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## Authors' contributions

Enma V. Hummer: analysis and interpretation of data; preparation of the manuscript; approved the final manuscript.

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## Funding:

This study was funded by the Center for Equine Health (CEH), University of California Davis, Davis, CA, 95616 (n° 18-31).

## Competing interests:

The authors declare no competing interests.

## Ethical Animal Research:

This study was approved by the Institutional Animal Use and Care Committee of the University of California Davis (n° 21270)

## Acknowledgements

We are grateful to Mr. Tibi Farca for valuable technical assistance during the experiments. This project was supported by the Center for Equine Health with funds provided by the State of California satellite wagering fund and contributions by private donors.

## Availability of supporting data and materials:

Data are available upon reasonable request.

## Items Masked for Review

Line 61: Institutional Animal Care and Use Committee of the University of California, Davis.

**Word count** 3890, including abstract, main text, Manufacturer's information, Figure legends and Supplementary material legends.

## Abstract

Background: The possibility of accurately and continuously measuring arterial oxygen partial pressure (PaO<sub>2</sub>) in horses may facilitate the management of hypoxaemia during general anaesthesia.

Objectives: The aim of this study was to evaluate the ability of a novel fiberoptic sensor to measure PaO<sub>2</sub> (PaO<sub>2Sensor</sub>) continuously and in real-time in horses undergoing ventilatory manoeuvres during general anaesthesia.

Study Design: *In vivo* experimental study.

Methods: Six adult healthy horses were anaesthetised and mechanically ventilated in dorsal recumbency. A fiberoptic sensor was placed in one of the facial arteries through a catheter to continuously measure and record PaO<sub>2Sensor</sub>. After an alveolar recruitment manoeuvre (ARM), a decremental positive end-expiratory pressure (PEEP) titration using 20-minute steps of 5 cmH<sub>2</sub>O from 20 to 0 cmH<sub>2</sub>O was performed. An arterial blood sample was collected at 15 minutes of ventilation at each PEEP level for PaO<sub>2</sub> measurement using an automated blood gas machine (PaO<sub>2ref</sub>). The agreement between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> was assessed by Pearson's correlation, Bland-Altman plot, and four-quadrant plot analysis. In the last minute of ventilation at each PEEP level, a slow tidal inflation/deflation manoeuvre was performed.

Results: The mean relative bias between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> was 4% with limits of agreement between -17 and 29%. The correlation coefficient between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> was 0.98 (*P* < 0.0001).

The PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> concordance rate for changes was 95%. Measurements of PaO<sub>2Sensor</sub> during the slow inflation/deflation manoeuvre at PEEP 15 and 10 cmH<sub>2</sub>O were not possible because of significant noise on the PaO<sub>2</sub> signal generated by a small blood clot.

Main limitations: Small sample size.

Conclusion: The tested fiberoptic probe was able to accurately and continuously measure PaO<sub>2Sensor</sub> in anaesthetised horses undergoing ventilatory manoeuvres. A heparinized system in the catheter used by the fiberoptic sensor should be used to avoid blood clots and artifacts in the PaO<sub>2</sub> measurements.

**Keywords:** Anaesthesia, arterial oxygenation, Arterial ventilation, Horse.

## 1. Introduction

Hypoxaemia is common during equine general anaesthesia, is a risk factor for poor recovery quality<sup>1</sup> and may be a potential risk factor for the higher anaesthesia-related mortality rate in horses compared to other domestic animal species.<sup>2,3</sup> Reduced muscle oxygenation<sup>4</sup> and increased incidence of surgical site infections<sup>5</sup> are associated with hypoxaemia in horses undergoing general anaesthesia (GA), but its effect on postanaesthetic outcome is poorly understood. The major cause of hypoxaemia in anesthetized horses is a ventilation/perfusion (V/Q) mismatch as a result ofatelectasis<sup>6</sup> and redistribution of pulmonary blood flow.<sup>7,8</sup> Ventilatory strategies including the application of positive end-expiratory pressure (PEEP) and alveolar recruitment manoeuvres (ARM) can be used to reduce atelectasis and improve V/Q matching, potentially minimizing hypoxaemia in anesthetized horses.<sup>9-11</sup> However, the treatment of hypoxaemia in the anesthetized horse can be extremely challenging and unsuccessful.<sup>12</sup> Intermittent arterial blood gas (ABG) sampling for the spot analysis of arterial partial pressure of oxygen (PaO<sub>2</sub>) is the current clinical standar to monitor arterial oxygenation in horses under GA. Recently,

