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- The effect on arterial oxygenation

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SUMMARY

The aim of this study was to evaluate the effect of pulsed inhaled nitric oxide (PiNO) during intermittent positive pressure ventilation (IPPV) on arterial oxygenation and right to left vascular shunt in colic horses undergoing abdominal surgery. Hypoxemia is a serious complication commonly occurring during general equine anaesthesia. Several different methods to improve oxygenation have been studied, where use of PiNO has introduced novel thinking. Previous studies have shown that PiNO is an effective method to treat low arterial oxygenation during general anaesthesia in both healthy horses and in spontaneously breathing colic horses. The combination of mechanical ventilation and PiNO has however not before been studied. This is a crucial step before the method can be adapted to clinical practise, as many horses require mechanical ventilation during general anaesthesia to avoid hypoventilation and respiratory acidosis. The results from the present study showed that the alveolar-arterial oxygen partial pressure difference ($P(A\text{-}a)O_2$) decreased in horses receiving PiNO during IPPV. A significant increase in partial pressure of oxygen (PaO_2), oxygen saturation of arterial blood (SaO_2) and oxygen content (CaO_2) was measured. Furthermore, the right to left vascular shunt decreased during PiNO. In conclusion, the present study showed that PiNO during IPPV results in an improved arterial oxygenation in colic horses undergoing abdominal surgery.

SAMMANFATTNING

Syftet med studien var att utvärdera effekten av pulsad inhalerad kvävemonoxid (PiNO) på arteriell syresättning och vaskulär shunt i samband med övertrycksventilering (IPPV) hos kolikhästar som genomgår bukkirurgi. Hypoxemi är en allvarlig och vanligt förekommande komplikation under allmän anestesi på hästar. Flera olika metoder för att förbättra syresättningen hos hästar under allmän anestesi har studerats, varav PiNO är en. Tidigare studier har visat att PiNO är en mycket effektiv metod för att behandla låg arteriell syresättning under allmän anestesi hos både friska hästar och hos kolikhästar som spontanandas. Dock har kombinationen av övertrycksventilering och PiNO ej tidigare studerats. Detta är ett viktigt steg innan metoden kan anpassas till klinisk praxis då många hästar är i behov av mekanisk ventilering under allmän anestesi för att undvika hypoventilation och respiratorisk acidos. Resultaten från den här studien visade att hästar som behandlades med PiNO i kombination med IPPV fick en minskad alveolär-arteriell syrgasdifferans ($P(A\text{-}a)O_2$), ökade i arteriellt syretryck (PaO_2), arteriell syremättnad (SaO_2) och arteriellt syreinnehåll (CaO_2). Jämfört med kontrollhästar som endast erhöll IPPV minskade även den vaskulära shunten när hästarna behandlades med PiNO. Sammanfattningsvis visade studien att behandling med PiNO i kombination med IPPV leder till en förbättrad arteriell syresättning hos kolikhästar som genomgår bukkirurgi.

CONTENT

ABBREVIATION

CaO_2	Oxygen content in arterial blood
CcO_2	Oxygen content in pulmonary capillaries
CvO_2	Oxygen content in venous blood
FiO_2	Fraction of inspired oxygen
Hb	Haemoglobin
iNO	Inhaled nitric oxide
IPPV	Intermittent positive pressure ventilation
N_2	Nitrogen gas
NO	Nitric oxide
NO_2	Nitrogen dioxide
$P(A\text{-}a)O_2$	Partial pressure difference between alveolar and arterial O_2
$PaCO_2$	Partial pressure of CO_2 in arterial blood
PAO_2	Partial pressure of oxygen in the alveolar gas
PaO_2	Partial pressure of oxygen in arterial blood
PEEP	Positive end-expiratory pressure
PIP	Peak inspiratory pressure
PiNO	Pulsed inhaled nitric oxide
Qs/Qt	Ratio of shunted blood (Qs) to total blood flow (Qt)
Qt	Cardiac output
SaO_2	Oxygen/haemoglobin saturation of arterial blood
V_A/Q	Ventilation/perfusion ratio

INTRODUCTION

It is a well-known fact in veterinary medicine that general anaesthesia in horses is associated with higher mortality and higher risk of complications than in other animals, and the risk is even higher when the horse is critically ill (Johnston *et al.,* 2002; Brodbelt *et al.,* 2008). Colic horses undergoing abdominal surgery have an almost ten times higher anaesthesia related mortality compared to healthy horses (Johnston *et al.,* 2002). One common and serious complication during anaesthesia is hypoxaemia. The main cause of hypoxaemia in anaesthetized horses is a right to left vascular shunt that develops in the lungs (Nyman *et al.,* 1989; 1990).

Several studies have shown that pulsed inhaled nitric oxide (PiNO) during the first part of inspiration is an effective and safe method to improve arterial oxygenation during general anaesthesia in spontaneously breathing healthy horses. The improvement is caused by a decrease of the right to left vascular shunt in the lungs (Heinonen *et al.,* 2001; Grubb *et al.,* 2008; Grubb *et al.,* 2012; Nyman *et al.,* 2012; Grubb *et al.,* 2013). A recent study by Wiklund et al. (2017) showed that the method effectively improved arterial oxygenation also in critically ill colic horses undergoing abdominal surgery. Since the horses in that study were breathing spontaneously a significant degree of hypoventilation and respiratory acidosis was evident. To avoid hypoventilation and respiratory acidosis, many horses require mechanical ventilation during inhalation anaesthesia (Day *et al.,* 1995; Blissit *et al.,* 2008). However, both ventilation and blood flow is altered by the positive airway pressure applied to the thorax during artificial ventilation (Steffey *et al.,* 1977; Steffey *et al.,* 1992; Mizuni *et al.,* 1994; Edner *et al.,* 2005). The effect of PiNO on pulmonary gas exchange may differ between mechanically and spontaneously breathing horses and before PiNO can be used routinely in clinical practise it is necessary to investigate the physiological effects during intermittent positive pressure ventilation (IPPV).

The aim of the study is to evaluate the effect of PiNO on arterial oxygenation and on vascular shunt during IPPV in horses undergoing abdominal surgery.

The hypothesis of the present study is that pulsed inhalation of nitric oxide during IPPV will decrease the right to left vascular shunt and improve arterial oxygenation in colic horses undergoing abdominal surgery, compared to control horses only receiving IPPV.

LITERATURE REVIEW

Complications during general anaesthesia

Horses that undergo anaesthesia are at higher risk of complications and mortality compared to other companion animals. The mortality rate reported in healthy individuals is 0.9% (Johnston *et al*., 2002) compared to 0.24% and 0.17% in cats and dogs (Brodbelt *et al.,* 2008). In colic horses undergoing abdominal surgery the mortality rate is as high as 8% (Johnston *et al.,* 2002). Some of the complications associated with general anaesthesia in horses are hypotension, hypercapnia due to hypoventilation, hypoxaemia, fractures during recovery, myopathies and pulmonary oedema (Wagner 2008).

Hypoxaemia is a common and serious complication that has been associated with a range of other severe complications such as sudden cardiac arrest (McGoldrick *et al.,* 1998), postanaesthetic cerebral necrosis (McKay *et al.,* 2002), decreased skeletal muscle oxygenation, lactic acidaemia (Taylor 1998), hepatic insult (Whitehair *et al.,* 1996) and surgical site infection (Costa-Farré *et al.,* 2014). Hypoxaemia is defined as an arterial partial pressure of oxygen (PaO_2) less than 8 kPa (60 mm Hg) (Hubbel *et al.,* 2015). There are five different main causes of hypoxaemia: hypoventilation, decreased fraction of inspired oxygen (FiO_2), impaired oxygen diffusion across the alveolar-arterial membrane, mismatch between the ventilation and the perfusion in the alveoli and shunting of blood through the lungs from the venous to the arterial circulation (Grubb 2012). Beside the huge impact of the right to left vascular shunt on arterial oxygenation in the anaesthetised horse, ventilation-perfusion mismatch contributes to some degree in both spontaneously and mechanically ventilated horses. Further, when the horses breathe spontaneously during general anaesthesia hypoventilation is commonly present (Nyman & Hedenstierna, 1989).

Shunt fraction

Shunting of blood through non-ventilated areas of the lung, leading to deoxygenated blood from the venous circulation being mixed with arterial circulation, is the major cause of hypoxaemia in anesthetized horses (Nyman *et al.,* 1989). Nyman et al. (1989*)* showed that in horses in dorsal recumbency, approximately 34% of the blood passes the lung without passing ventilated alveoli and therefore does not take part in gas exchange. The cause of this large shunt is compression atelectasis in dependent areas of the lung (Nyman *et al.,* 1990). When the horse is positioned in dorsal recumbency atelectasis develops because of the horse's dome shaped diaphragm, leading to intestines ending up on top of the caudal part of the lung (Sorenson & Robinson 1980; Nyman *et al.,* 1990).

