Oxygen supplementation before induction of general anaesthesia in horses

Journal:	*Equine Veterinary Journal*
Manuscript ID	EVJ-TN-15-150.R1
Wiley - Manuscript type:	Technical Notes
Discipline:	Anaesthesiology
Body System/Disorder:	Respiratory: lower airways and pleural cavity < Respiratory
Abstract:	Reasons for performing study: Hypoventilation or apnoea, caused by the induction of general anaesthesia, may cause hypoxaemia. Pre-oxygenation can be employed to lengthen the period before this happens. No scientific studies are published on pre-oxygenation in equine anaesthesia. This technical note describes a successful technique in this respect. Objectives: To determine whether supplementation of oxygen at a flow rate of 15 litres per minute for three minutes via a nasal cannula before induction of general anaesthesia is effective in elevating the arterial partial pressure of oxygen (PaO2) directly after induction. Study design randomized, prospective clinical trial Methods Eighteen adult horses, American Society of Anesthesiologists (ASA) physical status 1 or 2, undergoing elective anaesthesia were randomly allocated to one of two groups. The first group (control group) received no oxygen supplementation before induction of general anaesthesia, whereas the second group (oxygen group) did. All horses were anaesthetized with intravenous detomidine, butorphanol, ketamine, midazolam and isoflurane. Directly after induction and 30 minutes later, T=0 and T=30, respectively, an arterial blood sample was taken for blood gas analysis. For T=30 an estimate of intrapulmonary shunt fraction (Qs/Qt) was calculated. Results At T=0 arterial partial pressure of oxygen (PaO2) was significantly higher in the oxygen group compared to the control group (11.0 ± 2.6 kPa versus 7.4 ± 1.6 kPa; mean ± SD), (p=0.0048), at T=30 differences were not statistically significant. Partial pressure of carbon dioxide (PaCO2) and Qs/Qt did not differ between the groups. Conclusions: Supplementing oxygen by a nasal cannula before induction of general anaesthesia in horses is feasible and does effectively elevate the PaO2 immediately after induction. Future research is needed to determine whether supplementation of oxygen before induction of general anaesthesia in horses will affect outcomes.

1 **Oxygen supplementation before induction of general anaesthesia in horses.**

2

3 **Word count (including abstract, references, and table legend): 2590**

Abstract

Reasons for performing study: Hypoventilation or apnoea, caused by the induction of general anaesthesia, may cause hypoxaemia. Pre-oxygenation can be employed to lengthen the period before this happens. No scientific studies are published on pre-oxygenation in equine anaesthesia. This technical note describes a successful technique in this respect.

Objectives: To determine whether supplementation of oxygen at a flow rate of 15 litres per minute for three minutes via a nasal cannula before induction of general anaesthesia is effective in elevating the arterial partial pressure of oxygen (PaO_2) directly after induction.

Study design randomized, prospective clinical trial

Methods Eighteen adult horses, American Society of Anesthesiologists (ASA) physical status 1 or 2, undergoing elective anaesthesia were randomly allocated to one of two groups. The first group (control group) received no oxygen supplementation before induction of general anaesthesia, whereas the second group (oxygen group) did. All horses were anaesthetized with intravenous detomidine, butorphanol, ketamine, midazolam and isoflurane. Directly after induction and 30 minutes later, T=0 and T=30, respectively, an arterial blood sample was taken for blood gas analysis. For T=30 an estimate of intrapulmonary shunt fraction (Qs/Qt) was calculated.

Results At T=0 arterial partial pressure of oxygen (PaO_2) was significantly higher in the oxygen group compared to the control group (11.0 ± 2.6 kPa versus 7.4 ± 1.6 kPa; mean ± SD), (p=0.0048), at T=30 differences were not statistically significant. Partial pressure of carbon dioxide ($PaCO_2$) and Qs/Qt did not differ between the groups.

Conclusions: Supplementing oxygen by a nasal cannula before induction of general anaesthesia in horses is feasible and does effectively elevate the PaO_2 immediately after

induction. Future research is needed to determine whether supplementation of oxygen before induction of general anaesthesia in horses will affect outcomes.

Introduction

Pre-oxygenation is a common practice in human and small animal anaesthesia to lengthen the period before hypoxaemia, caused by hypoventilation or apnoea resulting from induction of general anaesthesia, develops. To the authors' knowledge, however, there are no scientific reports on techniques, strategies, benefits, and potential adverse effects of pre-oxygenating horses before induction of general anaesthesia.

Although a previous study has shown that supplementing oxygen through a nasal cannula in standing awake horses is effective in increasing the arterial partial pressure of oxygen (PaO_2) [1], it can be questioned whether supplementing oxygen to horses before induction of general anaesthesia is feasible and effective in preventing potential hypoxaemia.

The aim of the current study was to determine whether supplementing oxygen via a nasal cannula before induction of general anaesthesia in horses is effective in increasing the PaO_2 immediately after induction of general anaesthesia, whether it leads to disturbance of the induction process, and whether it affects venous admixture (Qs/Qt) by increasing resorption atelectasis.