a small fiberoptic sensor that can be inserted into a standard arterial cannula has been developed for continuous PaO<sub>2</sub> monitoring.<sup>13</sup> Its projected use has been primarily directed towards humans who experience considerable dynamic alterations in PaO<sub>2</sub> in the critical care setting.<sup>14</sup> The high prevalence of significant decrease in PaO<sub>2</sub> observed in horses during GA shares some pathophysiological features with these critically ill human patients. The use of this sensor in equine anaesthesia has the potential to detect rapid variations in PaO<sub>2</sub> in real time and inform more timely and precise strategies to improve pulmonary gas exchange, and possibly achieve better outcomes. In addition, the monitoring of dynamic changes in PaO<sub>2</sub> can be used as a research tool to better understand the effects of different strategies to improve V/Q inequalities in anaesthetised horses.

The aim of this study was to evaluate the ability of a novel fiberoptic sensor to provide real-time continuous measurement of PaO<sub>2</sub> in horses undergoing ventilatory manoeuvres during GA. It is hypothesized that the fiberoptic sensor will be able to continuously measure PaO<sub>2</sub> with acceptable accuracy and precision.

## 2. Methods:

2.1. Animals

This study of six healthy adult horses (5 Warmbloods and one Lusitano) weighing 581 ± 47 (mean ± standard deviation) was part of a larger study investigating the cardiovascular and respiratory effects of PEEP in horses, approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California Davis. The maximum duration of each experiment per IACUC protocol was 5 hours. Demographic data are presented in Table S1.

2.2. Anaesthesia and Instrumentation:

Anaesthesia and instrumentation details are provided in the supplementary material (Data S1). Anaesthesia was intravenously induced with propofol (3 mg/kg) and ketamine (2.2 mg/kg), and maintained during the experiments with an end-tidal concentration of isoflurane (FE'ISO) between 1.8 and 2.2% (approximately 1.5 minimum alveolar concentration).<sup>15</sup> With the horses in dorsal recumbency, volume control ventilation<sup>16</sup> was started with a tidal volume of 15 ml/kg, no PEEP (ZEEP), inspiratory-to-expiratory ratio (I:E) of 1:2, inspiratory oxygen fraction (FIO<sub>2</sub>) between 0.70 and 0.96, and respiratory rate adjusted to maintain end-tidal partial pressure of CO<sub>2</sub> (PE'CO<sub>2</sub>) between 30 and 40 mmHg (baseline ventilation).

Two 20-gauge arterial catheters<sup>17</sup> were placed, one in each facial artery (**Figure 1**): one to measure arterial blood pressures (ABP) with a calibrated pressure transducer and to collect blood samples for analysis, and the other as an introducer for the fiberoptic sensor<sup>18</sup> (< 200  $\mu$ m diameter) to continuously measure PaO<sub>2</sub> (PaO<sub>2Sensor</sub>). The ABP monitoring catheter was continuously flushed with 3 ml/hr of heparinized saline (1 ml/hr). Intravenous dobutamine (0.5 to 3 mcg/kg/min) was used to maintain mean arterial pressure (MAP) > 70 mmHg.