The degree of shunt is presented in relation to cardiac output (Qs/Qt) and is an oxygen-based index used to estimate to what degree venous blood passes the lungs without being fully oxygenated. Qs/Qt can be measured with different invasive techniques or calculated based on oxygen content in arterial and mixed venous blood, the latter being collected from the pulmonary artery (Laghi *et al*., 1989; Aaros *et al.,* 2012). In a clinical setting where it is not suitable to use invasive techniques or collect mixed venous blood, Qs/Qt can be substituted with the F-shunt. The F-shunt is an oxygen based index which is based on the formula for Qs/Qt but with a fixed value of 35 ml/L for the difference between oxygen content in arterial and mixed venous blood ($C(a\text{-}v)O_2$) (Briganti *et al.,* 2008; Aaros *et al.,* 2012). This gives a possibility to calculate shunt fraction without needing mixed venous blood from the pulmonary artery. Studies have shown that the F-shunt is the most reliable oxygen indices for estimating shunt fraction, independent of heamodynamic conditions of the patient (Briganti *et al.,* 2008; Aaros *et al.,* 2012).

Different methods on how to correct hypoxaemia have been studied with varying results. One example is selective mechanical ventilation of dependent lung regions, which has been shown to increase PaO_2 markedly (Nyman *et al.,* 1987; Moens *et al.,* 1992). A possible problem with the method is that it requires techniques not practical to use as a routine in clinical practise, for

example is tracheotomy and specialized endotracheal tubes necessary (Nyman *et al.,* 1987; Moens *et al.,* 1992). Another method that has been studied is changing FiO_2 during anaesthesia. Different studies have shown that a high FiO_2 leads to a greater arterial oxygenation compared to a lower FiO_2 (Marntell *et al.,* 2005; Hubbel 2011), however a high FiO_2 increased intrapulmonary shunt and caused hypoventilation (Marntell *et al.,* 2005). A third method is to use positive end-expiratory pressure (PEEP) during IPPV. Wilson & McFeely (1991) showed an increased arterial oxygenation in horses where PEEP was applied, but in a majority of the horses the high pressure led to cardiovascular depression and a decrease in arterial blood pressure that required medical treatment. More recent studies have shown improved PaO_2 in horses ventilated with IPPV with PEEP and alveolar recruitment manoeuvres (RM) (Hopster *et al.,* 2011; Hopster *et al.,* 2016). Even though PaO_2 was improved in the study, CaO_2 and SaO_2 was unchanged, and with increasing airway pressure there was a decreased oxygen delivery. Two disadvantages with the technique is that it required repeated recruitment manoeuvres to be able to keep the lung open (Hopster *et al.,* 2011) and the high intrathoracic pressure caused by PEEP led to decreased cardiac output, decreased oxygen delivery and impaired gastrointestinal perfusion (Hopster *et al.,* 2016).

Most of the previous studies on possible treatment methods of hypoxaemia have focused on altering the ventilation in the lung in different ways. A different approach is to alter perfusion in the lung, which may be done by the method of PiNO. A series of publications have proven PiNO to be an effective and safe way of improving oxygenation in horses during anaesthesia (Grubb *et al.,* 2008; Grubb *et al.,* 2012; Nyman *et al.,* 2012; Grubb *et al.,* 2013; Wiklund *et al.,* 2017).

Nitric oxide

The compound nitric oxide (NO) is a vasodilator that is produced endogenously by endothelial cells in veins and arteries (Ignarro *et al.,* 1987; Palmer *et al.,* 1987). NO is an unstable molecule which rapidly binds to haemoglobin in the blood, leading to inactivation of NO. This means that when NO is administered mixed in to the inhalation gas (iNO) it can act as a local vasodilator in the lung, without causing systemic arterial vasodilation (Frostell *et al.,* 1991). Since the discovery of the effect of NO it has been used in humans to treat hypoxic respiratory failure in neonates and acute respiratory distress syndrome (Dobyns *et al.,* 1999; Desande *et al.,* 2004; Bronicki *et al.,* 2015).

The first time iNO was administrated to horses it was given continuously during inspiration, which had no positive effect on arterial oxygenation (Young *et al.,* 1999). In 2000 the method of pulsing iNO (PiNO) was developed, first in pigs (Heinonen *et al.,* 2000) and later in horses (Heinonen *et al.,* 2001). By dilating the vessels in the well-ventilated areas of the lung, PiNO can initiate redistribution of the blood flow in the lung from the atelectatic, dependent regions to the ventilated, nondependent regions and thus lead to a reduced right to left vascular shunt, improved oxygenation and reduced hypoxemia (Heinonen *et al.,* 2001; Grubb *et al.,* 2014). Heinonen *et al*. (2001) showed that PiNO is an effective method for counteracting impaired gas exchange caused by anaesthesia in horses during spontaneous breathing. Since then several

studies throughout the years have continued to come to the same conclusion (Grubb *et al.,* 2008; Grubb *et al.,* 2012; Nyman *et al.,* 2012; Grubb *et al.,* 2013).

One possible side effect of administrating NO is the formation of the toxic form nitrogen dioxide (NO_2). Since NO is unstable it can spontaneously undergo oxidation in air to NO_2 (Weinberger *et al.,* 2001). NO_2 is known to irritate the airways and can in high doses cause damages to the lungs. Heinonen *et al.* (2002) showed that when NO is given during the first part of inspiration, most part of the nitric oxide is absorbed in the lung which prevent the formation of NO_2.

Previous studies in horses were made in healthy animals during experimental conditions. In 2014 the first clinical trial was conducted at the University of Agricultural Science in Uppsala, Sweden. It was shown that PiNO was also an effective way of improving arterial oxygenation and reducing blood lactate in colic horses undergoing acute abdominal surgery while breathing spontaneously (Wiklund *et al.,* 2017). So far no side effects have been observed in the studies made (Grubb *et al.,* 2008; Grubb *et al.,* 2012; Nyman *et al.,* 2012; Grubb *et al.,* 2013; Wiklund *et al.,* 2017).

Intermittent positive pressure ventilation - IPPV

During general anaesthesia many horses suffer from hypoventilation, due to the respiratory depressant effect of different drugs used, which causes hypercapnia (retention of carbon dioxide in the blood) and respiratory acidosis (Steffey *et al*., 1987; Grosenbaugh *et al.,* 1998). This in combination with compression atelectasis caused by pressure from the heavy abdominal organs on the dependent lung regions makes spontaneous breathing not optimal for many horses (Sorenson & Robinson 1980; Nyman *et al.,* 1990). To counteract these negative respiratory effects, mechanical ventilation is commonly used in today's equine practise (Day *et al.,* 1995; Blissit *et al.,* 2008).

One technique of providing ventilatory support in horses is using positive pressure to expand the lungs, most commonly delivered mechanically by a large animal ventilator. This technique is referred to as IPPV (Moens 2013). Even though IPPV is widely used in equine anaesthesia it has been shown to impair cardiovascular function and oxygen delivery compared to spontaneous ventilation (Steffey *et al.,* 1977; Steffey *et al.,* 1992; Mizuni *et al.,* 1993; Edner *et al.,* 2005). When spontaneously breathing, the intrathoracic pressure is subatmospheric, which promotes venous return to the heart. IPPV increases the intrathoracic pressure during inspiration, reducing venous return, which leads to impaired cardiac output (Steffey *et al.,* 1977; Steffey *et al.,* 1992; Mizuni *et al.,* 1993). The positive pressure in the airway can also cause compression of the capillaries in the lung and increase pulmonary vascular resistance (Kerr & McDonell 2009). Since IPPV affects cardiovascular function and ventilation, the effect of PiNO on mechanically ventilated colic horses undergoing abdominal surgery may be different compared to that in spontaneously breathing colic horses.

MATERIAL AND METHODS

The study was designed as a prospective randomized clinical trial and included horses undergoing abdominal surgery due to colic at the Equine Clinic of the University Animal Hospital (UDS) in Uppsala, Sweden, from October 2015 to November 2016. The study was approved by the local ethics committee for animal experiment, Uppsala, Sweden.

The collection of data was accomplished in collaboration with the anaesthesia team at the clinic. Before the surgery, horse owners signed a consent form agreeing to their horse being enrolled into the study and that samples taken from their horse may be used for research purposes.

Horses

The study comprises 30 horses, where 15 were given nitric oxide (PiNO group) during anaesthesia and 15 served as a control group (C group) that did not receive PiNO. The age, sex, breed, weight and diagnosis of all horses in the study are listed in Table 1. Horses in the PiNO group aged from 1 to 19 years with an average of 9.6 years. The control horses aged from 1 to 18 years with an average of 10.7 years. The average weight in the PiNO group was 521 kg (360 kg – 697 kg) and in the C group 570 kg (392 kg – 689 kg).

