± 0.07	15	
Glucose (mmol L^{-1})	Pulsed INO	11.3 ± 2.4	14	9.5 ± 2.0†	15	0.0121†
	C	11.3 ± 1.5	13	9.9 ± 1.9†	14	0.0014†
Hb (g L^{-1})	Pulsed INO	121 ± 14	14	114 ± 18	14	ns
	C	131 ± 20	14	123 ± 25	14	
pH	Pulsed INO	7.22 ± 0.07*	15	7.21 ± 0.06	15	0.0195*
	C	7.28 ± 0.05	15	7.25 ± 0.08	15	
$PaCO_2$ [kPa (mmHg)]	Pulsed INO	8.7 ± 1.9 (65 ± 14)	15	9.1 ± 2.3 (68 ± 17)	15	
	C	8.8 ± 1.6 (66 ± 12)	15	9.3 ± 1.9 (70 ± 14)†	15	0.0330†
$P(A\text{-}a)O_2$ [kPa (mmHg)]	Pulsed INO	56.4 ± 8.5 (423 ± 64)	15	48.3 ± 8.6 (362 ± 65)*†	15	<0.0001* 0.0003†
	C	60.9 ± 8.2 (457 ± 62)	15	62.7 ± 8.8 (470 ± 66)	15	
CaO_2 (mL L^{-1})	Pulsed INO	155 ± 22	14	160 ± 26*	14	0.0126*
	C	163 ± 26	13	142 ± 30†	13	0.0218†
O_2ER (%)	Pulsed INO	20 ± 14	14	22 ± 17*	14	0.0328*
	C	14 ± 5	12	11 ± 4	12	

HR, heart rate; ABP, mean arterial blood pressure; f_R, respiratory frequency; FIO_2, fraction of inspired oxygen; Hb, haemoglobin; $PaCO_2$, arterial carbon dioxide tension; $P(A\text{-}a)O_2$, alveolar–arterial gradient; CaO_2, oxygen content; O_2ER, oxygen extraction ratio. The baseline value is at the beginning of anaesthesia before pulsed INO delivery commenced, and the end value is at the end of anaesthesia.

*Significantly different compared to C.

†Significantly different from baseline value.

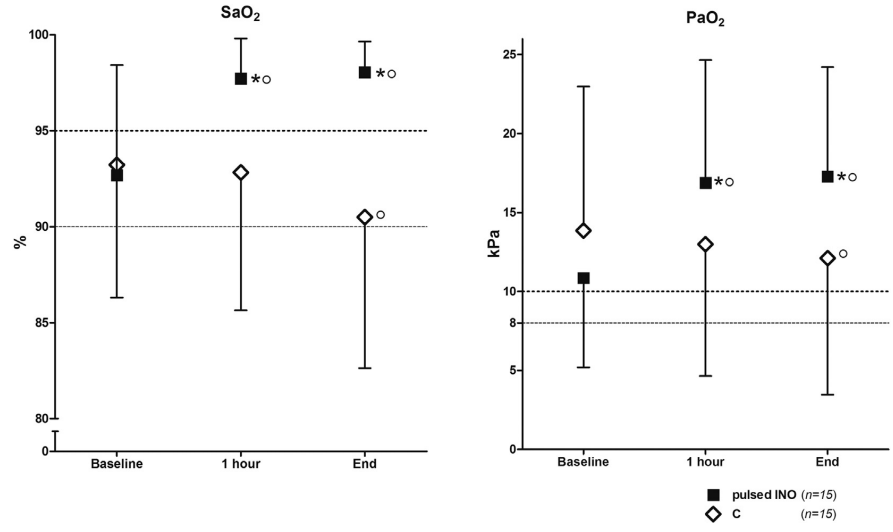


Figure 1 Arterial oxygen saturation (SaO_2) and arterial oxygen tension (PaO_2) in horses that were administered pulsed inhaled nitric oxide (pulsed INO group) and those that were not (C group) during anaesthesia. Data are presented as mean ± standard deviation (SD) at baseline (beginning of anaesthesia before pulsed INO delivery commenced), at 1 hour and at end of anaesthesia in pulsed INO group and C group, respectively. The dotted lines indicate 90% and 95% SaO_2, and 8 and 10 kPa (60 and 75 mmHg) PaO_2. *Significantly different compared to C; °significantly different from baseline value.

PaO_2 7.0 ± 1.1 kPa (53 ± 8 mmHg). In these horses, PaO_2 increased to 14.2 ± 5.0 kPa (106 ± 37 mmHg) during pulsed INO delivery, which improved SaO_2 from 86 ± 5% to 97 ± 2%. Twelve of the 15 horses in the C group presented a lower PaO_2 and SaO_2 at the end of anaesthesia compared to baseline. In four horses with a low baseline PaO_2 and SaO_2 [6.5 ± 0.7 kPa (49 ± 5 mmHg) and 83 ± 5%, respectively], PaO_2 was reduced to 6.1 ± 0.7 kPa (46 ± 5 mmHg) and SaO_2 to 81 ± 5%.

Calculated F-shunt

Comparison of each individual's change in F-shunt during anaesthesia, calculated in percent, showed a significant ($p < 0.0001$) difference between horses in the pulsed INO group ($n = 14$) and horses in the C group ($n = 14$) (Fig. 2). At baseline, there was no difference in F-shunt between the two groups ($p = 0.7652$); however, there was a difference at the end of anaesthesia ($p = 0.0014$). In the pulsed INO group, the F-shunt decreased 29 ± 17% during anaesthesia, from an actual value of 39 ± 10% at baseline to 27 ± 6% at the end of anaesthesia. In the C group, the F-shunt increased 11 ± 25%, from an actual value of 40 ± 12% at baseline to 44 ± 12% at the end of anaesthesia.

Blood lactate

Although individual variations were measured, the blood lactate concentration decreased 17 ± 21% in the pulsed INO group ($n = 15$) during anaesthesia, from 3.1 ± 2.6 to 2.3 ± 1.3 mmol L^{-1} ($p = 0.0119$). In the pulsed INO group, 10 horses had a decrease in blood lactate concentration during anaesthesia, two horses had an unchanged concentration and three horses had an increase (Fig. 3). There was no change (2 ± 31%) in blood lactate concentration in the C group ($n = 15$), the concentration went from 2.4 ± 1.2 to 2.3 ± 1.1 mmol L^{-1} ($p = 0.8201$). In the C group, eight horses had a decreased and seven horses had an increased concentration in blood lactate during anaesthesia. There was no difference ($p = 0.5462$) in fluid therapy between the groups.

Discussion

The positive effect of pulsed INO on arterial oxygenation was both instantaneous and sustained,

Table 2 Change in arterial oxygen tension after pulsed inhaled nitric oxide (INO) delivery. Individual change in arterial oxygen tension (PaO_2) in horses that were administered pulsed INO during anaesthesia. The baseline value is at the beginning of anaesthesia before pulsed INO delivery commenced. Horses with lower PaO_2 [<10 kPa (75 mmHg)] at baseline had a larger ($p = 0.0047$) increase in PaO_2 compared to horses with higher PaO_2 [>10 kPa (75 mmHg)] at baseline.

Horse	Baseline PaO_2 kPa (mmHg)	Highest PaO_2 kPa (mmHg)	Change
INO 13	5.6 (42)	10.4 (78)	86%
INO 8	6.0 (45)	11.0 (83)	84%
INO 6	7.8 (58)	11.6 (87)	50%
INO 3	7.9 (59)	15.5 (116)	97%
INO 1	7.9 (59)	22.3 (167)	182%
INO 7	8.8 (66)	24.3 (182)	175%
INO 5	9.5 (71)	24.9 (187)	164%
INO 2	9.5 (71)	14.6 (109)	54%
INO 12	9.7 (73)	19.2 (144)	98%
INO 15	10.0 (75)	32.0 (240)	222%
INO 11	11.2 (84)	13.7 (103)	23%
INO 4	11.4 (85)	18.5 (139)	62%
INO 14	13.6 (102)	20.7 (139)	53%
INO 9	15.3 (114)	17.9 (134)	17%
INO 10	29.1 (218)	37.3 (280)	28%
	Baseline PaO_2 kPa (mmHg)	*n*	Mean change
	<10 (75)	10	121%
	>10 (75)	5	37%

regardless of general condition, aetiology of colic and length of surgery.