A modified flow divider<sup>16</sup> was placed between the endotracheal tube and the breathing system to measure airway pressure (P<sub>aw</sub>), flow (V), PE'CO<sub>2</sub>, FE'ISO, and FIO<sub>2</sub>. A pressure transducer<sup>19</sup> measured P<sub>aw</sub> from a port close to the endotracheal tube, and V was measured by an adult pneumotachometer<sup>20</sup> connected to the flow divider. A mainstream/scan>sensor<sup>21</sup> was used to measure PE'CO<sub>2</sub> and a side-stream gas analyser<sup>22</sup> measured FIO<sub>2</sub> and FE'ISO. All equipment was calibrated before each experiment. The digital signals of ABP, P<sub>aw</sub> and V were recorded at 100 Hz, and PaO<sub>2Sensor</sub> at 10 Hz with LabChart Pro<sup>23</sup>. Volume (V) was calculated by the integral of V'.

2.3. Continuous measurement of PaO<sub>2Sensor</sub>

A fiberoptic sensor<sup>18</sup> measured PaO<sub>2Sensor</sub> during the experiments. The sensor's working principle is based on O<sub>2</sub> luminescence quenching. On exposure to light, electrons within a platinum (Pt) luminophore compound can be temporarily excited to higher energy levels. When electrons return to their base state, they release measurable quanta of energy as light. In the presence of O<sub>2</sub>, the amount of energy released is reduced ("quenched"). The degree of quenching is predictable based on the concentration of O<sub>2</sub> in the medium around the luminophore. The Pt-based luminophore is embedded in a polymethylmethacrylate matrix at the end of the fiberoptic probe for continuous PaO<sub>2</sub> measurement.<sup>13,17-19</sup> The sensor was introduced 1 - 5 cm beyond the tip of the arterial catheter. Fiberoptic sensors were calibrated at the manufacturer and their technical properties are presented elsewhere.<sup>20</sup>

2.4. Measurements

Fifteen minutes after instrumentation, cardiovascular and respiratory variables were recorded (baseline). Subsequently, an ARM was performed increasing PEEP every minute from ZEEP to 30 cmH<sub>2</sub>O, in increments of 10 cmH<sub>2</sub>O. Immediately after the ARM, PEEP was set to 20 cmH<sub>2</sub>O and titrated down to ZEEP in 20 minute-steps of 5 cmH<sub>2</sub>O (Figure 1B). At 15 minutes of ventilation in each PEEP step, an arterial blood sample (1 ml) was collected in pre-heparinized syringes<sup>24</sup> for the measurement of PaO<sub>2</sub>. These PaO<sub>2</sub> measurements (PaO<sub>2ref</sub>) were corrected for animal core temperature for reference.

The ABG machine, used in this project was routinely calibrated before and during the experiments and automatic quality control check performed every 8 hours. During the five last minutes at each PEEP level, a slow inflation (20 s) and deflation (20 s) manoeuvre programmed on the ventilator was performed to achieve a V<sub>T</sub> of 18 ml/kg. After this manoeuvre, V<sub>T</sub> was reset to 15 ml/kg and PEEP decreased to the next level. At the end of the experiment, all instrumental artefacts were removed, and the horse transferred to the recovery stall. Horses were returned to the institutional research horse herd on the day after the experiments.

2.5. Changes in PaO<sub>2</sub> caused by ventilation manoeuvres

Changes in PaO<sub>2Sensor</sub> associated with the ARM were calculated at each PEEP step (10, 20, and 30 cmH<sub>2</sub>O) and during the first minute after PEEP was lowered to 20 cmH<sub>2</sub>O. The PaO<sub>2Sensor</sub> measured at baseline was used as reference (100%) to calculate relative changes in PaO<sub>2Sensor</sub>, observed in every PEEP step of the ARM and at 30 and 60 seconds after PEEP was lowered to 20 cmH<sub>2</sub>O.

Rapid changes in PaO<sub>2Sensor</sub> resulting from the slow inflation and deflation manoeuvre performed in each step of the PEEP titration were calculated. The PaO<sub>2Sensor</sub> measured immediately before the manoeuvre was used as the reference value (100%) to calculate the relative changes of PaO<sub>2Sensor</sub> occurred every 10 seconds of the manoeuvre and at 10 seconds of tidal breathing after the manoeuvre.