The horses in the study were either referred to the clinic by a veterinarian who had examined and treated the horse in the field, or they arrived to the clinic without earlier examination. All horses were examined by a veterinarian when arriving to the clinic consisting of a general clinical examination, a rectal examination, nasogastric intubation and in most cases abdominocentesis. The clinical findings and response to medication determined if the horse should be continued to be treated medically or undergo surgery (laparotomy).

Table 1. *Individual data and diagnosis for each horse*

Horse	**Age (years)**	**Breed**	**Weight (kg)**	**Sex**	**Diagnosis**
PiNO 1	19	Swedish warmblood	600	G	Strangulating lipoma
PiNO 2	2	Swedish coldblood	360	S	Small colon impaction, retroflexion of large colon
PiNO 3	12	Swedish warmblood	697	G	Large colon displacement
PiNO 4	2	Standardbreed trotter	463	S	Large colon displacement
PiNO 5	1	Connemara	437	M	Large colon impaction
PiNO 6	10	Swedish warmblood	675	G	Large colon displacement
PiNO 7	19	Fjord horse	500	G	Large colon torsion and displacement
PiNO 8	7	Mixed breed	460	M	Cecocolic invagination, large colon displacement
PiNO 9	12	Swedish warmblood	604	G	Colon torsion (large amount of blood in abdomen)
PiNO 10	15	Arabian horse	450	G	Large colon torsion and displacement
PiNO 11	5	Tinker	550	G	Foramen epiploicum entrapment

PiNO 12	13	Friesian horse	524	M	Small intestine strangulation
PiNO 13	11	Swedish warmblood	560	G	Large colon displacement, proximal enteritis
PiNO 14	15	Hanoverian horse	535	M	Large colon displacement, adherens between small intestine and nephrosplenic ligament
PiNO 15	1	Mixed breed	400	M	Large colon displacement
C 1	8	Swedish warmblood	615	G	Foramen epiploicum entrapment, large colon impaction
C 2	2	Swedish warmblood	580	G	Jejunocecal invagination
C 3	7	Icelandic horse	392	M	Proximal enteritis
C 4	4	Swedish trotter	557	M	Large colon torsion
C 5	14	Swedish warmblood	689	G	Large colon torsion
C 6	18	Swedish warmblood	586	G	Large colon torsion
C 7	16	Swedish warmblood	634	G	Large colon torsion
C 8	12	Swedish warmblood	602	M	Large colon torsion
C 9	16	Dutch warmblood	526	S	Small intestine torsion
C 10	1	Swedish warmblood	395	S	Large colon displacement
C 11	12	Swedish warmblood	654	M	Large colon displacement
C 12	11	Oldenburg horse	609	M	Small intestine strangulation
C 13	12	Swedish warmblood	620	G	Large colon torsion
C 14	13	Swedish warmblood	565	G	Large colon torsion and impaction
C 15	15	Arabian horse	523	G	Strangulating lipoma

PiNO = horse receiving PiNO during anaesthesia, C = control horse, G = gelding, S = stallion, M = mare.

Anaesthesia

The horses were anaesthetized according to a standard protocol used at the equine clinic at UDS. Before surgery all horses received bensylpenicillin (20 mg/kg, Geepenil® vet, Orion Pharma Animal Health), gentamicin (6.6 mg/kg IV, Gentaject® vet, Ceva Animal Health), and flunixin meglumine (1.1 mg/kg IV, Flunixin® N-vet, N-vet). The jugular vein was catheterised before surgery. The horses were pre-medicated with romifidine (0.1 mg/kg IV, Sedivet® vet, Boehringer Ingelheim Vetmedica) and butorphanol (0.025/kg IV, Butomidor® vet, Richter pharma ag, Austria). Additionally, eight of the horses (six in the PiNO group and two in the control group) also received acepromazine (0.03 mg/kg IM, Plegicil® vet, Pharmaxim) as a premedication, given before administration of romifidine and butorphanol.

General anaesthesia was induced with diazepam (0.03 mg/kg, Diazepam-ratiopharm®, Ratiopharm) and ketamine (2.2 mg/kg IV, Ketaminol® vet, Intervet, Sweden). The horses were intubated with a cuffed tracheal tube and positioned in dorsal recumbency on the surgery table. The tube was connected to a large animal ventilator (Tafonius, Vetronic, Services Ltd) and anaesthesia was maintained with isoflurane (Attane® vet, VM Pharma) in 70-80% oxygen. All horses were ventilated with intermittent positive pressure ventilation (IPPV) during the surgery.

However, some horses were breathing spontaneously during surgery preparations, before IPPV was initiated. The facial artery was catheterised to be able to measure invasive blood pressure and for collection of arterial blood samples. All horses were instrumented with ECG electrodes placed for lead II analysis and measurement of heart rate, and with a pulse oximeter for monitoring oxygen saturation of the blood. During anaesthesia all horses were given an intravenous infusion of crystalloid fluids (Ringer-acetat Fresenius Kabi, Fresenius Kabi or Lactated Ringer's solution, B Braun). Dobutamine (Dobutamin Carino®, Carinopharm) and a colloid solution (Voluven®, Fresenius Kabi) were given symptomatically to treat low blood pressure. Most horses were given intravenous lidocaine (Xylocain®, Astra Zeneca) as additional pain management. Lidocaine was given as a bolus (2 mg/kg IV during 10-15 minutes) followed by a constant rate infusion (2 mg/kg/hr). The infusion was terminated 20-30 minutes before the end of anaesthesia. If the depth of anaesthesia suddenly was reduced the horse was given a bolus dose of thiopental IV (Thiopental Inresa®, 0.5-1 mg/kg, Inresa Arzneimittel GmbH).

At the end of anaesthesia, after the last samples were collected, the horses were weaned off the ventilator back to spontaneous breathing for 5-10 minutes before being moved to the recovery room. At the same time as the IPPV was turned off the treatment with PiNO was discontinued. A majority of the horses were given xylazine IV (0.1-0.2 mg/kg, Rompun® vet, Bayer) and phenylephrine intranasally (9-11 mg, Fenylefrin APL) before recovery. In the recovery stall a nasopharyngeal tube was inserted into one nostril and all horses were supplied with intranasal oxygen (15 L/minute) until attempts to move pulled the tube out. Extubation was performed when the horse started to swallow.

Collection of data

Blood samples were taken in pre-heparinized 2 ml syringes. A baseline sample of both arterial and venous blood was taken at the beginning of anaesthesia after completion of instrumentation and beginning of IPPV ("Baseline"). The time from induction to baseline ranged from 30 to 90 minutes, with a mean time of approximately 50 minutes in both groups. The time between induction and baseline sampling was dependent on how long time it took to prepare the horse and transport it to the surgery theatre.

After baseline sampling, blood samples were collected after 15, 30, 60, 90, 120, 150 and 180 minutes (named "15 minutes", "30 minutes" and so on). Arterial blood was collected at all times and venous blood was collected at baseline, 30, 90, 120 minutes and at the last sample ("End") before end of anaesthesia. The last samples were taken just before termination of IPPV. When both arterial and venous blood was collected the blood samples were taken simultaneously from the two catheters. Depending on the diagnosis and possible complications during surgery the total surgery time differed between the horses, and therefore the total number of samples obtained from each horse also varied.

The following was determined; the blood concentration of haemoglobin (Hb), the oxygen saturation of arterial and venous blood (SaO_2, SvO_2) and the oxygen and carbon dioxide

tensions in arterial blood (PaO_2, $PaCO_2$). Samples were analysed immediately after collection using an ABL90 (Radiometer).

The fraction of inspired oxygen (FiO_2) and the concentration of end-tidal isoflurane (Et ISO), peak inspiratory pressure (PIP), mean arterial blood pressure (MAP), tidal volume (VT), respiratory rate (RR) and heart rate (HR) were recorded from the monitoring screen at the same time as the blood samples were collected.

Nitric oxide

In the PiNO group delivery of PiNO was started immediately after the baseline sample was collected. Nitric oxide was added to the inhalation gas through a thin tube that went from a delivery device developed by Datex-Ohmeda Research Unit, Helsinki, Finland, (Heinonen *et al.*, 2000) to an adapter on the proximal end of the tracheal tube. Every time the ventilator gave the horse a breath, the positive pressure in the system triggered the delivery device to administer a pulse of nitric oxide. The device was set on giving nitric oxide during the first 45% of every breath.

The nitric oxide was supplied in a cylinder of 2000 ppm NO in N_2 (AGA AB, Sweden).

Calculated data

Partial pressure of alveolar oxygen - PAO_2

PAO_2 was calculated using the formula:

$PAO_2 = FiO_2 \times (\text{atmospheric pressure} - 6.27) - (PaCO_2/0.8)$

FiO_2 is the fraction of inspired oxygen. 6.27 is the water vapour pressure in the alveoli in kPa. 0.8 is the respiratory quotient. $PaCO_2$ is the partial pressure of arterial carbon dioxide.