The effect on PaO_2 and SaO_2 during pulsed INO was immediate, within 15 minutes, in all but one of the 15 horses. In line with previous studies, the positive effect of pulsed INO was present as long as NO was administered, even when the anaesthesia lasted more than 2.5 hours (Nyman et al. 2012; Grubb et al. 2013b). The effect of improved arterial oxygenation resulted in significantly higher CaO_2 at the end of anaesthesia in the pulsed INO group compared to the C group, but no effect on Hb concentration was observed. CO was not measured in this clinical study; however, no adverse effect of pulsed INO on CO has been measured in previous experimental studies (Heinonen et al. 2000, 2001, 2002; Nyman et al. 2012; Grubb et al. 2013b). Thus, increased CaO_2 in colic horses receiving pulsed INO possibly results in enhanced oxygen delivery to the tissues.

At the end of anaesthesia, seven of the 15 horses in the C group had a PaO_2 and SaO_2 less than 8 kPa (60 mmHg) and 87%, respectively, which is considered as severe hypoxaemia (Grimm et al. 2015). None of the horses in the pulsed INO group had severe hypoxaemia at the end of anaesthesia, and the positive effect of pulsed INO was greater in colic horses with PaO_2 below 10 kPa (75 mmHg) and SaO_2 below 95% at baseline compared to horses with a higher baseline value. This suggests that horses with hypoxaemia might benefit the most from pulsed INO.

Earlier studies have shown that pulsed INO effectively reduces the magnitude of right to left vascular shunt as a result of redistribution of pulmonary blood flow (Heinonen et al. 2001; Grubb et al. 2013b, 2014). Although mixed venous blood was not obtained in the present study with clinical cases, the F-shunt could be calculated. Based on the calculations, reduction in the magnitude of shunt seems to also be possible in colic horses with compromised general condition undergoing abdominal surgery.

Although Edner et al. (2007) did not find changes in blood lactate concentration during a 3-hour anaesthesia in colic horses subjected to abdominal surgery, lactate content increased in the gluteus muscle. Interestingly, in the present study a decline in blood lactate concentration during anaesthesia was evident in the pulsed INO group, whereas in the C group no changes occurred. The treatment of impaired circulation, based on infusion of crystalloid fluids and dobutamine during anaesthesia, did not differ between the two groups. However, decreasing lactate levels in the pulsed INO group and differences in O_2ER compared to the C group suggests improved peripheral perfusion and oxygenation. Still, many factors may influence lactate levels in individual horses, and the clinical effects of improved arterial oxygenation remains to be investigated.

Respiratory acidosis was evident in most cases owing to some respiratory depression, but there was no difference in $PaCO_2$ between horses administered pulsed INO and controls. As shown previously, pulsed INO does not affect the factors that determine minute ventilation (i.e. respiratory rate and tidal volume) (Nyman et al. 2012). In order to improve both oxygenation and ventilation, the next step towards clinical use is to evaluate pulsed INO during MV. Twelve horses in the pulsed INO group and 12 in the C group recovered from anaesthesia, but the quality of recovery was not evaluated in the present study.

In conclusion, the present study shows that pulsed INO effectively reduces the F-shunt and improves arterial oxygenation in colic horses during abdominal surgery. However, the effect of improved arterial oxygenation on quality of recovery and clinical outcomes still requires further exploration.

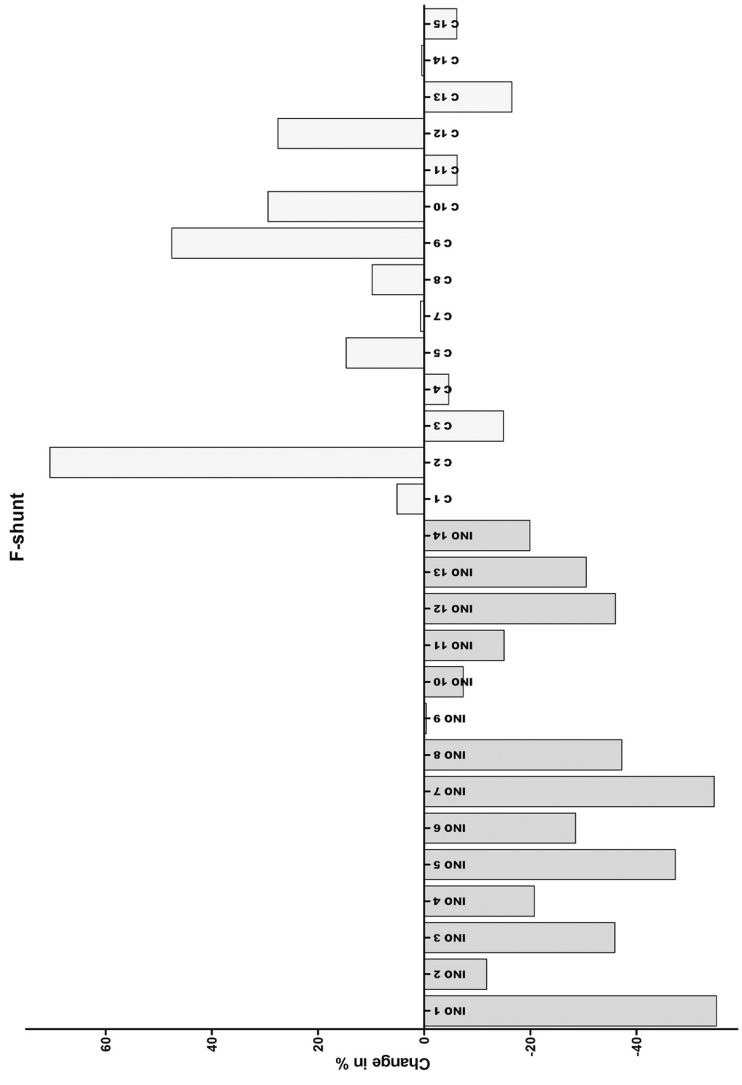


Figure 2 Individual change in F-shunt from baseline to end of anaesthesia in horses that were administered pulsed inhaled nitric oxide (INO) and those that were not (C). The baseline value is at the beginning of anaesthesia before pulsed INO delivery commenced. There was a significant ($p < 0.0001$) difference between horses that were administered pulsed INO and controls.

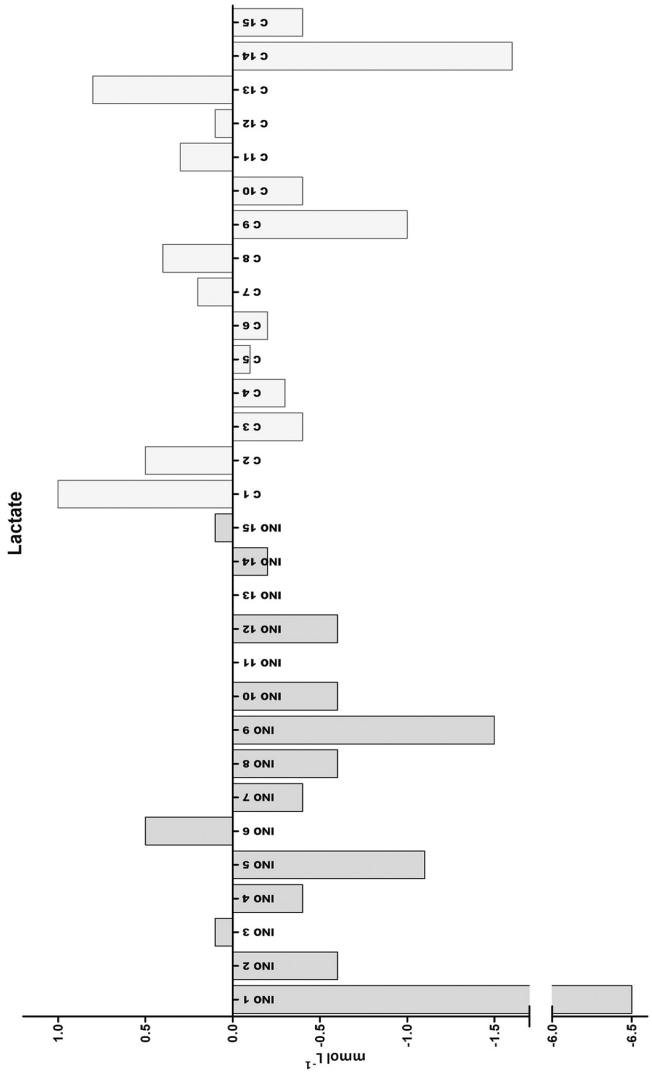


Figure 3 Individual change in blood lactate concentration (in mmol L^{-1}) from baseline to end of anaesthesia in horses that were administered pulsed inhaled nitric oxide (INO) and those that were not (C). The baseline value is at the beginning of anaesthesia before pulsed INO delivery commenced.