2.6. Statistical analyses:

Normality of the data was evaluated by the Shapiro-Wilk test. Normally distributed data are presented as mean (standard deviation), and non-normally distributed data as median (quartile 1 – quartile 3). Agreement between PaO<sub>2ref</sub> and PaO<sub>2Sensor</sub> was evaluated by Bland-Altman analysis corrected for repeated measurements,<sup>25</sup> and Pearson's correlation coefficient. A four-quadrant plot was used to evaluate the trending ability of PaO<sub>2Sensor</sub>. Concordance rate between the two methods was calculated by dividing the sum of data points in the four-quadrant plot that agreed with the trend divided by the number of all data points, using a 15% exclusion zone.<sup>26</sup> Pearson's correlation coefficients between PaO<sub>2Sensor</sub> - PaO<sub>2ref</sub> and body temperature and dobutamine rate at the time of measurement were calculated to verify their respective effect on the agreement between PaO<sub>2</sub> measurements. Statistical analyses were performed by MedCalc, version 20.008<sup>27</sup> and Microsoft Excel for Mac, version 16.9<sup>28</sup>.

## 3. Results

All six horses participated in the study. Horse 1 did not have PaO<sub>2</sub> measurements during the slow inflation/deflation at ZEEP because the experiment had to be finished before that timepoint. In Horse 3, no measurements of PaO<sub>2Sensor</sub> was performed during the slow inflation/deflation manoeuvres at PEEP 15 and 10 cmH<sub>2</sub>O because of significant signal instability created by a small blood clot at the tip of the fiberoptic sensor.

3.1. Agreement between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub>

Compared to PaO<sub>2ref</sub> (48 to 447 mmHg), PaO<sub>2Sensor</sub> mean relative bias was 4% with LOA between -17 and 29% ( **Figure 3** ). A significant correlation coefficient of 0.98 was observed between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> changes. The PaO<sub>2Sensor</sub> demonstrated a good ability to detect trend ( **Figure 4** ). A significant correlation coefficient of 0.46 was observed between PaO<sub>2Sensor</sub> – PaO<sub>2ref</sub> and body temperature (*P* = 0.009). From the 32 paired measurements of PaO<sub>2</sub>, 26 (81%) measurements of PaO<sub>2Sensor</sub> were within 15% of the PaO<sub>2ref</sub>.

3.2. PaO<sub>2</sub> responses to ventilatory manoeuvres

Figure 2A shows relative changes in PaO<sub>2Sensor</sub> in response to the ARM. The changes in PaO<sub>2Sensor</sub> in response to the ARM were greater at PEEP 30 cmH<sub>2</sub>O and in the first minute following a PEEP decrease to 20 cmH<sub>2</sub>O. The ARM resulted in PaO<sub>2Sensor</sub> increase in four horses (approximately four-fold increase in two of them), but did not alter PaO<sub>2Sensor</sub> in two other horses, in which PaO<sub>2Sensor</sub> decreased by approximately 10% at PEEP 30 cmH<sub>2</sub>O. An example of the real-time variation with PaO<sub>2Sensor</sub> and P<sub>aw</sub> during the ARM is presented in Figure S1.

The relative PaO<sub>2Sensor</sub> changes in response to the slow inflation/deflation manoeuvre from 6 horses are illustrated in Figure 2B. A typical response to the increase in P<sub>aw</sub> caused by the manoeuvre was a transient decrease in PaO<sub>2Sensor</sub> with its minimum value reached ~15 s following the maximum P<sub>aw</sub> (Figure S2). The largest decreases in PaO<sub>2Sensor</sub> (around 30%) were detected in horse 4 during PEEP of 10 and 15 cmH<sub>2</sub>O and horse 6 during PEEP of 15 cmH<sub>2</sub>O. Within approximately 10 seconds after P<sub>aw</sub> decrease to its pre-manoeuve level, PaO<sub>2Sensor</sub> typically returned to pre-manoeuve values.