Oxygen content in arterial blood - CaO_2

CaO_2 was calculated using the formula:

$CaO_2 = (Hb \times 1.36 \times SaO_2) / (100 + 0.227 \times PaO_2)$

1.36 is the amount of oxygen in mL that 1 gram of saturated haemoglobin can hold. SaO_2 is the oxygen saturation of arterial blood. 0.227 is a factor of how much oxygen dissolves in blood plasma. PaO_2 is the partial pressure of oxygen in arterial blood.

F-shunt

F-shunt was calculated using the formula:

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

$CcO_2 = (Hb \times 1.36 \times ScO_2/100) + (0.227 \times PaO2)$

$ScO_2 = 100\%$

CcO_2 is the oxygen content (ml L^{-1}) of capillary blood. ScO_2 is the oxygen saturation of capillary blood. 1.36 is the amount of oxygen in mL that 1 gram of saturated haemoglobin can hold. 0.227 is a factor of how much oxygen dissolves in blood plasma. PAO_2 is the partial pressure of alveolar oxygen. 35 is a fixed value of $C(a\text{-}v)O_2$ in ml L^{-1}.

Alveolar-arterial oxygen difference - $P(A\text{-}a)O_2$

$P(A\text{-}a)O_2$ was calculated using the formula:

$$P(A\text{-}a)O_2 = PAO_2 - PaO_2$$

Statistics

All raw data was entered and processed in Microsoft Excel 2011. GraphPad Prism 5 (GraphPad software, USA) was used for all statistical calculations. Mann Whitney test was used to compare the differences between the PiNO and the control group. Friedman test or Wilcoxon test were used to compare differences within the same group between the different time points. The difference was considered significant when $p<0.05$ with a confidence interval of 95%. Data are presented as mean ± SD.

RESULTS

Arterial oxygenation

There were no significant differences at baseline regarding $P(A\text{-}a)O_2$, PaO_2, SaO_2, F-shunt and CaO_2 between horses receiving PiNO and horses in the control group.

The change in $P(A\text{-}a)O_2$ calculated in percent, from baseline to the end of anaesthesia was significantly lower in the group receiving PiNO compared to controls ($p=0.0101$) (Figure 2). In the PiNO group $P(A\text{-}a)O_2$ decreased with 12 ± 22% from baseline to end of anaesthesia compared to an increase of 10 ± 29% in the control group. $P(A\text{-}a)O_2$ decreased during anaesthesia in 13 individuals in the PiNO group and increased in two. In four horses in the control group $P(A\text{-}a)O_2$ decreased during anaesthesia and in eleven horses $P(A\text{-}a)O_2$ increased (Figure 1).

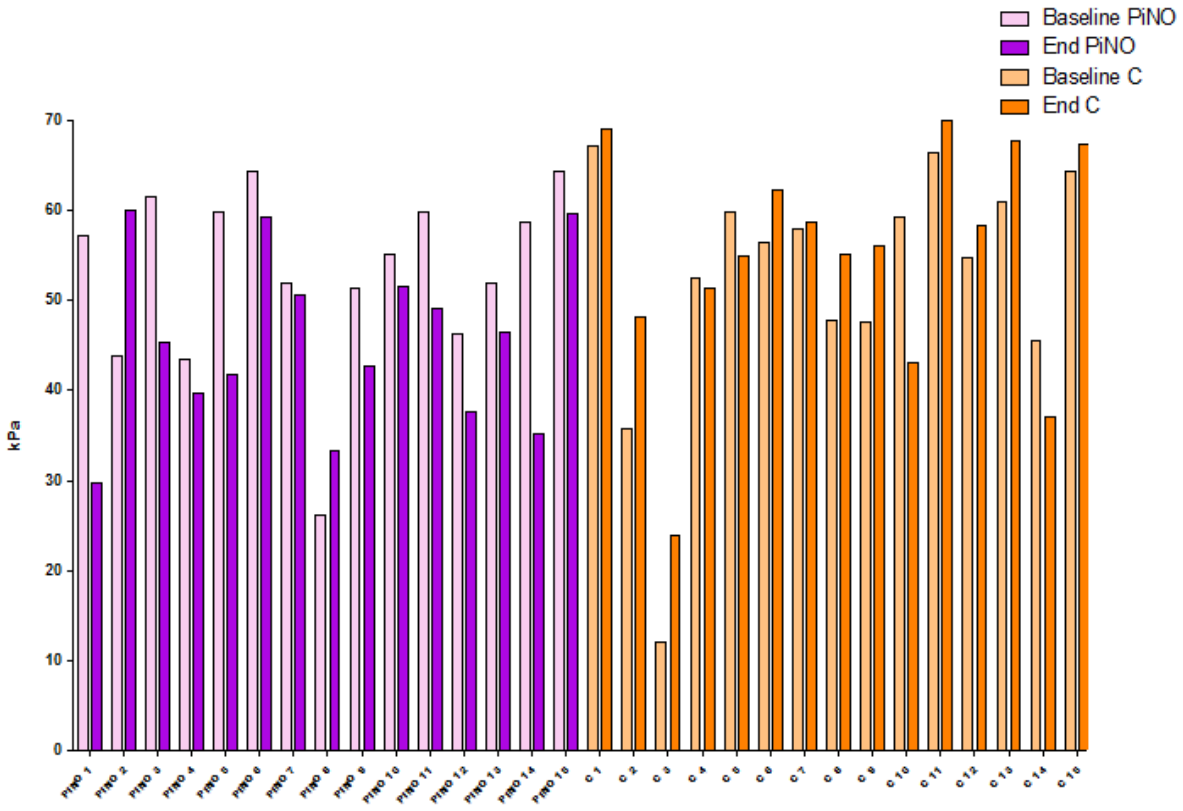

Figure 1. *P(A-a)O_2 at baseline and at the end of anaesthesia in individual horses. The left bar in each pair is illustrating baseline and the right bar is illustrating values at the end of anaesthesia. PiNO = horse receiving PiNO during anaesthesia, C = control horse.*

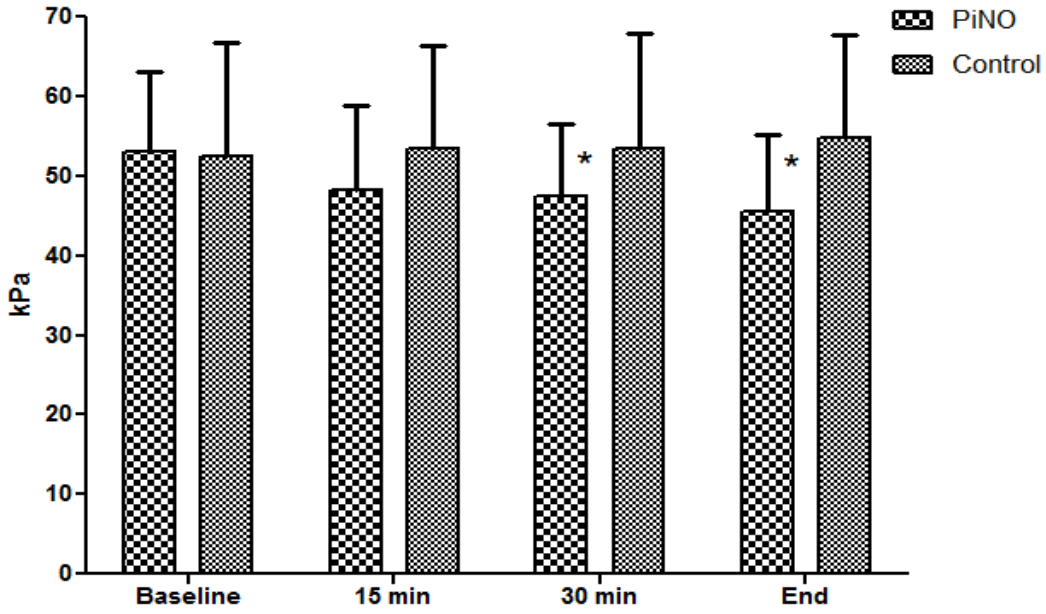

Figure 2. *Mean value and SD of P(A-a)O_2 at different time points during anaesthesia (Baseline, 15 minutes after baseline, 30 minutes after baseline and at the end of anaesthesia) in both groups. PiNO = horses receiving PiNO during anaesthesia, Control = control group. * Significantly different from control group.*

The PaO_2 increased from baseline to the end of anaesthesia in all horses receiving PiNO. In eight horses in each group PaO_2 was below 8 kPa at the beginning of anaesthesia. In seven of these horses in the PiNO group PaO_2 had increased to above 8 kPa at end of anaesthesia. In the eighth horse PaO_2 increased from 5.08 kPa to 6.56 kPa. The PaO_2 decreased in eight horses in the control group during anaesthesia. In eleven horses in the control group PaO_2 was below 10 kPa at end of anaesthesia, and in nine of them PaO_2 was below 8 kPa (Table 2).