Acknowledgements

The authors are grateful to Anneli Rydén for skilful technical assistance and to Brandon Velie for comments that greatly improved the manuscript. The study was supported by grants from the Swedish–Norwegian Foundation for Equine Research (H1047287).

Conflict of interest statement

Authors declare no conflict of interest.

Authors' contributions

MW: Design, data collection, statistical analysis, and writing of the manuscript; IG: Design, data collection, statistical analysis, and writing of the manuscript; GN: Design, statistical analysis, and writing of the manuscript.

References

Araos JD, Larenza MP, Boston RC et al. (2012) Use of the oxygen content-based index, Fshunt, as an indicator of pulmonary venous admixture at various inspired oxygen fractions in anesthetized sheep. Am J Vet Res 73, 2013–2020.

Briganti A, Portela DA, Grasso S et al. (2015) Accuracy of different oxygenation indices in estimating intrapulmonary shunting at increasing infusion rates of dobutamine in horses under general anaesthesia. Vet J 204, 351–356.

Clark RH, Kueser TJ, Walker MW et al. (2000) Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. N Engl J Med 342, 469–474.

Dobson A, Gleed RD, Meyer RE et al. (1985) Changes in blood-flow distribution in equine lungs induced by anesthesia. Q J Exp Physiol 70, 283–297.

Dobyns EL, Cornfield DN, Anas NG et al. (1999) Multicenter randomized controlled trial of the effects of inhaled nitric oxide therapy on gas exchange in children with acute hypoxemic respiratory failure. J Pediatr 134, 406–412.

Edner A, Nyman G, Essén-Gustavsson B (2007) Metabolism before, during and after anaesthesia in colic and healthy horses. Acta Vet Scand 49, 34–50.

Frostell C, Fratacci MD, Wain JC et al. (1991) Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83, 2038–2047.

Grimm KA, Lamont LA, Tranquilli WJ et al. (2015) Veterinary anesthesia and analgesia: the fifth edition of Lumb and Jones. John Wiley & Sons, Inc. pp. 102–103.

Grubb T, Edner A, Frendin JH et al. (2013a) Oxygenation and plasma endothelin-1 concentrations in healthy horses recovering from isoflurane anaesthesia administered with or without pulse-delivered inhaled nitric oxide. Vet Anaesth Analg 40, 9–18.

Grubb T, Frendin JH, Edner A et al. (2013b) The effects of pulse-delivered inhaled nitric oxide on arterial oxygenation, ventilation–perfusion distribution and plasma endothelin-1 concentration in laterally recumbent isoflurane-anaesthetized horses. Vet Anaesth Analg 40, 19–30.

Grubb T, Hogman M, Edner A et al. (2008) Physiologic responses and plasma endothelin-1 concentrations associated with abrupt cessation of nitric oxide inhalation in isoflurane-anesthetized horses. Am J Vet Res 69, 423–430.

Grubb T, Lord P, Berger M et al. (2014) Effects of pulse-delivered inhaled nitric oxide administration on pulmonary perfusion and arterial oxygenation in dorsally recumbent isoflurane-anesthetized horses. Am J Vet Res 75, 949–955.

Heinonen E, Hogman M, Merilainen P (2000) Theoretical and experimental comparison of constant inspired concentration and pulsed delivery in NO therapy. Intensive Care Med 26, 1116–1123.

Heinonen E, Nyman G, Hedenstierna G et al. (2001) Pulsed delivery of nitric oxide counteracts hypoxaemia in the anaesthetized horse. Vet Anaesth Analg 28, 3–11.

Heinonen E, Nyman G, Merilainen P et al. (2002) Effect of different pulses of nitric oxide on venous admixture in the anaesthetized horse. Br J Anaesth 88, 394–398.

Hopster K, Wogatzki A, Geburek F et al. (2016) Effects of positive end-expiratory titration on intestinal oxygenation and perfusion in isoflurane anaesthetised horses. Equine Vet J. http://dx.doi.org/10.1111/evj.12555.

Hopster K, Kästner SBR, Rohn K et al. (2011) Intermittent positive pressure ventilation with constant positive end-expiratory pressure and alveolar recruitment manoeuvre during inhalation anaesthesia in horses undergoing surgery for colic, and its influence on the early recovery period. Vet Anaesth Analg 38, 169–177.

Lester GD, DeMarco VG, Norman WM (1999) Effect of inhaled nitric oxide on experimentally induced pulmonary hypertension in neonatal foals. Am J Vet Res 60, 1207–1212.

Moens Y, Gootjes P, Lagerweij E (1992) A tracheal tube-in-tube technique for functionally separation of the lungs in the horse. Equine Vet J 24, 103–106.

Moens Y, Lagerweij E, Gootjes P et al. (1994) Differential artificial ventilation in anesthetized horses positioned in lateral recumbency. Am J Vet Res 55, 1319–1326.

Nyman G, Frostell C, Hedenstierna G et al. (1987) Selective mechanical ventilation of dependent lung regions in the anesthetized horse in dorsal recumbency. Br J Anaesth 59, 1027–1034.

Nyman G, Funkquist B, Kvart C et al. (1990) Atelectasis causes gas-exchange impairment in the anesthetized horse. Equine Vet J 22, 317–324.

Nyman G, Grubb T, Heinonen E et al. (2012) Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses. Vet Anaesth Analg 39, 480–487.

Nyman G, Hedenstierna G (1989) Ventilation–perfusion relationships in the anesthetized horse. Equine Vet J 21, 274–281.

Rossaint R, Falke KJ, Lopez F et al. (1993) Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328, 399–405.

Wagner AE (2008) Complications in equine anesthesia. Vet Clin North Am Equine Pract 24, 735–752.

Young LE, Marlin DJ, McMurphy RM et al. (1999) Effects of inhaled nitric oxide 10 ppm in spontaneously breathing horses anaesthetized with halothane. Br J Anaesth 83, 321–324.

Received 20 August 2016; accepted 24 November 2016.

Available online 17 April 2017

III

BEVA Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.13129

Effects of pulsed inhaled nitric oxide on arterial oxygenation during mechanical ventilation in anaesthetised horses undergoing elective arthroscopy or emergency colic surgery

M. WIKLUND[†]*, M. KELLGREN[†], S. WULCAN[†], T. GRUBB[‡] and G. NYMAN[†]

[†]Faculty of Veterinary Medicine and Animal Science, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
[‡]Washington State University's College of Veterinary Medicine, Pullman, Washington, USA.

*Correspondence email: maja.wiklund@uds.slu.se; Received: 21.05.18; Accepted: 12.04.19

Summary

Background: Administration of pulsed inhaled nitric oxide (PiNO) improves arterial oxygenation in spontaneously breathing anaesthetised healthy horses and in horses undergoing colic surgery. However, because hypoventilation commonly occurs, horses are often mechanically ventilated to prevent hypercarbia.
Objectives: To evaluate the effects of PiNO on arterial oxygenation during anaesthesia in mechanically ventilated healthy horses and horses undergoing colic surgery.
Study design: Prospective nonblinded clinical trial.
Methods: Fifty horses undergoing elective arthroscopy (Group A) and 30 horses undergoing colic surgery (Group C) in dorsal recumbency were included in the study. Every second horse in each group received PiNO (A-INO, C-INO), the others served as controls (A-CN, C-CN). All horses were mechanically ventilated and anaesthesia was maintained with isoflurane. PiNO was mechanically delivered at the proximal end of the endotracheal tube as a pulse during the first part of each inspiration. Data were collected at the start (baseline, before PiNO) and at the end of inhalation anaesthesia. The Tukey method was used to compare baseline and end values for each parameter.
Results: Arterial oxygen tension (PaO_2) increased from (median [IQR]) 13.6 (9.3, 30.1) at baseline to 24.2 (18.6, 37.0) kPa at the end of anaesthesia in A-INO ($P = 0.005$) and from 7.7 (6.4, 8.5) to 15.5 (9.9, 26.9) kPa in C-INO ($P = 0.007$). Mean (95% CI) difference in F-shunt between baseline and end were −6 (−10; −1) and −11 (−22; −1) % in A-INO ($P = 0.005$) and C-INO ($P = 0.04$) respectively. There was no change in PaO_2 or F-shunt from baseline to end of anaesthesia in A-CN or C-CN.
Main limitations: Cardiac output was not measured, thus O_2 delivery could not be calculated.
Conclusions: The combination of mechanical ventilation and PiNO improved pulmonary gas exchange during anaesthesia by a simultaneous decrease in F-shunt and improved alveolar ventilation.