However, Horse 2 had ~10% increase in PaO<sub>2Sensor</sub> within 10 s following the slow inflation/deflation manoeuvre at PEEP of 20 cmH<sub>2</sub>O. There was no apparent correlation between the level of PEEP and the magnitude of change in PaO<sub>2Sensor</sub> during the slow inflation/deflation manoeuvre.

Changes in PaO<sub>2Sensor</sub> during changes in the infusion rate of dobutamine were also observed and are presented in Data S1, Table S2 and Figure S3. A non-significant correlation of -0.11 was observed between the relative bias of PaO<sub>2Sensor</sub> and the dose of dobutamine at the time of measurement.

## 4. Discussion

This study demonstrated the feasibility of measuring PaO<sub>2</sub> using a fiberoptic sensor and is the first to presents the dynamic PaO<sub>2</sub> responses to ventilatory manoeuvres in the mechanically ventilated horse in dorsal recumbency. Arterial oxygenation responses varied, suggesting that continuous monitoring of PaO<sub>2</sub> may be useful in informing the effectiveness of ventilatory manoeuvres in anesthetized horses.

### 4.1. Temporal resolution

Hypoxaemia is a common and important complication in horses undergoing GA, especially those placed in dorsal recumbency.<sup>2,23</sup> Conventional monitoring of PaO<sub>2</sub> is achieved by intermittent sampling from an arterial catheter and analysis by a blood gas analyser. This method constrains ABG to snapshot measurements, which does not allow for real-time evaluation of acute and transient changes in PaO<sub>2</sub>, as those that were instead captured by the fiberoptic sensor during the ventilatory manoeuvres. The high temporal resolution achieved with this sensor allows the evaluation of short-term trends and the immediate effect of therapeutic interventions in horses undergoing anaesthesia.

### 4.2. Agreement between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub>

Bland-Altman analysis was used to assess agreement between relative changes, because the magnitude of the bias between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> was proportional to PaO<sub>2</sub>.<sup>21,24</sup> The agreement between PaO<sub>2Sensor</sub> and PaO<sub>2ref</sub> was considered acceptable based on the small relative bias of 4% and LOA below 30%. Acceptable precision of PaO<sub>2Sensor</sub> can be indicated by having more than 80% of the measurements within less than 15% difference from PaO<sub>2ref</sub>, as defined in previous studies.<sup>25,26</sup> In addition, PaO<sub>2Sensor</sub> demonstrated a good ability to detect trends based on the four-quadrant plot analysis, which is an important feature of any monitoring technology.<sup>20</sup> The manufacturer's calibration of the fiberoptic sensor was performed in 39°C and may have contributed to a larger bias and LOA of PaO<sub>2Sensor</sub>, because the horses' body temperature were below 39°C, with the lowest being 35.4°C. The moderate but significant correlation between PaO<sub>2</sub> and body temperature corroborates with this possible influence of body temperature, which has already been demonstrated in an *in vitro* study<sup>27</sup>. The combination of good trend ability and acceptable precision over a range of PaO<sub>2</sub> indicates the suitability of this instrument to reliably measure PaO<sub>2</sub> in the clinical and experimental settings of equine anaesthesia.