Materials and methods

Animals

Eighteen horses, ASA-1 and ASA-2, scheduled to undergo elective anaesthesia were enrolled. Written consent was obtained from all owners, and all animals were treated in accordance with our institutional ethical guidelines. An a priori sample size calculation, using the expected PaO_2 values of Wilson et al [1], showed that one animal per group was needed for a power of 0.8 and alpha of 0.05. However, more animals were included in the study to allow for statistical testing and subsequent generalisation of the results.

The animals were randomly allocated to either the control group or the oxygen group, which was concealed by drawing pre-written lots from an opaque envelope. Table 1 outlines details on group composition.

Procedures

All horses were premedicated with butorphanol (Dolorex)[a] 20.0 µg/kg bwt IV and detomidine (Domosedan)[b] 10.0 µg/kg bwt IV.

After adequate sedation (low head position and decreased responsiveness), the animals were placed behind a swing door for induction. A nasal cannula (silicon tube with an external diameter of 10 mm and 50 cm in length) was held over the face with the tip at the medial canthus of the left eye and a mark was placed on the tube at the level of the left nostril. After marking, the cannula was advanced into the left ventral nasal meatus until the mark on the tube was at the level of the nostril. Acceptance of the cannula was subjectively scored on a three point scale (good, moderate, poor) by the same observer. When in place, the oxygen flow was turned on and immediately increased to 15 litres per minute in the oxygen group. In the control group the oxygen flow was not turned on. The cannula was left in place for three minutes before the induction agents were given.

In all horses, anaesthesia was induced with ketamine (Narketan)[c] 2.2 mg/kg bwt IV and midazolam (Midazolam Actavis)[d] 0.05 mg/kg bwt IV. Directly after induction (T=0, all horses in left lateral recumbency), an arterial blood sample was drawn anaerobically from the right facial artery, collected in a balanced heparinised syringe[e] and directly analysed on a blood gas analyser[f]. All samples were obtained within 1 minute after the facial artery could be safely accessed. After oro-tracheal intubation the endotracheal tube was attached to a circle breathing system and a fresh gas mixture of isoflurane in oxygen and air was given to both groups at a total flow rate of 4-6 litres per minute and with an inspiratory oxygen

fraction (FiO_2) of 50%. End tidal isoflurane concentration (ET-Iso) was targeted at 1.3%. Thirty minutes after taking the first arterial blood sample, a second arterial blood sample was taken (T=30) and directly analysed for blood gasses and haemoglobin content. The latter was analysed on a haematology analyser[g]. At this point the horses were either in lateral or dorsal recumbency as stated in table 1. The actual FiO_2, measured by our anaesthesia monitor[h] and atmospheric pressure, measured by the blood gas analyser[f], were noted. Based on these parameters, an estimate of shunt fraction (Qs/Qt) was calculated, using equation 1 [2, 3, 4]. During the 30 minute period in which blood samples were taken, the animals were breathing spontaneously. After this period, the anaesthetist involved could change ventilation, fresh gas flow and ET-Iso as needed for appropriate maintenance of anaesthesia. All procedures in the first 30 minutes period were carried out by the first author of the manuscript.

Equation 1:

$Qs/Qt = (CcO_2-CaO_2)/(CcO_2-(CaO_2-3.5))$

With:

Qs/Qt; estimated shunt fraction

CcO_2; Pulmonary capillary oxygen content, calculated by:

$CcO_2 = 1.31xHbxSaO_2 + 0.0225xPAO_2$

CaO_2; Arterial oxygen content (ml/dl), calculated by:

$CaO_2 = (1.31xSaO_2xHb) + (0.0225xPaO_2)$

CaO_2-3.5; estimated mixed venous oxygen content (CvO_2), with fixed oxygen extraction of 3.5 ml of oxygen per dl of blood

Hb; Haemoglobin concentration (g/dl)

SaO_2; fraction of saturated arterial blood

PaO_2; partial pressure of oxygen in arterial blood (kPa), obtained from blood gas analysis

PAO_2; Alveolar partial pressure of oxygen (kPa), calculated by the alveolar gas equation:

$PAO_2 = FiO_2$ x (Patm – Pwater)-($PaCO_2$/RQ)

FiO_2; inspired fraction of oxygen

Patm; atmospheric pressure (kPa)

Pwater; saturated water vapour pressure (6.25 kPa at body temperature)

$PaCO_2$; obtained from blood gas analysis

RQ; respiratory quotient: 0.8

Data and statistical analysis

Data were analysed using Microsoft Excel 2010[i] and SPSS 20[j].

Values for age, weight, PaO_2, $PaCO_2$, and Qs/Qt (calculated using equation 1) were tested using an independent samples T-test (two-sided), as assumptions on normality and homogeneity of variance were met. Bonferroni correction was used to correct for multiple testing (*p*-value x 2). Group distribution regarding gender, ASA-physical status, and position during the maintenance phase of anaesthesia was tested using a Fisher's exact test. Differences were considered statistically significant when $p \leq 0.05$.

Results

Results are summarized in table 1.

No statistically significant differences in data on group composition were found.

In two horses (one in each group), acceptance to the silicon tube in the nose was scored as 'moderate', the observed reactions were: sneezing, head shaking and head lift. In all other animals it was scored as 'good'.