The Summary is available in Portuguese – see Supporting information

Keywords: horse; anaesthesia; PiNO; colic; arthroscopy

Introduction

Hypoxaemia and hypoventilation are two common complications in the anaesthetised horse [1–3]. The incidence of anaesthesia-induced hypoxaemia, which is more difficult to treat than hypoventilation, is not well defined but is suggested to be around 12–17% [4–6]. Mechanical ventilation is commonly used to treat and prevent hypoventilation. Hypercarbia and acid-base balance can be normalised with mechanical ventilation in most cases [7–9]. However, there is no guarantee that hypoxaemia will be alleviated with the improved ventilation [10,11].

Pulsed inhaled nitric oxide (PiNO) has been used to improve arterial oxygen tension (PaO_2) and arterial oxygen content (CaO_2) in spontaneously breathing research horses [12–15]. Nitric oxide causes local vasodilation in the pulmonary vessels, thereby enable a shift in blood flow from the dependent (atelectatic) to the nondependent (aerated) regions of the lung [14]. Using this technique, hypoxaemia is treated by redirecting blood flow, or pulmonary perfusion, to the ventilated regions of the lung rather than by changing ventilation of the lung [14]. However, PiNO does not affect ventilation and hypercarbia developed in all or most horses in previous studies [12–15]. These findings were also confirmed in a clinical study where spontaneously breathing horses undergoing colic surgery treated with PiNO remained hypercarbic despite improved arterial oxygenation [16].

The aim of this study was to determine and compare the effect of PiNO on oxygenation in anaesthetised mechanically ventilated horses that were anaesthetised for either elective or emergency surgery. The hypothesis was that PiNO in combination with mechanical ventilation would improve oxygenation from baseline in each group while providing normoventilation as evidenced by normocarbia in both healthy horses and horses undergoing colic surgery.

Materials and methods

The design of the study was a prospective nonblinded clinical trial. Practical aspects and time limit influenced the final sample size which became 50 healthy horses undergoing elective arthroscopy (25 receiving PiNO and 25 controls) and 30 horses undergoing emergency colic surgery (15 horses receiving PiNO and 15 controls). Sample size calculations based on improvement in PaO_2 with PiNO in previous studies [13–18] indicated that with a power of 80 and 5% Type I error rate a sufficient number of horses in each group was 11 to detect an effect size of 50%. With the present sample size smaller effect sizes can be detected with the same power.

Horses

Inclusion criteria for the healthy group were determination of clinical health (ASA status I-II) by physical examination and admission to the hospital for elective arthroscopy (Group A) in dorsal recumbency. Exclusion criteria were any horses not ASA I-II. Inclusion criteria for the colic group were all adult horses with signs of abdominal pain (colic) and admission to the hospital for acute colic surgery (Group C) in dorsal recumbency. Horses under the age of 6 months were excluded from both groups. Arthroscopic procedures were performed at the Equine Clinic at the University Animal

Hospital in Uppsala, Sweden and at Mälaren Equine Practice in Sigtuna, Sweden, between September 2015 and September 2017. Colic surgeries were performed at the Equine Clinic at the University Animal Hospital in Uppsala, between October 2015 and November 2016.

The horses which, according to the approved owner consent, could be included in the study were divided so that every other horse received PiNO, designated A-INO and C-INO. Control horses, not receiving PiNO, were designated A-CN and C-CN. All horses were scheduled for mechanical ventilation.

Anaesthesia

After aseptic preparation, a 14 gauge catheter (Milacath)[a] was placed in the jugular vein for administration of intravenous (i.v.) drugs and fluids and collection of blood for venous blood gas analysis. For all horses premedication included 1.1 mg/kg bwt flunixin meglumine (Flunixin N-vet)[b], 0.1 mg/kg bwt romifidine (Sedivet)[c] and 0.025 mg/kg bwt butorphanol (Butador)[d] i.v. Group A horses also received 0.03 mg/kg bwt acepromazine (Plegicil)[e] i.m. For induction of anaesthesia, 0.03 mg/kg bwt diazepam (Diazepam-ratiopharm)[f] and 2.2 mg/kg bwt ketamine (Ketaminol)[g] were administered i.v. Following endotracheal intubation, anaesthesia was maintained with isoflurane (IsoFlo)[h] in oxygen (fresh gas flow 1 L/100 kg bwt/min). Mechanical ventilation began when the horse was connected to the large animal anaesthetic machine (Tafonius)[i]. In Group A, mechanical ventilation was initiated within 5 min after intubation and in Group C after 5–30 min. The initial settings for mechanical ventilation were a tidal volume of 10 ml/kg bwt and a respiratory frequency of 6-8 breaths/min. The settings were changed during anaesthesia in response to blood gas values.

Low blood pressure was treated with i.v. crystalloid fluids (Ringer-acetat)[j], colloid fluids (Voluven)[j] and 0.5-2 μg/kg bwt/min dobutamine (Dobutamin Carino)[k] with the goal to maintain mean arterial blood pressure (MAP) > 65 mmHg. Horses in Group C received a 2 mg/kg bwt lidocaine (Xylocain)[l] bolus administered over 20 min immediately after induction and followed by a lidocaine constant rate infusion (2 mg/kg bwt/h) until 20–30 min before recovery.

TABLE 1: Descriptive data of parameters at baseline (beginning of anaesthesia before PiNO delivery commenced) and at end of inhalation anaesthesia in horses undergoing elective arthroscopy (Group A) or colic surgery (Group C)

Parameter	Group	Treatment	Baseline	End
PaO_2 kPa	A	INO	13.6 (9.3, 30.1)	24.2 (18.6, 37.0)
		CN	14.1 (10.2, 21.5)	11.3 (9.5, 22.9)
	C	INO	7.7 (6.4, 8.5)	15.5 (9.9, 26.9)
		CN	6.9 (5.4, 14.6)	7.4 (5.7, 15.9)
SaO_2%	A	INO	94 ± 3	96 ± 1
		CN	95 ± 2	93 ± 3
	C	INO	86 ± 8	94 ± 5
		CN	85 ± 10	85 ± 9
F-shunt%	A	INO	34 ± 7	29 ± 5
		CN	33 ± 6	37 ± 7
	C	INO	46 ± 9	35 ± 7
		CN	47 ± 14	49 ± 14
$PaCO_2$ kPa	A	INO	8.0 ± 1.0	8.3 ± 0.8
		CN	7.6 ± 0.5	8.0 ± 0.5
	C	INO	7.6 ± 1.4	7.9 ± 1.1
		CN	7.8 ± 1.2	8.0 ± 1.4
CaO_2 ml/L	A	INO	160 ± 26	165 ± 24
		CN	160 ± 18	158 ± 21
	C	INO	128 ± 28	160 ± 34
		CN	146 ± 28	152 ± 23
HR beats/min	A	INO	35 ± 5	37 ± 8
		CN	37 ± 5	40 ± 6
	C	INO	39 ± 7	42 ± 10
		CN	37 ± 6	41 ± 9
MAP mmHg	A	INO	64 ± 10	71 ± 13
		CN	58 ± 10	73 ± 12
	C	INO	72 ± 20	82 ± 19
		CN	78 ± 21	87 ± 15
$P(A\text{-}a)O_2$ kPa	A	INO	43.7 ± 11.5	36.9 ± 10.4
		CN	42.6 ± 9.4	47.9 ± 10.7
	C	INO	53.1 ± 10.1	45.5 ± 9.6
		CN	52.0 ± 14.0	54.9 ± 12.8
pH	A	INO	7.34 ± 0.04	7.34 ± 0.03
		CN	7.36 ± 0.02	7.36 ± 0.03
	C	INO	7.33 ± 0.07	7.33 ± 0.05
		CN	7.31 ± 0.06	7.32 ± 0.06
Lactate mmol/L	A	INO	1.2 ± 0.3	1.4 ± 0.3
		CN	1.3 ± 0.4	1.4 ± 0.4
	C	INO	2.0 (1.7, 4.5)	1.9 (1.4, 3.3)
		CN	1.9 (1.2, 3.1)	2.1 (1.8, 2.8)

The horses were either given PiNO (A-INO, n = 25; C-INO, n = 15) or served as controls (A-CN, n = 25; C-CN, n = 15). Data are expressed as mean ± s.d. or median (IQR) if normally or not normally distributed respectively.