Table 2. *Number of horses in each group with a PaO_2 < 8 kPa, 8-10 kPa and > 10 kPa at Baseline and at the end of anaesthesia ("End"). PiNO = horses receiving PiNO during anaesthesia, Control = control group*

	PiNO		**Control**	
	Baseline	End	Baseline	End
> 10 kPa	2	11	4	4
8-10 kPa	5	3	3	2
< 8 kPa	8	1	8	9

There was a significant difference in PaO_2 between the groups at the end of anaesthesia ($p=0.0144$). The PaO_2 increased from Baseline to End in the PiNO group with 110 ± 92%, from an actual value of 9.4 ± 7.2 kPa to 18.4 ± 10.4 kPa. There was no significant increase in PaO_2 in the control group from Baseline to End.

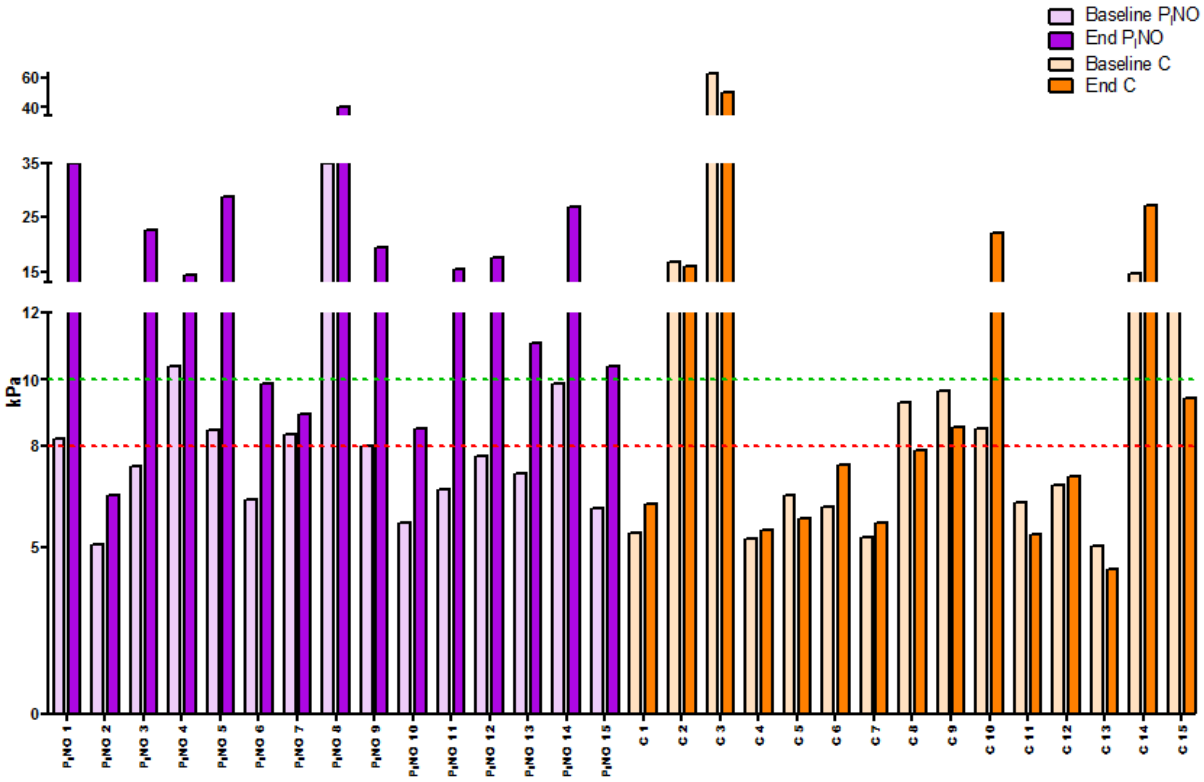

Figure 3. *PaO_2 at baseline and at the end of anaesthesia in individual horses. The left bar in each pair is illustrating baseline and the right bar is illustrating the end of anaesthesia. PiNO = horse receiving PiNO during anaesthesia, C = control horse. Red line PaO_2 = 8 kPa, green line PaO_2 = 10 kPa.*

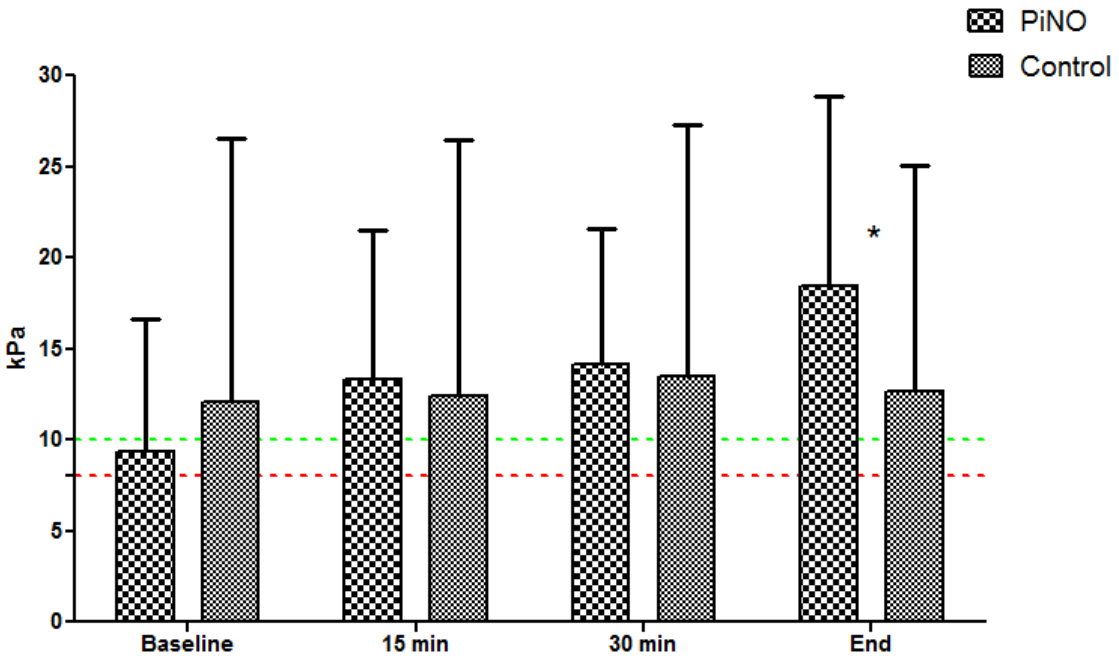

Figure 4. *Mean value and SD of PaO_2 at different time points during anaesthesia (Baseline, 15 minutes after baseline, 30 minutes after baseline and at the end of anaesthesia). PiNO = horses receiving PiNO during anaesthesia, Control = control group. * Significantly different compared to control group. Red dotted line PaO_2 = 8 kPa, green dotted line PaO_2 = 10 kPa.*

There was a significant difference in SaO_2 between the two groups at all measured times after baseline during anaesthesia (Table 3). In the PiNO group SaO_2 improved from baseline to the end of anaesthesia in all horses, from a mean value of 86 ± 8% to 94 ± 5%. In the control group there was no difference in SaO_2 at the end of anaesthesia compared to baseline (85 ± 9% at both baseline and end of anaesthesia) (Table 3, Figure 6).

In the PiNO group SaO_2 was below 90% in ten horses at baseline. In nine of these horses SaO_2 improved to above 90% at the end of anaesthesia. In the tenth horse the SaO_2 increased from 65% to 78%. In total, SaO_2 was above 90% at the end of anaesthesia in 14 horses in the PiNO group. In the control group SaO_2 was below 90% at baseline in ten horses. SaO_2 increased to above 90% at the end of anaesthesia in one of these and stayed below 90% in the other nine control horses. In one horse in the control group SaO_2 decreased from 92% to 89% during anaesthesia. In total, five horses in the control group had a SaO_2 above 90% at the end of anaesthesia (Figure 5).