At T=0, the PaO_2 of the oxygen group was significantly higher compared to the control group ($t_{16} = 3.5951$ $p = 0.0048$). For the $PaCO_2$ and Qs/Qt there were no significant differences between groups.

Discussion

At T=0, in all control group animals, but one, PaO_2 values were below the cut-off point at which intervention to treat the hypoxaemia is deemed necessary, i.e. <8-9.3 kPa [5]. In contrast, in the oxygen group only one animal had a PaO_2 value <8-9.3 kPa. This shows that horses who breathe room air before induction of general anaesthesia are likely to develop hypoxaemia in the early phase after induction and that supplementing oxygen, using the technique described in this study, may prevent this.

There is limited scientific literature on the effect of hypoxaemia on short and long term outcome in anaesthetized horses. Although this lack of evidence does not mean that hypoxaemia does not affect outcome in horses, we know that horses possess a large cardiopulmonary reserve capacity which may allow them to be less affected by hypoxaemia than other species. This large reserve results from a superior O_2 delivery system consisting of 1) a large heart (up to 2% of body weight) with high stroke volumes, 2) a high maximum heart rate (up to 250 beats per minutes), 3) a highly contractive spleen that can increase haematocrit levels up to 60-70%, and 4) a slightly left shifted O_2 dissociation curve (P50 of ~3.3 kPa) [6]. However, stroke volume, myocardial inotropy, venous return, heart frequency, sympathetic tone, splenic contraction and local tissue perfusion pressure are negatively affected by anaesthetics and recumbency. As a consequence, general anaesthesia can decrease the cardiopulmonary reserve of both healthy and sick horses impairing the capacity to cope with hypoxaemia. A recent study shows that hypoxaemia (next to duration of anaesthesia and type of suture material) is a risk factor for developing wound infection in

152 emergency colic horses [7]. This supports the view that it is relevant to prevent hypoxaemia
153 during general anaesthesia in horses, as it may affect outcome.

154 It could be argued that, for better comparison between the groups, instead of no flow,
155 air (FiO_2 0.21) at a flow rate of 15 litres/min should have been given to the control group. In
156 the oxygen group, the flow itself might have stimulated the animals, leading to increased
157 ventilation and improved oxygenation. However, we assume that this effect was minimal as
158 in both groups none of the animals but one were disturbed by the placement of the nasal
159 cannula and all animals were adequately sedated for safe and uneventful induction of general
160 anaesthesia. In addition, no differences in $PaCO_2$ were found between groups, suggesting that
161 the oxygen group did not have an increased alveolar minute volume compared to the control
162 group.

163 In theory, oxygen flow rates of higher than 15 litres per minute could have been given
164 to further increase the PaO_2 at T=0. Unfortunately, our oxygen flow meter did not allow for
165 accurate measurement of higher oxygen flow rates. Another option to further increase the
166 PaO_2 is to deliver the oxygen bilaterally in the nose [1].

167 Pre-oxygenation could potentially increase intrapulmonary shunt by increasing
168 resorption atelectasis [8]. Our results show no significant difference in intrapulmonary shunt
169 fraction (Qs/Qt) between the oxygen and control group at T=30, making it less likely that the
170 oxygen supplementation before induction of general anaesthesia as described in this study
171 increases intrapulmonary shunt by promoting resorption atelectasis. In this study Qs/Qt was
172 estimated by using equation 1. Calculating the true Qs/Qt, requires mixed venous blood
173 samples, obtained via a pulmonary artery catheter. Placing a pulmonary artery catheter is not
174 without risk and therefore considered non-feasible in studies using client owned horses.
175 Therefore, alternatives, such as the equation used in the present study are employed to non-

invasively calculate Qs/Qt. The equation used, assumes a fixed oxygen content difference between arterial and mixed venous blood of 3.5 ml/dl. This assumption is based on previous work in humans [2], and allows to calculate Qs/Qt without the need for mixed venous blood samples. Although this way of calculating Qs/Qt has been published in equine studies [3, 4], the formula is not yet validated in this species. However, at present it seems the best surrogate to non-invasively calculate Qs/Qt in client owned horses.

Supplementing oxygen to horses before induction of general anaesthesia could potentially lead to hypercapnia and respiratory acidosis in the early maintenance phase after induction, due to absence of a hypoxic respiratory drive. This phenomenon was observed previously in anaesthetized elk [9]. However, the PaO_2 of the elk that demonstrated hypoxaemic respiratory drive (~4 kPa) was lower than the mean PaO_2 in our control group (7.4 kPa). Also, in awake horses, hypoxaemic respiratory drive was shown at a PaO_2 of 5 kPa [10], which is below the PaO_2 in our control group. As our results show that the arterial carbon dioxide concentration does not differ between groups, we conclude that supplementing oxygen to horses before induction of anaesthesia in itself does not lead to hypercapnia. The hypercapnia found in this study is most likely due to the respiratory depressant effect of general anaesthesia induced with ketamine and midazolam and maintained by an inhalant [11].

We conclude that supplementing oxygen to horses before induction of general anaesthesia is effective in increasing the PaO_2 during the early anaesthesia maintenance phase, does not disturb the induction process and does not increase Qs/Qt. However, future research is needed to determine whether this technique is equally effective in non-healthy horses and whether preventing hypoxaemia during the early maintenance phase will improve overall short and long term outcome.