PaO_2, arterial oxygen tension; SaO_2, arterial oxygen saturation; $PaCO_2$, arterial carbon dioxide tension; CaO_2, arterial oxygen content; HR, heart rate; MAP, mean arterial blood pressure; $P(A\text{-}a)O_2$, alveolar-arterial gradient.

TABLE 2: Comparison of baseline values and values at the end of anaesthesia of various cardiovascular and respiratory parameters in horses undergoing elective arthroscopy and that were given pulsed inhaled nitric oxide (INO, n = 25) and those that were not (CN, n = 25)

Parameter	Group A	Difference of means	Simultaneous 95% CI	P-value
PaO_2 log data	Baseline INO–Baseline CN	0.0	(−0.3; 0.3)	>0.9
	End CN–Baseline CN	−0.1	(−0.4; 0.2)	0.8
	End INO–Baseline INO	0.4	(0.1; 0.8)	0.005
	End INO–End CN	0.6	(0.2; 0.9)	<0.001
SaO_2%	Baseline INO–Baseline CN	−1	(−2; 1)	0.6
	End CN–Baseline CN	−1	(−3; 0)	0.1
	End INO–Baseline INO	2	(0; 4)	0.005
	End INO–End CN	3	(1; 4)	<0.001
F-shunt%	Baseline INO–Baseline CN	1	(−3; 6)	0.9
	End CN–Baseline CN	3	(−1; 8)	0.2
	End INO–Baseline INO	−6	(−10; -1)	0.005
	End INO–End CN	−8	(−12; -4)	<0.001
$PaCO_2$ kPa	Baseline INO–Baseline CN	0.4	(−0.1; 0.8)	0.2
	End CN–Baseline CN	0.4	(−0.1; 0.9)	0.2
	End INO–Baseline INO	0.3	(−0.2; 0.8)	0.3
	End INO–End CN	0.3	(−0.2; 0.8)	0.4
CaO_2 ml/L	Baseline INO–Baseline CN	1	(−9; 10)	>0.9
	End CN–Baseline CN	−2	(−11; 7)	>0.9
	End INO–Baseline INO	5	(−4; 14)	0.5
	End INO–End CN	8	(−1; 17)	0.1
HR beats/min	Baseline INO–Baseline CN	−2	(−5; 2)	0.5
	End CN–Baseline CN	3	(0; 7)	0.1
	End INO–Baseline INO	2	(−1; 6)	0.3
	End INO–End CN	−3	(−6; 1)	0.2
MAP mmHg	Baseline INO–Baseline CN	6	(−2; 14)	0.2
	End CN–Baseline CN	15	(7; 23)	<0.001
	End INO–Baseline INO	6	(−2; 14)	0.2
	End INO–End CN	−3	(−11; 5)	0.8
$P(A\text{-}a)O_2$ kPa	Baseline INO–Baseline CN	1	(−6; 8)	>0.9
	End CN–Baseline CN	5	(−2; 12)	0.2
	End INO–Baseline INO	−7	(−14; 0)	0.06
	End INO–End CN	−11	(−18; −4)	0.001
pH	Baseline INO–Baseline CN	−0.02	(−0.04; 0.00)	0.08
	End CN–Baseline CN	0.00	(−0.02; 0.02)	>0.9
	End INO–Baseline INO	0.00	(−0.02; 0.02)	>0.9
	End INO–End CN	−0.02	(−0.04; 0.00)	0.2
Lactate log data	Baseline INO–Baseline CN	0.0	(−0.2; 0.2)	>0.9
	End CN–Baseline CN	0.1	(−0.1; 0.3)	0.7
	End INO–Baseline INO	0.1	(−0.1; 0.3)	0.3
	End INO–End CN	0.0	(−0.2; 0.2)	>0.9

PaO_2, arterial oxygen tension; SaO_2, arterial oxygen saturation; $PaCO_2$, arterial carbon dioxide tension; CaO_2, arterial oxygen content; HR, heart rate; MAP, mean arterial blood pressure; $P(A\text{-}a)O_2$, alveolar-arterial gradient.

Delivery of PiNO

The NO was delivered by a device[m] that was triggered by the positive pressure at the beginning of each breath from the ventilator. The pulse was delivered during the first part of inspiration, as determined in previous studies [13,15,16]. The delivery device was connected with a plastic line to an adapter located at the proximal end of the endotracheal tube, and once started the horse would receive a pulse of NO with every mechanical breath. The NO was supplied from a cylinder of 2000 p.p.m. NO in nitrogen gas[n].

Monitoring and collection of data

After aseptic preparation, a catheter was placed in the facial artery for blood pressure monitoring and for collection of arterial blood. Monitoring during anaesthesia was performed with a multi-parameter monitor (Tafonius)[l] and included arterial blood pressure, heart rate, electrocardiogram, ventilation parameters and continuous respiratory gas analysis including measurement of the volatile anaesthetic agent (isoflurane) concentration. Arterial and jugular venous blood samples were obtained for assessment of PaO_2, jugular venous oxygen tension, arterial carbon dioxide tension ($PaCO_2$), arterial oxygen saturation (SaO_2), pH, haemoglobin (Hb) and lactate concentrations. An automated whole-blood gas analyser (ABL90 FLEX)[o] was used for the blood gas analysis. All blood samples were stored on ice for up to two hours if not analysed immediately. The respiratory gas and blood gas analysers were calibrated routinely according to the manufacturers' guidelines.

Once completely instrumented (after 15-25 min of inhalation anaesthesia in Group A and after 25–60 min in Group C), data were collected and used as anaesthesia baseline data. After baseline data collection, PiNO delivery commenced for horses in the PiNO groups. Data were then collected at the end of inhalation anaesthesia.

Calculated data

F-shunt was calculated as follows:

F-shunt = $(CcO_2 - CaO_2)/([CcO_2 - CaO_2] + 35)$, where 35 is a fixed value of $C(a\text{-}v)O_2$ in ml/L [19].

TABLE 3: Comparison of baseline values and values at the end of anaesthesia of various cardiovascular and respiratory parameters in horses undergoing colic surgery and that were given pulsed inhaled nitric oxide (INO, n = 15) and those that were not (CN, n = 15)