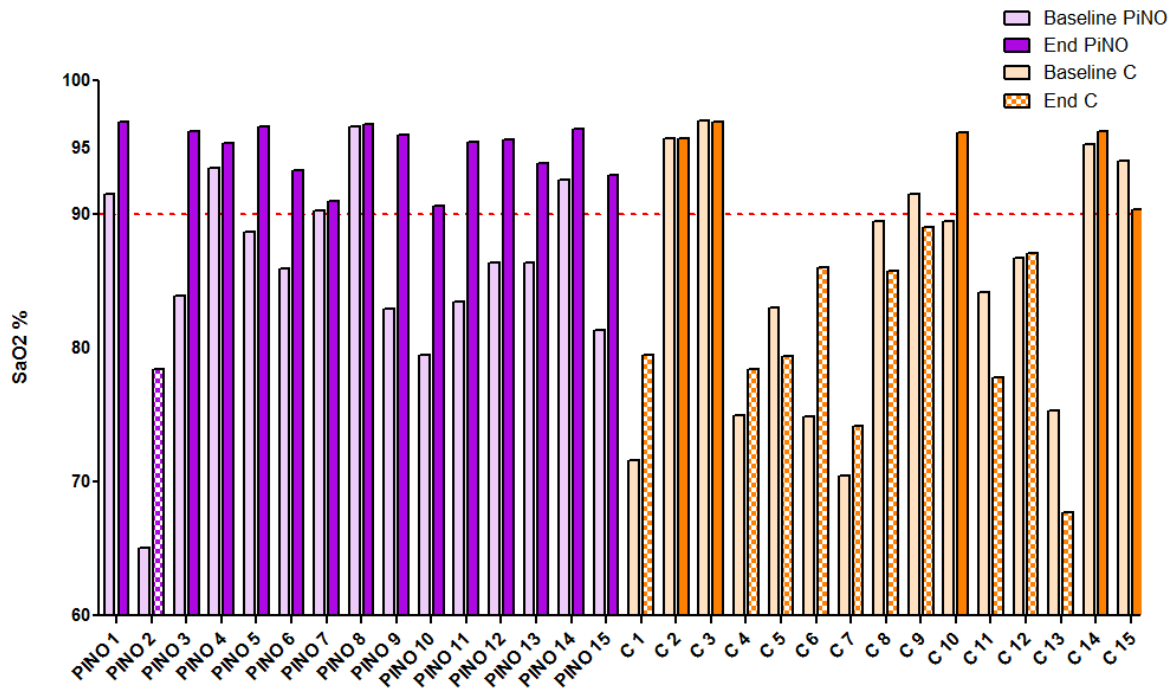

Figure 5. *SaO_2 at baseline and at the end of anaesthesia for each individual horse. The left bar in each pair is illustrating baseline and the right bar is illustrating the end of anaesthesia. PiNO = horse receiving PiNO during anaesthesia, C = control horse. Red dotted line SaO_2 = 90%.*

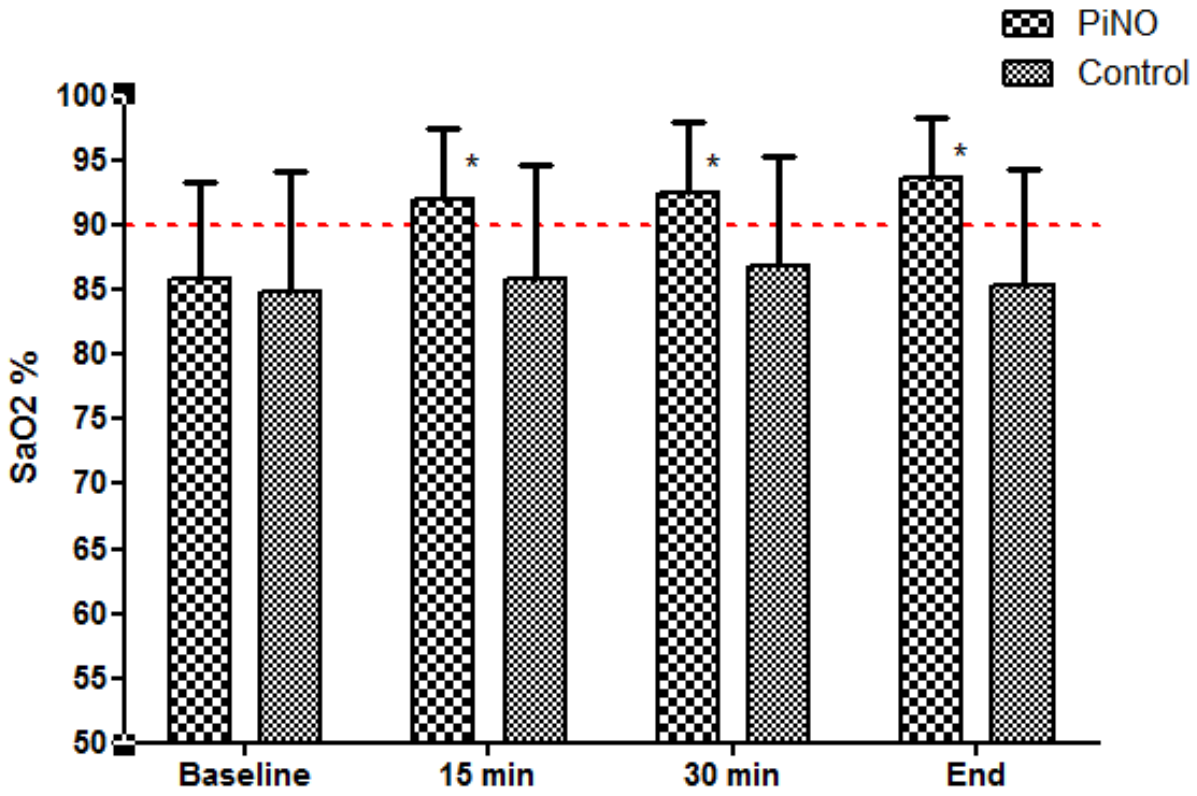

Figure 6. *Mean value and SD of SaO_2 at each measured time point during anaesthesia (Baseline, 15 minutes after baseline, 30 minutes after baseline and at the end of anaesthesia) in both groups. Red dotted line SaO_2 = 90%. PiNO = horses receiving PiNO during anaesthesia, Control = control horses. * Significantly different compared to control group ($p<0.05$).*

In the PiNO group CaO_2 increased significantly from baseline to the end of anaesthesia ($p<0.01$). Comparison of changes in CaO_2 between the groups, calculated in percent during anaesthesia showed a significant difference at 30 minutes after baseline ($p=0.0279$) and a tendency to significance at the end of anaesthesia ($p=0.0512$) (Table 3).

There was a significant difference in F-shunt between horses receiving PiNO and the control horses at the end of anaesthesia ($p=0.0062$). In the PiNO group the F-shunt decreased with 23 ± 16% during anaesthesia, from an actual value of 46 ± 9% to 35 ± 7%. In the control group the F-shunt increased with 3 ± 15%, from an actual value of 48 ± 14% to 49 ± 14%. The F-shunt decreased in 13 horses in the PiNO group from baseline to the end of anaesthesia, compared to seven horses in the control group (Figure 7).

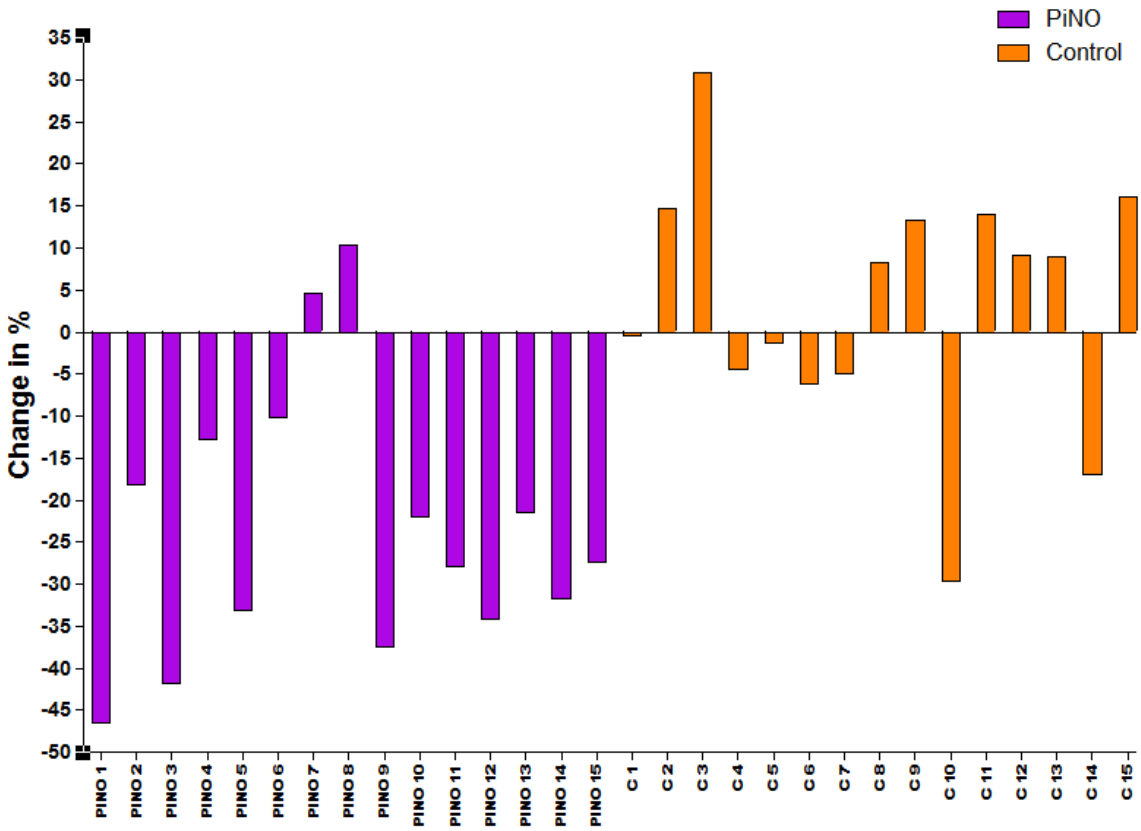

Figure 7. *Individual changes in F-shunt in percent, from baseline to the end of anaesthesia. PiNO = horse receiving PiNO during anaesthesia, C = control horse.*