Table 1. Results for distribution of demographic data, PaO_2, $PaCO_2$, and Qs/Qt.

		Control group	Oxygen group
Gender		Female: 3 Male: 6	Female: 3 Male: 6
Age (years; mean ± SD)		8.1 ± 5.4 (range: 3-18)	9.2 ± 5.3 (range: 3-18)
Weight (kg; mean ± SD)		487 ± 58.7 (range:400-560)	569 ± 84.9 (range: 430-680)
ASA classification		ASA-1: 6 ASA-2: 3	ASA-1: 6 ASA-2: 3
Breed		Royal Dutch Warmblood: 4 Arabian: 2 Haflinger: 1 Oldenburger: 1 Frisian: 1	Royal Dutch Warmblood: 5 Arabian: 1 Haflinger: 1 Shire: 1 Hungarian: 1
Acceptance to nasal cannula		Good: 8 Moderate: 1	Good: 8 Moderate: 1
Position during maintenance		Lateral: 7 Dorsal: 2	Lateral: 7 Dorsal: 2
PaO_2 (kPa) (mean ± SD)	T=0 [a]	7.4 ± 1.6 (range: 5.0-10.9)	11.0 ± 2.6 (range: 7.0-15.7)
	T=30	15.9 ± 6.9 (range: 7.9-27.2)	14.2 ± 5.7 (range: 8.21-24.8)
PaCO2 (kPa) (mean ± SD)	T=0	6.8 ± 0.6 (range: 5.5-7.6)	7.5 ± 1.3 (range: 6.5-9.4)
	T=10	7.8 ± 1.4 (range: 5.3-9.7)	8.3 ± 1.2 (range: 6.1-10.3)
Qs/Qt (%) (mean ± SD)		20 ± 11 (range: 6-41)	24 ± 9 (range: 12-40)

PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide; Qs/Qt, estimate of pulmonary shunt fraction. [a]Significant difference between groups $p \leq 0.05$.

Manufacturers' details

[a] MSD Animal Health, Boxmeer, the Netherlands

[b] Zoetis Animal Health, Capelle a/d IJssel the Netherlands

[c] Vetoquinol, Breda, the Netherlands

[d] Actavis, Hafnarfjordur, Iceland

[e] Rapidlyte; Siemens Healthcare Diagnostics, Tarrytown, NY, USA

[f] Rapidlab 1200 series, Siemens Healthcare Diagnostics, Tarrytown, NY, USA

[g] Medonic CA 530 Vet analyzer, Boule Medical AB, Spånga, Sweden

[h] Datex S/5, Datex Ohmeda, Helsinki, Finland.

[i] Microsoft, Redmond, WA, USA.

[j] IBM software, Armonk, NY, USA.

References

[1] Wilson, D.V., Schott, H.C. 2nd, Robinson, N.E., Berney, C.E. and Eberhart, S.W. (2006) Response to nasopharyngeal oxygen administration in horses with lung disease. *Equine Vet J* **38**, 219-223.

[2] Harrison, R.A., Davison, R., Shapiro, B.A. and Meyers, S.N. (1975) Reassessment of the assumed A-V oxygen content difference in the shunt calculation. *Anesth Analg* **54**, 198-202.

[3] Briganti, A., Portela, D.A., Sgorbini, M., Tayari, H., Fusar Bassini, R., Romano, M.S., Breghi, G. and Staffieri, F. (2011) Comparison of different oxygenation indices for the estimation of intrapulmonary shunt in horses under general anaesthesia. *Vet Anaesth Analg* **38**, 5-6.

[4] Mosing, M., Rysnik, M., Bardell, D., Cripps P.J. and MacFarlane P.D. (2013) Use of continuous positive airway pressure (CPAP) to optimise oxygenation in anaesthetised horses - a clinical study. *Equine Vet J* **45**, 414-418.

[5] Haskins, S.C. (2007) Monitoring anesthetized patients. In: *Lumb and Jones' veterinary anesthesia and analgesia*, 4th edn., Eds: W.J. Tranquilli, J.C. Thurmon, K.A. Grimm, Blackwell Publishing, Ames, IA, USA. pp 533-560.

[6] Poole, D.C. and Erickson, H.H. (2011) Highly athletic terrestrial mammals: horses and dogs. *Compr Physiol* **1**, 1-37.

[7] Costa-Farré, C., Prades, M., Ribera, T., Valero, O. and Taurà P. (2014) Does intraoperative low arterial partial pressure of oxygen increase the risk of surgical site infection following emergency exploratory laparotomy in horses? *Vet J* **200**, 175-180.

[8] Hedenstierna, G., Edmark, L., and Aherdan, K.K. (2000) Time to reconsider the pre-oxygenation during induction of anaesthesia. *Minerva Anestesiol* **66**, 293-296.

[9] Paterson, J.M., Caulkett, N.A. and Woodbury, M.R. (2009) Physiologic effects of nasal oxygen or medical air administered prior to and during carfentanil-xylazine anesthesia in North American elk (Cervus Canadensis manitobensis). *J Zoo Wildl Med* **40**, 39-50.