### 4.3. Response to ventilatory manoeuvres

Ventilatory manoeuvres were performed in the horses to test the ability of the fiberoptic sensor to capture real-time variations in PaO<sub>2Sensor</sub>. The fiberoptic sensor was able to continuously monitor changes in PaO<sub>2Sensor</sub> promoted by the ARM followed by PEEP of 20 cmH<sub>2</sub>O. An increase in PaO<sub>2Sensor</sub> following the ARM was observed in four of the six horses, suggesting recruitment of previously collapsed alveoli, assuming unaltered pulmonary perfusion and its distribution. Two of these horses showed a large increase in PaO<sub>2</sub> while the other two only a minor increase. This result is in general agreement with other studies that evaluated ARM in dorsally recumbent anesthetized horses.<sup>9-11</sup> However, a small decrease followed by no change in PaO<sub>2Sensor</sub> was detected in two horses. The variability of this PaO<sub>2Sensor</sub> response is is part of the challenge to manage hypoxaemia in the anesthetized horse,<sup>12</sup> and is probably related to different states of alveolar recruitability in different horses, as demonstrated in humans with acute respiratory distress syndrome.<sup>28</sup> The clinical use of this fiberoptic sensor can possibly improve the ventilatory management of anesthetized horses by readily identifying recruitable and nonrecruitable individuals and the earlier triggering of alternative therapies to improve oxygenation such as aerosolized albuterol<sup>29</sup> or pulsed nitric oxide.<sup>30</sup>

During the slow inflation/deflation manoeuvres performed in all PEEP levels (20 to 0 cmH<sub>2</sub>O), PaO<sub>2Sensor</sub> generally decreased following peak airway pressures, with most increasing back to baseline PaO<sub>2</sub> levels when P<sub>aw</sub> was returned to pre-manoeuve levels. While the experiments were not designed to study the mechanisms responsible for these PaO<sub>2Sensor</sub> changes, we assume that the decrease in PaO<sub>2Sensor</sub> during the inflation part of the manoeuvre may be related to redistribution of pulmonary blood flow from ventilated to non- or poorly-ventilated alveoli,<sup>26</sup> especially if the ARM was not effective in opening the lung.<sup>31</sup> Capturing the real-time dynamics of PaO<sub>2</sub>, as during the inflation/deflation manoeuvre, can provide novel understanding of the mechanisms of gas exchange derangements as well as on methods to improve gas exchange in the anesthetized horse.

### 4.4 Limitations

Only six horses were studied. This study aimed at demonstrating the ability of a novel fiberoptic sensor to measure PaO<sub>2Sensor</sub> continuously and in real-time in horses, for which a larger sample size may have been ethically justifiable. Only the facial artery was used for placement of the sensor, and it is unknown whether other arteries, for example those commonly catheterized for ABP measurement such as the metatarsal and transverse facial arteries, would also yield similar results. Given the anatomy of the arterial circulation and the associated oxygen cascade, it is likely that PaO<sub>2Sensor</sub> values would have been similar in similar arterial vessels. The lack of a heparinized system connected to the fiberoptic probe likely contributed to the formation the small blood clot that impaired the measurements of PaO<sub>2Sensor</sub> during PEEP 10 and 15 cmH<sub>2</sub>O in Horse 3. This effect was unexpected since no blood clots were visualize under electron microscopy after this type of fiberoptic sensor being in contact with non-heparinised swine blood for 24 hours.<sup>13</sup> Because horses are considered less procoagulant than pigs,<sup>32,33</sup> it is likely that the clot was more related to the non heparinized arterial catheter than the fiberoptic probe itself. Lastly, the performance of the fiberoptic sensor was not verified for PaO<sub>2Sensor</sub> below 48 mmHg, which limits the conclusion of this study for more severe hypoxaemia cases.

In terms of future development, the sensor connectors should be designed so that the sensor can be placed in the same arterial catheter as used for direct blood pressure measurement, to avoid the need for placement of a dedicated arterial catheter and thus increase the ease of the sensor's use in clinical practice. An additional advantage of this option is to have the system constantly heparinized avoiding the formation of blood clots as observed in Horse 3.

## 4.5. Conclusion

In conclusion, it was possible to continuously measure PaO<sub>2Sensor</sub> in the mechanically ventilated horse, and recorded values presented acceptable agreement with standard ABG analysis. In addition, the fiberoptic sensor detected trends and tracked dynamic changes in PaO<sub>2</sub> associated with ARM and a slow inflation/deflation manoeuvre. A heparinized system is recommended for the future use of this sensor in horses to avoid the formation of intra-arterial blood clots and artifacts on PaO<sub>2</sub> measurements.