Parameter	Group C	Difference of means	Simultaneous 95% CI	P-value
PaO_2 log data	Baseline INO–Baseline CN	−0.1	(−0.6; 0.4)	>0.9
	End CN–Baseline CN	0.0	(−0.5; 0.5)	>0.9
	End INO–Baseline INO	0.7	(0.1; 1.2)	0.007
	End INO–End CN	0.5	(0.0; 1.0)	0.05
$SaO_{2\%}$	Baseline INO–Baseline CN	1	(−7; 8)	>0.9
	End CN–Baseline CN	0	(−7; 8)	>0.9
	End INO–Baseline INO	8	(0; 15)	0.04
	End INO–End CN	8	(1; 16)	0.03
F-shunt%	Baseline INO–Baseline CN	−1	(−12; 9)	>0.9
	End CN–Baseline CN	2	(−9; 12)	>0.9
	End INO–Baseline INO	−11	(−22; −1)	0.04
	End INO–End CN	−14	(−24; −3)	0.005
$PaCO_2$ kPa	Baseline INO–Baseline CN	−0.2	(−1.1; 0.7)	>0.9
	End CN–Baseline CN	0.1	(−0.8; 1.0)	>0.9
	End INO–Baseline INO	0.3	(−0.6; 1.2)	0.8
	End INO–End CN	0.0	(−0.9; 0.9)	>0.9
CaO_2 mL/L	Baseline INO–Baseline CN	−17	(−41; 7)	0.2
	End CN–Baseline CN	6	(−18; 30)	>0.9
	End INO–Baseline INO	32	(8; 56)	0.005
	End INO–End CN	9	(−15; 32)	0.8
HR beats/min	Baseline INO–Baseline CN	2	(−6; 10)	>0.9
	End CN–Baseline CN	4	(−4; 12)	0.6
	End INO–Baseline INO	3	(−5; 11)	0.8
	End INO–End CN	1	(−7; 9)	>0.9
MAP mmHg	Baseline INO–Baseline CN	−6	(−24; 13)	0.8
	End CN–Baseline CN	9	(−9; 28)	0.5
	End INO–Baseline INO	11	(−8; 29)	0.4
	End INO–End CN	−5	(−23; 14)	0.9
$P(A\text{-}a)O_2$ kPa	Baseline INO–Baseline CN	1	(−9; 11)	>0.9
	End CN–Baseline CN	3	(−7; 13)	0.9
	End INO–Baseline INO	−8	(−17; 2)	0.2
	End INO–End CN	−9	(−19; 0)	0.07
pH	Baseline INO–Baseline CN	0.02	(−0.03; 0.07)	0.6
	End CN–Baseline CN	0.02	(−0.03; 0.07)	0.8
	End INO–Baseline INO	0.00	(−0.05; 0.05)	>0.9
	End INO–End CN	0.00	(−0.05; 0.05)	>0.9
Lactate log data	Baseline INO–Baseline CN	0.1	(−0.4; 0.6)	>0.9
	End CN–Baseline CN	0.0	(−0.5; 0.6)	>0.9
	End INO–Baseline INO	−0.1	(−0.6; 0.5)	>0.9
	End INO–End CN	0.0	(−0.5; 0.5)	>0.9

PaO_2, arterial oxygen tension; SaO_2, arterial oxygen saturation; $PaCO_2$, arterial carbon dioxide tension; CaO_2, arterial oxygen content; HR, heart rate; MAP, mean arterial blood pressure; $P(A\text{-}a)O_2$, alveolar-arterial gradient.

CcO_2 is oxygen content in ml/L of capillary blood and calculated as: $CcO_2 = (Hb \times 1.36 \times 1) + (0.227 \times PaO_2)$.

For horses in Group A the mean value of the Hb during anaesthesia was used in the calculations, for horses in Group C the measured Hb for each time point was used.

Data analysis

The raw data collected were entered in Microsoft Excel 2010[P] and then processed. For all statistical calculations, the statistical package Minitab 18[Q] was used, data from Group A and data from Group C were analysed separately. A general linear model, with horse ID as a random effect, was used. Residual plots were used to determine if residual are normally distributed with equal variances. PaO_2 and lactate were not normally distributed and the raw data for these parameters were log transformed before analysed. The Tukey method with a family error rate of 0.05 (equal to a 95% simultaneous confidence level) was used to compare baseline and end values for each parameter. Data are presented as mean ± standard deviation (s.d.), mean and range or as median and interquartile range (IQR) for normally or not normally distributed data respectively.

Results

Horses

All 50 horses in Group A and 30 horses in Group C completed the study. The most common breeds were Warmblood (Group A and C) and Standardbred trotter (Group A). There were no differences in age, weight and anaesthesia time between treatments within each group. In Group A mean (range) age were 4 (1–12) and 4 (1–15) years (P>0.9), weight 480 (330–690) and 480 (340–700) kg (P>0.9), anaesthesia time 96 (40–215) and 75 (45–140) min (P = 0.1) in A-INO and A-CN respectively. In Group C mean age were 10 (1–19) and 11 (1–18) years (P = 0.6), weight 520 (360–700) and 570 (390–690) kg (P = 0.2), anaesthesia time 216 (100–325) and 197 (135–310) min (P = 0.4) in C-INO and C-CN respectively. Descriptive data, shown as mean ± s.d. or median (IQR), are presented in Table 1 and statistical results are presented in Table 2 and Table 3 for Group A and Group C respectively. Details of diagnosis in the group of horses that underwent colic surgery are presented in Supporting Item 1.

Arterial oxygen tension and saturation

Compared with baseline values, median (IQR), 13.6 (9.3, 30.1) and 7.7 (6.4, 8.5) kPa, significant increased PaO_2 was measured at the end of anaesthesia, 24.2 (18.6, 37.0) and 15.5 (9.9, 26.9) kPa, in A-INO ($P = 0.005$) and C-INO ($P = 0.007$) respectively (Fig 1). There were no change in PaO_2 from baseline to end of anaesthesia in A-CN ($P = 0.8$) or C-CN ($P>0.9$). Baseline values were 14.1 (10.2, 21.5) and 6.9 (5.4, 14.6) kPa and end values were 11.3 (9.5, 22.9) and 7.4 (5.7, 15.9) kPa in A-CN and C-CN respectively.

Compared with baseline, SaO_2 was significantly higher at end of anaesthesia in A-INO ($P = 0.005$) and C-INO ($P = 0.04$). Mean (95% CI) difference between baseline and end were 2 (0; 4) and 8 (0; 15)% in A-INO and C-INO respectively. There were no change in SaO_2 from baseline to end of anaesthesia in A-CN ($P = 0.1$) or C-CN ($P>0.9$).

F-shunt

At the end of anaesthesia the F-shunt had decreased significantly compared with baseline values in A-INO ($P = 0.005$) and C-INO ($P = 0.04$). Mean (95% CI) difference between baseline and end were −6 (−10; −1) and −11 (−22; −1)% in A-INO and C-INO respectively (Fig 2). There were no change in F-shunt from baseline to end of anaesthesia in A-CN ($P = 0.2$) or C-CN ($P>0.9$).

Arterial carbon dioxide tension

Compared with baseline, there were no changes in $PaCO_2$ at the end of anaesthesia in any of the four groups.

Discussion

Based on PiNO-induced increases in PaO_2, SaO_2 and decreases in F-shunt, the results of this study show that delivery of PiNO is an effective method to improve oxygenation in horses undergoing arthroscopy and colic surgery that are mechanically ventilated during anaesthesia. The improvement in arterial oxygenation and decrease in shunt was expected based on the results from previous studies [12–16]. However, horses in those studies were hypercarbic due to hypoventilation. During inspiration, mechanical ventilation contributes to an increased intrathoracic pressure, which can force pulmonary blood from nondependent (aerated) to dependent (atelectatic) areas of the lung [20,21], and hence it was not certain whether or not PiNO would be effective. When compared with the Group A horses, Group C horses appeared to have greater cardiorespiratory depression as indicated by the elevated lactate, high F-shunt, and low PaO_2 and SaO_2. Even though Group C horses showed clinical signs of impaired circulation, the MAP was above 70 mmHg at the beginning of anaesthesia. This is likely due to increased vascular resistance (SVR), since HR was not elevated. Although there are no research studies in horses, in other species gastric distension [22] and pain [23], both of which can occur with colic, can contribute to increased SVR. Haemodynamic compromise, advanced age and higher weight can all negatively affect the ventilation/perfusion matching [24,25]. Nevertheless, PiNO caused a significant increase in arterial oxygenation in the colic horses with a magnitude comparable to the improvement in arterial oxygenation induced by PiNO in healthy horses. This finding is important because many horses undergoing colic surgery, including many of the horses in this study, have oxygen deficiency at the induction of anaesthesia [26,27].

It has been shown in previous studies that improvement in arterial oxygenation depends on decrease in right to left pulmonary shunt in horses receiving PiNO [14]. The magnitude of the F-shunt in the present clinical study decreased with PiNO in both Group A and Group C. A possible explanation for the high F-shunt at the beginning of anaesthesia in Group C horses might be gas- or digesta-distended abdomen that causes increased compression atelectasis due to a cranial displacement of the diaphragm [24,26].

The improvement in oxygenation and decrease in F-shunt were not only statistically significant but also clinically relevant as some of the Group C horses had dangerously low PaO_2 and SaO_2 levels (<8 kPa [<60 mmHg] and <90% respectively) prior to PiNO delivery but all were at or above that level during PiNO delivery. Administration of oxygen alone is unlikely to improve indices of oxygenation when the pulmonary shunt is >30–40% [28]. In all horses, the shunt % was >30% and the shunt % increased and indices of oxygen decreased in horses not receiving PiNO.