[10] Pelletier, N. and Leith, D.E. (1995) Ventilation and carbon dioxide exchange in exercising horses: effect of inspired oxygen fraction. *J Appl Physiol (1985)* **78**, 654-662.

[11] Luna, S.P., Taylor, P.M. and Massone, F. (1997) Midazolam and ketamine induction before halothane anaesthesia in ponies: cardiorespiratory, endocrine and metabolic changes. *J Vet Pharmacol Ther* **20**, 153-159.

Point to Point response letter to the reviewers' comments.

We would like to thank both reviewers for their detailed and useful comments on our manuscript. We feel that addressing the comments improved the quality of our manuscript. Below we provide, per reviewer, each individual comment and our response to that comment.

Peer Reviewer: 1

Abstract:
#1:
Please keep the format the same throughout (colons, italics).
Response:
We have gone through the abstract and removed the italic fonts in lines 6 and 10.

#2:
Pre-oxygenation is usually in direct reference to apnoea following induction of anaesthesia, not just the process of induction.
Response:
We agree with the reviewer that hypoxaemia results from either hypoventilation or apnoea induced by the induction of general anaesthesia and not from the induction process itself. We therefore replaced the sentence:
"*Pre-oxygenation is a common process to lengthen the period before hypoxaemia develops after induction of general anaesthesia*"
by
"*Hypoventilation or apnoea, caused by the induction of general anaesthesia, may cause hypoxaemia. Pre-oxygenation can be employed to lengthen the period before this happens."* (lines 6-8 revised manuscript)

#3
Topics in Equine Anesthesia, An Issue of Veterinary Clinics: Equine Practice, by Stuart Clark-Price discusses pre-oxygenation in colic horses (citing it is standard of practice in some hospitals), so the statement that no techniques are described is incorrect.
Response:
Although there may exist descriptions on the use of pre-oxygenation in horses, there are no scientific studies showing the efficacy of employing any type of pre-oxygenation technique in equine anaesthesia. We therefore replaced
"*For equine anaesthesia no techniques are described to prevent hypoxaemia during the induction process. This study shows the efficacy of a potential method for doing so.*"
by
"*No scientific studies are published on pre-oxygenation in equine anaesthesia. This technical note describes a successful technique in this respect*" (lines 8-9 revised manuscript)

#4
"Shows" a technique or describes a technique?
Response:
In the revised version of the manuscript (line 9) the word "describes" is used.

#5
It does elevate the PaO2, but only in the immediate period. The fact that it is not sustained needs to be stated in the conclusion.
Response:
In the revised version of the manuscript (line 27) it is now stated that the PaO_2 is elevated in the immediate period after induction.

#6
Improve the short and long term outcome, or simply affect the outcomes?
Response:
We agree with the reviewer that it is more prudent to speak of 'affecting the outcomes' as it can be either positive or negative. This is changed accordingly in the revised version of the manuscript (line 29).

Introduction
#7
Again, I would expand the definition of pre-oxygenation to include the risk of hypoxemia secondary to apnea commonly seen with induction agents like propofol and thiopental.
Response:
We agree with the reviewer that we could be clearer on the definition of pre-oxygenation. However we do not agree that apnoea and hypoventilation are only seen with induction agents like propofol or thiopental. In our experience hypoventilation and apnoea is seen with all induction agents including alfaxalone, ketamine, etomidate and volatile anaesthetics.
In response to the reviewer's comment, we changed the manuscript as follows:
"*Pre-oxygenation is a common practice in human and small animal anaesthesia*"
is changed to:
"*Pre-oxygenation is a common practice in human and small animal anaesthesia to lengthen the period before hypoxaemia, caused by hypoventilation or apnoea resulting from induction of general anaesthesia, develops.*" (lines 31-33 revised manuscript)

#8
Again, there are written descriptions of pre-oxygenating horses. Clark-Price's book, Muir and Hubbell's Equine Anesthesia briefly mentions it. Maybe no studies, but descriptions can be found.
Response:
Although there may be descriptions of pre-oxygenation in textbooks or expert opinion reviews, there are no studies showing the effectiveness, benefits or negative effect of pre-oxygenation in equine anaesthesia. Therefore we changed "*no reports on techniques, strategies, benefits, and potential adverse effects of pre-oxygenating….*"
to
"*no scientific reports on techniques, strategies, benefits, and potential adverse effects of pre-oxygenating……*" (lines 33-35 in the revised manuscript)

Materials and Methods:
#9
How were you sure the nasal cannula (being silicon) did not bend in the nasal meatus?
Response:
If the nasal cannula would bend it would have occluded, and this would have been detected by either hearing a high pitched whistling sound or by the nasal cannula popping of the oxygen supply line due to the pressure build-up, neither of which did happen. Although there could still have been

a very small chance that the cannula placement was incorrect, we did not check for this. As this study was designed to assess the clinical efficacy of the technique it was not feasible to assess correct placement by imaging (e.g. X-ray) as this would be outside the normal clinical routine.