Table 3. *Mean value ± SD at each measured time point during anaesthesia (Baseline, 15 minutes after baseline, 30 minutes after baseline and at the end of anaesthesia) in both groups*

Group	Parameter	Baseline	*n*	15 min	*n*	30 min	*n*	End	*n*
PiNO	$P(A\text{-}a)O_2$ kPa	53.1 ± 10.1	15	48.3 ± 10.6	15	47.4 ± 9.1 *■	15	45.5 ± 9.6 ***■	15
C	$P(A\text{-}a)O_2$ kPa	52.6 ± 14.1	15	53.4 ± 13.0	15	53.5 ± 14.5	15	54.9 ± 12.8	15
PiNO	PaO_2 kPa	9.4 ± 7.2	15	13.3 ± 8.2 *	15	14.2 ± 7.4 **	15	18.4 ± 10.4 ***■	15
C	PaO_2 kPa	12.1 ± 14.5	15	12.4 ± 14.0	15	13.5 ± 13.8	15	12.6 ± 12.4	15
PiNO	SaO_2 %	86 ± 8	15	92 ± 5 *	15	93 ± 5 **	15	94 ± 5 ***■	15
C	SaO_2 %	85 ± 9	15	86 ± 9	15	87 ± 8	15	85 ± 9	15
PiNO	CaO_2 mL L^-	132 ± 32	15	136 ± 28	15	155 ± 40 ** ■	15	160 ± 35 **	15
C	CaO_2 mL L^-	147 ± 29	15	147 ± 29	15	152 ± 27	15	153 ± 22	15
PiNO	F-shunt %	46 ± 9	15	37 ± 7 ** ■■	15	37 ± 8 **	15	35 ± 7 **■■	15
C	F-shunt %	48 ± 14	15	47 ± 13	15	46 ± 13	15	49 ± 14	15

PiNO = horses receiving PiNO during anaesthesia, C = control horses. * Significantly different compared to baseline. ■ Significantly different compared to control group. */■ $p<0.05$, **/■■ $p<0.01$, ***/■■■ $p<0.001$.

Ventilation and circulation

There was no significant difference between the two groups regarding ventilation parameters (PIP, V_E, $PaCO_2$, Et ISO and FiO_2) during anaesthesia. Mean values of the different parameters are presented in Table 4. In both groups the horses were hypercapnic ($PaCO_2$ > 6 kPa) (Moens 2013) during the whole course of anaesthesia.

There was a significant difference in the concentration of haemoglobin (Hb) between horses in the PiNO group and in the control group at baseline and at 15 minutes after baseline, control horses having a higher Hb than PiNO horses. Regarding heart rate (HR) and mean arterial pressure (MAP) there was no significant difference between the two groups at any time during anaesthesia.

Table 4. *Ventilation and circulation data presented in mean value ± SD for both groups at each measured time point during anaesthesia (Baseline, 15 minutes after baseline, 30 minutes after baseline and at the end of anaesthesia)*

Group	Parameter	Baseline	*n*	15 min	*n*	30 min	*n*	End	*n*
PiNO	V_E L min^{-1}	35 ± 9	15	33 ± 7	15	34 ± 7	15	35 ± 8	15
C	V_E L min^{-1}	36 ± 8	15	38 ± 9	15	39 ± 11	15	40 ± 10	15
PiNO	PIP cmH_2O	28 ± 7	15	26 ± 5	15	26 ± 7	15	26 ± 6	15
C	PIP cmH_2O	29 ± 5	15	28 ± 6	15	27 ± 6	15	26 ± 7	15
PiNO	$PaCO_2$ kPa	7.6 ± 1.4	15	7.8 ± 1.4	15	7.7 ± 1.2	15	7.9 ± 1.1	15
C	$PaCO_2$ kPa	7.8 ± 1.2	15	8.0 ± 1.2	15	7.8 ± 1.1	15	8.0 ± 1.4	15
PiNO	Hb g L^{-1}	110 ± 20 ■	15	106 ± 18 ■	15	120 ± 28	15	121 ± 24	15
C	Hb g L^{-1}	124 ± 18	15	123 ± 18	15	126 ± 15	15	130 ± 16	15
PiNO	HR beats min^{-1}	39 ± 7	15	42 ± 12	15	42 ± 9	15	42 ± 10	15
C	HR beats min^{-1}	37 ± 5	15	39 ± 5	15	41 ± 5 *	15	41 ± 9	15
PiNO	F_iO_2	0.73 ± 0.08	15	0.72 ± 0.08	15	0.72 ± 0.07	15	0.75 ± 0.07	15
C	F_iO_2	0.76 ± 0.09	15	0.77 ± 0.08	15	0.78 ± 0.08 *	15	0.79 ± 0.07 *	15
PiNO	Et ISO	1.0 ± 0.3	15	1.0 ± 0.3	15	0.9 ± 0.3	15	1.1 ± 0.3	15
C	Et ISO	1.0 ± 0.2	15	0.9 ± 0.2	15	1.0 ± 0.2	15	1.1 ± 0.2 *	15
PiNO	MAP mmHg	72 ± 20	15	79 ± 20 *	15	83 ± 17 **	15	82 ± 18 **	15
C	MAP mmHg	76 ± 22	15	84 ± 16	15	86 ± 14	15	87 ± 15 *	15

PiNO = horses receiving PiNO during anaesthesia, C = control horses. * Significantly different compared to baseline. ■ Significantly different compared to control group. */■ $p<0.05$, **/■■ $p<0.01$, ***/■■■ $p<0.001$.

Outcome

In Table 5 the outcome of each horse is presented. The horses were classified as “survived” if they were alive seven days post-surgery. Of the 30 horses included in the study 24 were sent home from the hospital, 13 horses in the PiNO group and eleven horses in the control group. One horse was euthanized during surgery due to bad prognosis, one horse was euthanized in recovery due to an abdominal hernia, one horse died in recovery and three horses were euthanized within seven days after surgery due to complications or bad prognosis.

The length of anaesthesia ranged from 100-320 minutes in the PiNO group, with a mean length of 220 minutes and from 140-310 minutes in the control group with a mean length of 200 minutes.

Post-surgery complications seen in both groups included colic, diarrhoea, fever, impaction, laminitis, colitis, ileus, endotoxaemia and wound infection.

Table 5. *Length of anaesthesia, post-surgery complications (if any) and final outcome of surgery for individual horses*

Horse	**Length of anaesthesia (min)**	**Complications**	**Outcome**
PiNO 1	178		Survived
PiNO 2	268	Colic, diarrhoea, fever, small colon impaction	Survived
PiNO 3	167		Survived
PiNO 4	100		Survived
PiNO 5	222		Survived
PiNO 6	323	Colic, ileus	Survived
PiNO 7	300	Laminitis	Euthanized post surgery
PiNO 8	197		Survived
PiNO 9	165		Survived
PiNO 10	260	Colic, ileus	Survived
PiNO 11	325	Fever	Survived
PiNO 12	110		Euthanized during surgery
PiNO 13	235	Colitis	Survived
PiNO 14	237		Survived
PiNO 15	155	Colic	Survived
C 1	275		Died in recovery
C 2	150		Survived
C 3	157		Survived
C 4	200		Survived
C 5	145		Survived
C 6	310		Euthanized in recovery

C 7	219	Repeated abdominal surgery due to large colon impaction, laminitis, fever	Survived
C 8	180	Colic, ileus, endotoxaemia, laminitis	Euthanized post surgery
C 9	250	Ileus	Survived
C 10	138		Survived
C 11	140		Survived
C 12	253		Survived
C 13	137	Colic, diarrhoea	Survived
C 14	215	Fever, surgical site infection, colic	Survived
C 15	189	Colic, ileus	Euthanized post surgery

PiNO = horses receiving PiNO during anaesthesia, C = control horses

DISCUSSION

The aim of the study was to evaluate the effect of PiNO on arterial oxygenation and vascular shunt during IPPV in colic horses undergoing abdominal surgery. The results showed an improved arterial oxygenation and a decreased vascular shunt in horses receiving PiNO during IPPV compared to control horses only receiving IPPV. These results confirm the hypothesis of the study.