The horses in this current study were, despite mechanical ventilation, to some extent hypercarbic, but the lowest pH averaged 7.31 and was

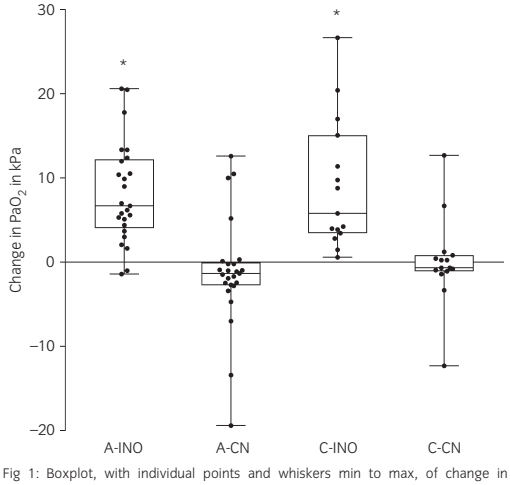


Fig 1: Boxplot, with individual points and whiskers min to max, of change in arterial oxygen tension (PaO_2) in kPa from baseline (beginning of anaesthesia before PiNO delivery commenced) to end of inhalation anaesthesia in horses that were administered pulsed inhaled nitric oxide (PiNO) and underwent arthroscopy (A-INO) or colic surgery (C-INO) and controls (A-CN, C-CN). *Indicates significant change ($P<0.05$) from baseline to end within respective group.

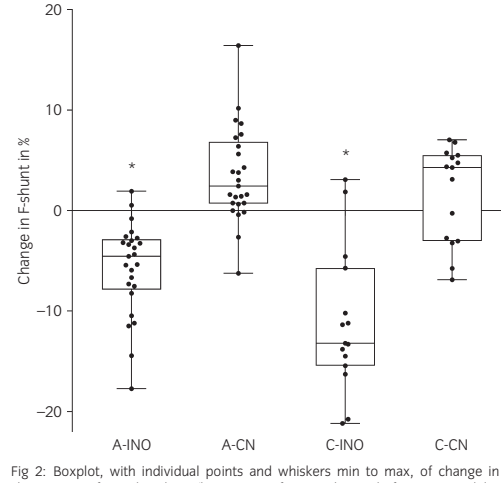


Fig 2: Boxplot, with individual points and whiskers min to max, of change in F-shunt in % from baseline (beginning of anaesthesia before PiNO delivery commenced) to end of inhalation anaesthesia in horses that were administered pulsed inhaled nitric oxide (PiNO) and underwent arthroscopy (A-INO) or colic surgery (C-INO) and controls (A-CN, C-CN). *Indicates significant change ($P<0.05$) from baseline to end within respective group.

not considered clinically important. Although the impact of different levels of $PaCO_2$ was not compared in our study, mild hypercarbia has been shown to be beneficial for cardiovascular function in anaesthetised horses in other studies [29,30]. In addition, ventilation to completely return the patient to normocarbia, including the use of positive end-expiratory pressure and/or recruitment manoeuvres, can be detrimental [31,32]. These techniques improve PaO_2 and decrease $PaCO_2$, but the high airway pressures required for the recruitment of alveoli decreased the cardiac output (CO) and oxygen delivery (DO_2) to the tissues [31,32]. As evidenced in this study, PaO_2 can be improved without high airway pressures by administering PiNO, and mild hypercarbia may be advantageous for maintaining circulation and tissue oxygen delivery [30]. Although no changes in the average lactate concentration were measured between horses treated with PiNO compared with controls, differences were noted on an individual basis. It has been shown previously that the increase in plasma lactate concentration is not paralleled by similar increases in muscle lactate [33]. Dissimilarities may be due to individual differences in venous drainage and an accumulation of produced lactate within the muscle during anaesthesia, thus, more studies are needed to investigate if the effect of PiNO can influence muscle lactate concentration.

Limitations of this study include a slight difference in anaesthesia protocols between Groups A and C, and lack of cardiac output measurements. The anaesthetic protocol in Group A, but not Group C, included acepromazine. Oxygenation was higher and shunt fraction lower in horse receiving acepromazine plus romifidine as premedicants when compared with horses receiving romifidine alone [34]. This might have facilitated PiNO effects on oxygenation and shunt fraction in the Group A horses but a control group without acepromazine would be required to make that conclusion. Although the Group C horses did not receive acepromazine, it is not an appropriate control group. The type of surgery and the fact that horses presented for emergency surgery may have more physiologic abnormalities which make these groups too dissimilar for meaningful statistical comparison. Since cardiac output was not measured DO_2 could not be calculated. Although previous studies have shown that the CO is not affected by PiNO [12,15,17,18,35], the effect on DO_2 under clinical circumstances needs to be studied. Presumably, avoidance of forceful expansion of the alveoli which would have been caused by positive end-expiratory ventilation and administration of adequate fluid therapy preserved CO and DO_2 in the horses in this current study. This was a clinical study to determine the clinical utility of PiNO in anaesthetised healthy and colic patients, not research horses, so these limitations did not influence the goal of the study.

In conclusion, the combination of mechanical ventilation and PiNO improves the pulmonary gas exchange during anaesthesia by a simultaneous decrease in F-shunt and improved alveolar ventilation.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

The study was approved by the local ethics committee for animal experiments, Uppsala, Sweden, approval number C 201/14.

Source of funding

This study was supported by grants from The Swedish-Norwegian Foundation for Equine Research.

Acknowledgements

The authors thank the staff at the Equine Clinic at the University Animal Hospital and at Mälaren Equine Practice for their support, and Claudia von Brömssen at Swedish University of Agricultural Sciences for assistance with statistical analysis.

Owner informed consent

Owners gave consent for their animals' inclusion in the study.

Authorship

M. Wiklund and G. Nyman contributed to study design, study execution, data analysis and interpretation, and preparation of the manuscript. M. Kellgren and S. Wulcan contributed to study design and study execution. T. Grubb contributed to preparation of the manuscript. All authors gave their final approval of the manuscript.

Manufacturers' addresses

[a]Mila International Inc., Erlanger, Kentucky, USA.
[b]N-vet AB, Sweden.
[c]Boehringer Ingelheim Vetmedica, Sweden.
[d]Vetoquinol, Sweden.
[e]Pharmaxim, Sweden.
[f]Ratiopharm, Germany.
[g]Intervet, Sweden.
[h]Orion Pharma Animal Health, Sweden.
[i]Vetronic Services, Devon, UK.
[j]Fresenius Kabi, Sweden.
[k]Carinopharm GmbH, Germany.
[l]AstraZeneca, Sweden.
[m]Datex-Ohmeda Research Unit, Finland.
[n]AGA AB, Sweden.
[o]Radiometer, Denmark.
[p]Microsoft, Redmond, Washington, USA.
[q]Minitab, Ltd., Coventry, Warwickshire, UK.

References

1. Nyman, G., Funkquist, B., Kvart, C., Frostell, C., Tokics, L., Strandberg, A., Lundquist, H., Lundh, B., Brismar, B. and Hedenstierna, G. (1990) Atelectasis causes gas exchange impairment in the anaesthetised horse. *Equine Vet. J.* **22**, 317-324.
2. Nyman, G. and Hedenstierna, G. (1989) Ventilation-perfusion relationships in the anaesthetised horse. *Equine Vet. J.* **21**, 274-281.
3. Wagner, A.E. (2008) Complications in equine anesthesia. *Vet. Clin. North Am. Equine Pract.* **24**, 735-752.
4. Auckburally, A. and Nyman, G. (2017) Review of hypoxaemia in anaesthetized horses: predisposing factors, consequences and management. *Vet Anaesth. Analg.* **44**, 397-408.
5. Hubbell, J.A.E. and Muir, W.W. (2015) Oxygenation, oxygen delivery and anaesthesia in the horse: Oxygenation, oxygen delivery and anaesthesia. *Equine Vet. J.* **47**, 25-35.
6. Pascoe, P.J., McDonell, W.N., Trim, C.M. and Van Gorder, J. (1983) Mortality rates and associated factors in equine colic operations—a retrospective study of 341 operations. *Can. Vet. J.* **24**, 76.
7. Day, T.K., Gaynor, J.S., Muir, W.W., Bednarski, R.M. and Mason, D.E. (1995) Blood gas values during intermittent positive pressure ventilation and spontaneous ventilation in 160 anesthetized horses positioned in lateral or dorsal recumbency. *Vet. Surg. VS* **24**, 266-276.
8. Edner, A., Nyman, G. and Essén-Gustavsson, B. (2005) The effects of spontaneous and mechanical ventilation on central cardiovascular function and peripheral perfusion during isoflurane anaesthesia in horses. *Vet Anaesth. Analg.* **32**, 136-146.
9. Hubbell, J.A.E. (2010) Review of support of ventilation in the anesthetized horse. *Proc. 56th Annu. Conv. Am. Assoc. Equine Pract*. Baltimore, Maryland, USA 4-8 Dec. 2010 33-37.
10. Trim, C.M. and Wan, P.Y. (1990) Hypoxaemia during anaesthesia in seven horses with colic. *Vet Anaesth. Analg.* **17**, 45-49.
11. Whitehair, K.J. and Willits, N.H. (1999) Predictors of arterial oxygen tension in anesthetized horses: 1,610 cases (1992-1994). *J. Am. Vet. Med. Assoc.* **215**, 978-981.
12. Grubb, T. (2012) Evaluation of efficacy and safety of pulsed inhaled nitric oxide in the anesthetized horse preparing for clinical use, Dept. of Animal