#10
Did the same individual perform the induction each time? Did the same individual pass the cannula? Did the same individual draw the arterial samples?
Response:
Yes, all inductions, nasal cannula placements and drawing of the arterial blood samples were performed by the first author. The following sentence is added to the revised version of the manuscript (lines 88-89): "*All procedures in the first 30 minutes period were carried out by the first author of the manuscript.*"

#11
What were the horses respiratory rates following induction? Were any apnoeic?
Response:
As the horses were placed behind a swing door during the induction process, it was difficult to count their respiratory rates during the induction process. When the horses were safely induced and approached to obtain the arterial blood samples, they were usually hypoventilating 2-3 breaths per minute but not apnoeic.

#12
Was there ever difficultly in drawing an arterial sample, prolonged time to acquiring the sample?
Response:
All samples were obtained within 1 minute after the facial artery could be safely accessed. This information is added to the revised manuscript (lines 76-77).

#13
How were the horses placed on the surgical table? Were they hoisted and, if so, was respiratory support provided during that time?
Response:
The horses were indeed hoisted to the surgical table, but only after the arterial blood samples were drawn. During this time no respiratory support was provided. As stated in the manuscript, the horses were breathing spontaneously during the 30 minutes study period.

#14
Which lateral were horses placed in for surgery? If they were initially in left lateral but then flipped to right lateral, which could affect your study.
Response:
Given the set-up of our induction area and swing door, all horses were in left lateral recumbency when the arterial blood gas was taken from the facial artery at T=0. We therefore feel this would not have affected the results of our study as the positioning was equal for all horses included in the study. With respect to the data obtained at T=30 we also feel that the lateral side at which the horses were positioned would not have greatly affected the results, because hoisting and positioning on the surgery table would commence directly after the first arterial blood gas was drawn, i.e. within 3 minutes of induction. Therefore the initial left lateral recumbency was very short and compression atelectasis was unlikely to have occurred that quickly. Furthermore, during hoisting, all horses were hanging in dorsal recumbency making the positioning during this phase equal for all horses included in the study.

#15
Why were the horses allowed to breathe spontaneously for the first 30 minutes?
Response:
The horses were allowed to breathe spontaneously for the first 30 minutes as this was our study period. To avoid bias between horses, induced by some horses being ventilated and some horses not being ventilated, all horses were maintained under general anaesthesia whilst breathing spontaneously. In our clinic, directly commencing mechanical ventilation in horses undergoing general anaesthesia is not standard practice.

Results
#16
Did the head shaking result in a change in sedation level?
Response:
Levels of sedation were not objectively scored in this study. It could however be assumed that there is a difference in level of sedation between horses that do shake their head and horses that do not. If for example sedation is scored, using the 4-point scale of Taylor et al. (2014), the difference between score 2 and 3 is based on whether the horse gives a slight or no response to intervention. Consequently a horse shaking its head could be classified with a lower sedation score than a horse not shaking its head. One could argue that a less sedated horse is hypoventilating less and therefore arterial oxygen levels may be elevated compared to non-responsive horses that are more sedate and hypoventilating more. This study was too small, however, to investigate whether there was a relation between reaction to the nasal cannula and post-induction PaO_2 levels.

Discussion
#17
See questions raised in the M&M section. Address these concerns (positioning, hoisting, lateral versus dorsal, spontaneous breathing versus ventilator for first 30 minutes) in the discussion.
Response:
The lateral vs dorsal recumbency should not have played a role in the outcomes of this study as the initial arterial samples were drawn directly after induction, where all horses were in left lateral recumbency. At T=30 the positioning, lateral versus dorsal, could have had an effect, however as shown in table 1 the distribution for lateral and dorsal recumbency was equal between groups and therefore no effects of positioning on the results between groups was expected.
As explained above and stated in the manuscript, all horses were breathing spontaneously during the study period. Therefore, we feel that we should not address the concern of spontaneous ventilation versus mechanical ventilation during the study period, as mechanical ventilation was not used.

#18
Why did the PaO2 become equal between groups?
Response:
The PaO_2 became equal between the two groups because, shortly after induction, both groups were spontaneously ventilating and breathing a FiO_2 of ~50%. Therefore, no differences should be expected between the two groups, unless there was a significant difference in shunting and/or alveolar minute volume between the groups. Based on calculated shunt estimates and $PaCO_2$ values, neither was the case.

Peer Reviewer: 2

#1
Line 7-8: In equine anesthesia no techniques are described....
Don't think you can state that no techniques are described. Rather would state that these are not supported by data that show that the technique is effective
Response:
We agree with the reviewer that it is not correct to state that no techniques are described, as is also indicated by comments #3 and #8 of reviewer 1. Accordingly we state in the revised manuscript that: "No scientific studies are published on pre-oxygenation in equine anaesthesia" (lines 8-9 in the revised manuscript).

#2
Line 12-13: ..is effective in elevating the arterial partial pressure of oxygen (PaO2) during the induction process. No measurements were made during supplementation nor induction. Therefore would be more correct to state: elevating PaO2 directly after induction.
Response:
This is changed accordingly (line12) in the revised version of the manuscript.

#3
Line 27-28: Supplementing oxygen by a nasal cannula before induction of general anaesthesia in horses is feasible and does effectively elevate the PaO2.
Response:
This is changed accordingly (lines 26-28) in the revised version of the manuscript.