In line with a previous study by Wiklund et al. (2017) where the effect of PiNO was studied in spontaneously breathing colic horses during abdominal surgery, the PaO_2 and SaO_2 increased in all horses receiving PiNO during IPPV. Eight horses in each group had a PaO_2 at baseline that would be defined as hypoxaemia (<8 kPa) (Hubbel & Muir, 2015). At the end of anaesthesia only one horse in the PiNO group were hypoxaemic compared to nine horses in the control group.

There was a significant difference in CaO_2 between the groups at 30 minutes of anaesthesia and although there was a tendency to significant effect at the end of anaesthesia ($p=0.0512$) it was not as apparent as previously reported during spontaneous breathing (Wiklund *et al*., 2017). Cardiac output was not measured in the clinical cases and although CaO_2 was improved in the PiNO treated horses no conclusions on the effect on oxygen delivery to the tissues can be made in this study. In the present study the CaO_2 mean value increased significantly by 23% in the PiNO group and slightly but not significantly with 8% in the control group from baseline to end of anaesthesia, while in the study by Wiklund et al. (2017) the CaO_2 in the PiNO group increased with 3% and the control group decreased with 13%. When comparing these results regarding CaO_2 in the two studies it indicates that the differences may be affected by the different ventilation modes used, with IPPV leading to higher baseline CaO_2 in both groups in the present study compared to the previous study, where horses were breathing spontaneously. This is in line with the results in a study made by Edner et al (2005), comparing the effect of IPPV and spontaneous breathing on physiological parameters in horses during general anaesthesia. SaO_2, PaO_2 and Hb determine CaO_2, and in the study by Edner et al. (2005) the group receiving IPPV showed higher PaO_2 and SaO_2 compared to the spontaneously breathing

horses, but there was no difference in Hb between the groups. In the present study, the Hb concentration is a factor that also may have affected the CaO_2, control horses having a higher Hb through the course of anaesthesia. Despite this, the CaO_2 in the control horses was lower. Possible reasons for the differences in Hb between the groups are many. Since horses have the ability to accumulate and also release a large portion of the red blood cells in the spleen, large variations can be seen. Low Hb is usually caused by anaemia, but can also be caused by an accumulation of red blood cells in the spleen caused by drugs (Whitney 2012). Premedication and induction of anaesthesia in this study followed a predetermined protocol used at the clinic, but not all horses received exactly the same premedication. Some horses received acepromazine as a part of the premedication, which is not included in the clinic's standard protocol for colic horses since the drug impair the vascular function, including vasodilatation and accumulation of red blood cells in the spleen. Even though six horses in the PiNO group were given acepromazine compared to only two horses in the control group, these six horses did not have a lower mean Hb concentration than the rest of the horses in the PiNO group. Thus, although acepromazine can lead to a lower Hb concentration it was not the reason for the significantly lower Hb in the PiNO group in this study. Causes of high Hb are dehydration, endotoxaemia and contraction of the spleen due to excitement, exercise, stress or pain (Whitney 2012). One study has also shown that horses with strangulating obstruction compared to healthy horses and to horses with non-strangulating obstruction had a significantly higher level of Hb (Kyaw *et al.*, 2008). Given that Hb would have been equal in both groups in the present study, the effect on CaO_2 in the PiNO group probably would have been greater.

Although IPPV might have a positive effect on oxygenation one must take into account the negative effects that IPPV has on circulation when evaluating the results in the present study. It has been reported that IPPV impairs cardiovascular function and oxygen delivery compared to spontaneous breathing (Steffey *et al.*, 1977; Steffey *et al.*, 1992; Mizuni *et al.*, 1993; Edner *et al.*, 2005). IPPV may thus alter the effect of PiNO negatively compared to the effect in spontaneously breathing horses.

Ventilation parameters were within normal ranges for horses in dorsal recumbency in both groups (Kerr & McDonell, 2009). Slight hypercapnia was measured in both groups during the entire anaesthesia, with a mean $PaCO_2$ of 7.6-8 kPa, but these values are within the expected range of $PaCO_2$ in mechanically ventilated horses in dorsal recumbency (Kerr & McDonell, 2009; Moens 2013). Mild hypercapnia (7.3-8.7 kPa (Khanna *et al.*, 1995)), as seen in this study, is likely beneficial during anaesthesia. Studies have shown that mild hypercapnia has a stimulating effect on the cardiovascular system and reduces the vasodilating effect of the inhalant drug (Wagner *et al.*,1990, Khanna *et al.*, 1997). Since there was no significant difference between the two groups regarding ventilation parameters it indicates that PiNO does not affect ventilation, which is in line with previous studies (Nyman *et al.*, 2012).

One factor regarding ventilation that may influence the results is the time that passed from induction of anaesthesia to start of IPPV. Several horses in both groups were initially spontaneously breathing during surgical preparations before IPPV was initiated. The reasons for the variations between individuals were different preferences of staff, time taken for

preparations, complications occurring at the beginning of anaesthesia and the current clinical situation. This is likely a factor that could influence the results for some individuals, since it has been shown that an immediate start of IPPV after induction of anaesthesia is advantageous for the gas exchange, compared to a delayed start of IPPV (Day 1995; Wolff & Moens, 2010).

One factor that complicated the delivery of NO during surgery was the occurrence of spontaneous breaths during IPPV in some horses. The device that delivered NO could not deliver NO on every breath if the respiratory rate was more than 8 per minute, because the timespan between the breaths became too short. Since the actual dose of NO delivered to the horses was not measured it is not possible to exactly know the amount of NO for every horse, and whether the spontaneous breaths affected the delivered amount of NO. To ensure the best effect when using PiNO and to ensure that the method works in all horses regardless of respiratory rate and spontaneous breaths during IPPV, the delivery device needs further adjustments to be able to trigger properly even at higher respiratory rates and to trigger on both positive and negative pressure.

Possible sources of error in the present study are that the horses had a variety of diagnoses, which resulted in varying clinical states and lengths of anaesthesia. Factors including how long the horses had been compromised, the treatment before presentation to the clinic and the treatment at the clinic before surgery also varied between individuals. Since the horses were randomly allocated to one of the two groups when entering the study these factors have most likely affected both groups equally and therefore should not have affected the groups differently. Another possible source of error is that all horses were clinical patients at the hospital and a part of the regular clinical work. Thus, different members of the staff supervised the anaesthesia and performed the surgery, which in theory can affect both surgery and quality of anaesthesia. This factor has not likely affected the results in this study since no member of staff predominantly anaesthetized either group. Additionally, the duration from induction to sampling of “Baseline” varied between individuals, resulting in some horses having been anaesthetized for almost one and a half hour before the baseline sample was collected, compared to only 30 minutes in other horses. This may have affected the results in individual horses since atelectasis develops fast after induction during general anaesthesia in horses, with concomitant vascular shunt in the lungs and an impaired gas exchange (Nyman *et al.,* 1989; Nyman *et al.,* 1990), but should not have affected the results differently between the groups since the mean time from induction to sampling of baseline was the same in both groups. All of the above stated problems may have caused problems for the performance of this study but represents a true clinical situation, which is what PiNO is meant for, and despite all of these problems PiNO led to a significantly greater oxygenation.

PiNO has been proven to be an easy and effective treatment of low PaO_2, without any known negative side effects (Grubb *et al.,* 2008, Nyman *et al.,* 2012; Grubb *et al.,* 2012; Grubb *et al.,* 2013; Wiklund *et al.,* 2017), compared to that caused by PEEP during IPPV, another method used to treat hypoxaemia. Several studies on PEEP during IPPV have shown negative effects on the cardiovascular system, leading to decreased cardiac output, low arterial blood pressure and impaired oxygen delivery (Wilson & McFeely, 1991; Hopster *et al.,* 2011; Hopster *et al.,*

2016). Unlike selective mechanical ventilation of dependent lung regions, another method earlier discussed (Nyman *et al.*, 1987; Moens *et el.*, 1992), PiNO is minimally invasive and easy to use in clinical practise. Even if certain horses may not respond to PiNO as well as others, it is a harmless method to try. PiNO has been proven to effectively improve oxygenation in colic horses during both spontaneous breathing and during IPPV, and with further adjustment to the delivery device PiNO will most likely be a method used in clinical practise for treatment of hypoxaemia in the future.

Conclusion

In conclusion, pulsed inhaled nitric oxide during intermittent positive pressure ventilation reduces the right to left vascular shunt and improves arterial oxygenation in colic horses undergoing abdominal surgery.

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