Environment and Health, Swedish University of Agricultural Sciences, Uppsala. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-e-732.
13. Grubb, T., Frendin, J.H.M., Edner, A., Funkquist, P., Hedenstierna, G. and Nyman, G. (2013) The effects of pulse-delivered inhaled nitric oxide on arterial oxygenation, ventilation-perfusion distribution and plasma endothelin-1 concentration in laterally recumbent isoflurane-anaesthetized horses. *Vet Anaesth. Analg.* **40**, e19-e30.
14. Grubb, T.L., Lord, P.F., Berger, M., Larsson, C., Rydén, A., Frendin, J., Funkquist, P., Edner, A. and Nyman, G. (2014) Effects of pulse-delivered inhaled nitric oxide administration on pulmonary perfusion and arterial oxygenation in dorsally recumbent isoflurane-anesthetized horses. *Am. J. Vet. Res.* **75**, 949-955.
15. Nyman, G., Grubb, T.L., Heinonen, E., Frendin, J., Edner, A., Malavasi, L.M., Frostell, C. and Högman, M. (2012) Pulsed delivery of inhaled nitric oxide counteracts hypoxaemia during 2.5 hours of inhalation anaesthesia in dorsally recumbent horses. *Vet Anaesth. Analg.* **39**, 480-487.
16. Wiklund, M., Granswed, I. and Nyman, G. (2017) Pulsed inhaled nitric oxide improves arterial oxygenation in colic horses undergoing abdominal surgery. *Vet Anaesth. Analg.* **44**, 1139-1148.
17. Heinonen, E., Hedenstierna, G., Meriläinen, P., Högman, M. and Nyman, G. (2001) Pulsed delivery of nitric oxide counteracts hypoxaemia in the anaesthetized horse. *Vet Anaesth. Analg.* **28**, 3-11.
18. Heinonen, E., Nyman, G., Meriläinen, P. and Högman, M. (2002) Effect of different pulses of nitric oxide on venous admixture in the anaesthetized horse. *Br. J. Anaesth.* **88**, 394-398.
19. Briganti, A., Portela, D.A., Grasso, S., Sgorbini, M., Tayari, H., Bassini, J.R.F., Vitale, V., Romano, M.S., Crovace, A., Breghi, G. and Staffieri, F. (2015) Accuracy of different oxygenation indices in estimating intrapulmonary shunting at increasing infusion rates of dobutamine in horses under general anaesthesia. *Vet. J.* **204**, 351-356.
20. Moens, Y. (2013) Mechanical ventilation and respiratory mechanics during equine anesthesia. *Vet. Clin. North Am. Equine Pract.* **29**, 51-67.
21. Nyman, G. and Hedenstierna, G. (1988) Comparison of conventional and selective mechanical ventilation in the anaesthetized horse; effects on central circulation and pulmonary gas exchange. *J. Vet. Med. Ser. A* **35**, 299-314.
22. Longhurst, J.C., Spilker, H.L. and Ordway, G.A. (1981) Cardiovascular reflexes elicited by passive gastric distension in anesthetized cats. *Am. J. Physiol.-Heart Circ. Physiol.* **240**, H539-H545.
23. Biber, B., Martner, J. and Werner, O. (1983) Modification by baroreceptor feedback of circulatory responses to noxious stimuli during anaesthesia in cats. *Acta Anaesthesiol. Scand.* **27**, 391-395.
24. Moens, Y., Lagerweij, E., Gootjes, P. and Poortman, J. (1995) Distribution of inspired gas to each lung in the anaesthetised horse and influence of body shape. *Equine Vet. J.* **27**, 110-116.
25. Kerr, C.L. and McDonell, W.N. (2009) Chapter 17 - Oxygen Supplementation and Ventilatory Support. In: *Equine Anesthesia (Second Edition)*, Eds: Muir, W.W., Hubbell, J.A.E., W.B. Saunders, St Louis. pp 332-352.
26. Boesch, J.M. (2013) Anesthesia for the Horse with Colic. *Vet. Clin. North Am. Equine Pract.* **29**, 193-214.
27. Edner, A.H., Nyman, G.C. and Essén-Gustavsson, B. (2007) Metabolism before, during and after anaesthesia in colic and healthy horses. *Acta Vet. Scand.* **49**, 34.
28. Petersson, J. and Glenny, R.W. (2014) Gas exchange and ventilation–perfusion relationships in the lung. *Eur. Respir. J.* **44**, 1023-1041.
29. Kalchofner, K.S., Picek, S., Ringer, S.K., Jackson, M., Hässig, M. and Bettschart-Wolfensberger, R. (2009) A study of cardiovascular function under controlled and spontaneous ventilation in isoflurane–medetomidine anaesthetized horses. *Vet Anaesth. Analg.* **36**, 426-435.
30. Khanna, A.K., McDonell, W.N., Dyson, D.H. and Taylor, P.M. (1995) Cardiopulmonary effects of hypercapnia during controlled intermittent positive pressure ventilation in the horse. *Can. J. Vet. Res.* **59**, 213-221.
31. Hopster, K., Kästner, S.B., Rohn, K. and Ohnesorge, B. (2011) Intermittent positive pressure ventilation with constant positive end-expiratory pressure and alveolar recruitment manoeuvre during inhalation anaesthesia in horses undergoing surgery for colic, and its influence on the early recovery period. *Vet Anaesth. Analg.* **38**, 169-177.
32. Hopster, K., Wogatzki, A., Geburek, F., Conze, P. and Kästner, S.B.R. (2017) Effects of positive end-expiratory pressure titration on intestinal oxygenation and perfusion in isoflurane anaesthetised horses. *Equine Vet. J.* **49**, 250-256.
33. Edner, A., Nyman, G. and Essén-Gustavsson, B. (2002) The relationship of muscle perfusion and metabolism with cardiovascular variables before and after detomidine injection during propofol–ketamine anaesthesia in horses. *Vet Anaesth. Analg.* **29**, 182-199.
34. Marntell, S., Nyman, G., Funkquist, P. and Hedenstierna, G. (2005) Effects of acepromazine on pulmonary gas exchange and circulation during sedation and dissociative anaesthesia in horses. *Vet Anaesth. Analg.* **32**, 83-93.
35. Heinonen, E., Högman, M. and Meriläinen, P. (2000) Theoretical and experimental comparison of constant inspired concentration and pulsed delivery in NO therapy. *Intensive Care Med.* **26**, 1116-1123.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Summary in Portuguese
Supplementary Item 1: Colic diagnosis.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2023:11

Hypoxemia is a common complication during equine anaesthesia that is challenging to treat. The main results of this thesis showed that Pulsed Inhaled Nitric Oxide (PiNO) is an effective and easily performed method to improve arterial oxygenation in horses during general anaesthesia and surgery under clinical conditions. During mechanical ventilation, adequate circulation is required to optimise the pulmonary gas exchange. The results suggest that good oxygenation during anaesthesia can have a positive effect on the quality of recovery in horses.

Maja Wiklund received her postgraduate education at the Department of Clinical Sciences. Her undergraduate degree in veterinary medicine was obtained at the Swedish University of Agricultural Sciences (SLU).

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-8046-074-3
ISBN (electronic version) 978-91-8046-075-0