#4
Line 29-30: ...whether supplementation of oxygen before induction of general anaesthesia in horses will improve (the) short and long term outcome (of the anaesthetic). Delete between ()
Response:
Also in response to comment #6 of reviewer 1, this sentence was changed to: "whether supplementation of oxygen before induction of general anaesthesia in horses will affect outcomes" (lines 28-29 in the revised manuscript)

#5
Line 37-38: questioned whether supplementing oxygen to horses before induction of general anaesthesia is feasible, effective, and/or necessary.
Why questioning feasibility if shown in a previous study that it can be done in standing awake horses?
Effective: should be stated more precisely in which way effective.
Necessary: same, indicate more precisely necessary for what?
Response:
Although showed to be successful in awake horses, it is unknown yet whether it is effective in horses undergoing general anaesthesia as well, as the anaesthetic drugs used will significantly affect the horse's physiology (e.g. reduction in cardiac output and reduction in alveolar minute volume) which may make the technique ineffective.

The word effective was aimed at whether the process of supplementing oxygen via the nose would lead to any increase in PaO_2 after induction of general anaesthesia at all. The word necessary was aimed at the fact that it is not known whether horses in general are hypoxaemic after induction of general anaesthesia and therefore whether there is any necessity of supplementing oxygen before induction of general anaesthesia. To make this more clear in the manuscript, the sentence:

"*…supplementing oxygen to horses before induction of general anaesthesia is feasible, effective, and/or necessary*"
is changed to:
"*…supplementing oxygen to horses before induction of general anaesthesia is feasible, and effective in preventing potential hypoxaemia*" (lines 38-39 in the revised manuscript)

#6
Line 40-41: effective in increasing the PaO2. Specify which time points.
Response:
The following was added to this sentence: "*immediately after induction of general anaesthesia*" (line 42 in the revised manuscript)

#7
Line 59-60: (low head position and decreased responsiveness in all horses). delete: in all horses
Response:
The revised version of the manuscript (line 61) was changed accordingly.

#8
Line 64: until the mark on the tube reached the nostril > was at the level of the nostril.
Response:
The revised version of the manuscript (line 66) was changed accordingly.

#9
Line 65: HvO > the same observer
Response:
The revised version of the manuscript (line 67) was changed accordingly.

#10
Line 73: dedicated? > calibrated?
Response:
In the revised version of the manuscript the word dedicated (line 74-75) was removed.

#11
Line 119: (All) results are summarized in table 1. delete ()
Response:
The revised version of the manuscript (line 122) was changed accordingly.

#12
Line 125: t16 = 3.5951. What does this mean?
Response:
We apologize if this is not a normal annotation, however we feel that it is. The meaning is that the value of the T-test with 16 degrees of freedom is 3.5951.

#13
Line 129-131: At T=0, in all control group animals, but one, PaO2 values were below the cut-off point at which intervention to treat the hypoxaemia is deemed necessary, i.e. <8-9.3 kPa [5]. In contrast, in the oxygen group only one animal had a PaO2 value <8-9.3 kPa.
These are results, so should be put in that section. I would suggest to show these data in a diagram with cut-off point indicated. From a clinical relevance point of view, this is important information.
Response:

We agree with the reviewer that we present/repeat a small part of our results here. However, we feel that part of this passage is interpreting the result in terms of clinical relevance and that interpretation of results should be in the discussion section instead of the results section. Therefore we would prefer to leave this passage as it is.

#14
Line 131-134: this section discusses occurrence of hypoxemia. Discussion on the relevance of hypoxemia should follow (Line 147-161)
Response:
We thank the reviewer for this suggestion and have moved the paragraph discussing the relevance of hypoxaemia upward in the discussion in the revised version (lines 138-153) of the manuscript.

#15
Line 135: It could be argued that, for better comparison between....
Response:
The revised version of the manuscript (line 154) was changed accordingly.

#16
Line 138: propose > assume?
Response:
The revised version of the manuscript (line 157) was changed accordingly.

#17
Line 147: There is limited scientific literature on effects of hypoxaemia in anaesthetized horses considering short and long term outcome after anaesthesia.
Rephrase: There is limited scientific literature on the effect of hypoxaemia on short and long term outcome in anaesthetized horses.
Response:
The revised version of the manuscript (lines 138-139) was changed accordingly.

#18
Line 148: Although this lack of (literature) > lack of evidence
Response:
The revised version of the manuscript (line 139) was changed accordingly.

#19
Line 149: they do possess a large cardiopulmonary reserve capacity > we know that horses possess a large...
Response:
The revised version of the manuscript (lines 140-141) was changed accordingly.

#20
Line 151: This (increased) reserve > large reserve
Response:
The revised version of the manuscript (line 142) was changed accordingly.

#21
Line 155-157: However, (as) stroke volume,... are (largely) > negatively affected by anaesthetics and recumbency.
Response:
The revised version of the manuscript (lines 146-148) was changed accordingly.

#22
Line 157: As a consequence, general anaesthesia can decrease....
Response:
The revised version of the manuscript (lines 148-149) was changed accordingly.

#23
Line 159-160: Indeed, a recent study shows a worse outcome in terms of wound infection in horses that were hypoxaemic during anaesthesia [7]. It should be mentioned that this was a study in emergency colic horses and that other factors also played a role in occurrence of wound infection.
Response:
In line with the reviewer's comment, we changed:
"*Indeed, a recent study shows a worse outcome in terms of wound infection in horses that were hypoxaemic during anaesthesia [7]. Therefore, preventing hypoxaemia during general anaesthesia in horses does seem clinically relevant.*"
to:
"*A recent study shows that hypoxaemia (next to length of anaesthesia and type of suture material) is a risk factor for developing wound infection in emergency colic horses [7]. This supports the view that it is relevant to prevent hypoxaemia during general anaesthesia in horses, as it may affect outcome.*"
(lines 150-153 in the revised manuscript)

#24
Line 160-161: it's a bit weak to draw this conclusion from ref [7] only. Please add additional refs, eventually from human literature or other species.
Response:
We agree with the reviewer that concluding that hypoxaemia is detrimental, based on one study is not very strong. However, we do not try to make the point that in general hypoxaemia is detrimental to patients, but we try to explain that, despite their extraordinary physiology, there now is evidence that outcome for horses may be affected by the presence of hypoxaemia. Unfortunately there is very limited scientific evidence showing a direct relationship between hypoxaemia and outcome in horses. To keep the discussion focussed on horses and try to make our point for this species and to adhere to the limited word count for a technical note, we prefer to avoid to extend this discussion to other species in the revised manuscript.

#25
Line 162: the FiO2 during oxygen supplementation: unclear here whether oxygen supplementation pre-induction or intra-operatively is meant. Should be specified!
Response:
We agree with the reviewer that this was unclear. In the revised version of the manuscript (line 169) it is now stated that it concerns the oxygen supplementation before induction of general anaesthesia.

#26
Line 162-164: should further explain why FiO2 expected around 0.49 based on results of Wilson
Response:
In response to comment #27, this passage was rewritten in the revised version of the manuscript (lines 167-171)

#27
Line 163-164: ...which is far below the FiO2 of >0.95 that induces an increase in resorption atelectasis in horses [8]. If stated like this suggestion is made that resorption atelectasis would only occur if FiO2 > 0.95. This conclusion cannot be drawn from ref [8] as it only compared FiO2 0.21 to FiO2 0.95.
Response:
We agree that this passage is misleading. We therefore replaced the following two sentences:
"*Based on the results of Wilson et al [1] the FiO2 during oxygen supplementation in the present study was expected to be around 0.49 which is far below the FiO2 of >0.95 that induces an increase in resorption atelectasis in horses [8]. Our results indeed show no significant difference in intrapulmonary shunt fraction (Qs/Qt) between the oxygen and control group at T=30*."
by:
"*Pre-oxygenation could potentially increase intrapulmonary shunt by increasing resorption atelectasis [8]. Our results show no significant difference in intrapulmonary shunt fraction (Qs/Qt) between the oxygen and control group at T=30, making it less likely that the oxygen supplementation before induction of general anaesthesia as described in this study increases intrapulmonary shunt by promoting resorption atelectasis*." (lines 167-171 in revised manuscript)
Note: in this revised text reference [8] originally referring to the study by Marntell et al., is now replaced by a publication of Hedenstierna et al.

#28
Line 164-166: Our results indeed show no significant difference in intrapulmonary shunt fraction (Qs/Qt) between the oxygen and control group at T=30.
Is this surprising, given the fact that Qs/Qt was measured after 30 min and intra-operative O_2 administration was same for both groups? Comment should be added to explain
Response:
Although the horses received the same FiO_2 during the 30 minute period after induction, in theory, pre-oxygenation could have led to a significant nitrogen washout in the oxygenation group. This loss of nitrogen could have resulted in increased resorption atelectasis adding to intrapulmonary shunt. As this is a comment often employed by opponents of pre-oxygenation we felt it was prudent to look whether with the technique presented such an effect was significantly present. We hope that this explanation and the revision of the text in response to comment #26 sufficiently addresses the comment of the reviewer.

#29
Line 166: Calculating the true Qs/Qt, requires mixed venous blood samples, obtained via a pulmonary artery catheter. Add a sentence before this one indicating that you used an adapted formula.
Response:
In the revised version of the manuscript (lines 171-172) the following sentence was added: "*In this study Qs/Qt was estimated by using equation 1.*"

#30
Line 182: As our results show that (the) hypercapnia... Delete ()
Response:
In the revised version of the manuscript (lines 188-189) this sentence was changed to: *"As our results show that the arterial carbon dioxide concentration does not differ between groups,…".*

#31
Line 168: using patients > client owned horses
Response:
The revised version of the manuscript (line 181) was changed accordingly.

#32
Line 193-198: Title of table one could be shorter. Most of it is already described in Materials & Methods
Response:
The revised version of the manuscript (line 200) was changed accordingly.

Reference:
Taylor P, Coumbe K, Henson F, Scott D, Taylor A. Evaluation of sedation for standing clinical procedures in horses using detomidine combined with buprenorphine. Vet Anaesth Analg. 2014;41:14-24