## Manufacturers' addresses

a Tatonius, Hallowell EMC and Vetronic USA

b BD Infusion Therapy Systems, UT, USA

c Oxford Optronix, UK

d MPX, Hugo Sacks Elektronik, Germany

e NM3, Philips Respironics, USA

f Capnostat, Philips Respironics, USA

g E-CAIOV, GE Healthcare, USA

h v8.1.16, ADInstruments, New Zealand

i PICO70, Radiometer America, USA

j ABL 825, Radiometer America, USA

k MedCalc Software, Belgium

l Microsoft, USA

## List of figures legends:

**Figure 1:** Illustration of the two arterial catheters positioned in each facial artery: one for the continuous measurement of partial pressure of oxygen (PaO<sub>2</sub>) and the other for blood pressure measurement and sampling for blood gas analysis.

**Figure 2:** Timeline of the study. ABG = arterial blood gas, ARM = alveolar recruitment manoeuvrer, arterial partial pressure of oxygen measured by the fibroptic sensor (PaO<sub>2Sensor</sub>).

**Figure 3:** Bland-Altman plot showing the relative bias (bold line) and the 95% limits of agreement (dashed lines) between the arterial partial pressure of oxygen measured by the fibroptic sensor (PaO<sub>2Sensor</sub>) and with standard blood gas analysis (PaO<sub>2ref</sub>). SD = standard deviation.

**Figure 4:** Evaluation of the trending ability of the fibroptic sensor by the four-quadrant plot showing the relation between the relative changes in arterial partial pressure of oxygen measured by standard blood gas analysis (deltaPaO<sub>2ref</sub>) and the fibroptic sensor (deltaPaO<sub>2Sensor</sub>). The bold line is the regression line, the dashed lines the 95% confidence intervals for the linear regression and the square in the centre is the exclusion zone of 15%. Values for a and b within parenthesis are the 95% confidence intervals.

**Figure 5:** Panel A: Relative changes in arterial partial pressure of oxygen measured with the fibroptic sensor (PaO<sub>2Sensor</sub>) in response to the alveolar recruitment manoeuvre (10, 20 and 30 cmH<sub>2</sub>O). Panel B: Relative changes in arterial partial pressure of oxygen measured by the three steps of positive end-expiratory pressure (PEEP) used during the ARM (10, 20 and 30 cmH<sub>2</sub>O) and the first minute of PEEP of 20 cmH<sub>2</sub>O, using as a reference (100%) the PaO<sub>2Sensor</sub> recorded immediately before the ARM in the absence of PEEP. Data are from six horses, where each horse is indicated with a different colour. Panel B: Relative changes in PaO<sub>2Sensor</sub> (normalised PaO<sub>2Sensor</sub>) during a slow pulmonary inflation and deflation manoeuvre performed in the absence (PEEP 0) and in four levels of PEEP (5, 10, 15, and 20 cmH<sub>2</sub>O). The relative changes in PaO<sub>2Sensor</sub> were calculated in 10-second intervals during the manoeuvre and at the first 10 seconds of tidal breathing following the manoeuvre, using as the reference (100%) the PaO<sub>2Sensor</sub> recorded immediately before the manoeuvre. The top left panel shows the variation in airway pressure during the manoeuvre (blue line). The other graphs of the panel illustrate the relative changes in PaO<sub>2Sensor</sub> in all horses [Horses 1-6].

## Supplementary Items:

Data S1: Detailed description of the anaesthesia and instrumentation and changes in arterial partial pressure of oxygen (PaO<sub>2</sub>) caused by changes in dobutamine infusion rate.

Table S1: Demographic data of the horses and cardiovascular and respiratory variables at baseline.

Table S2: Variations in intravenous dobutamine dosage ([Dobutamine]) and the oxygen arterial partial pressure ([PaO<sub>2Sensor</sub>]) measured by the fibroptic sensor (PaO<sub>2Sensor</sub>) positioned in the facial artery of anesthetized horses in dorsal recumbency (n = 6).

Figure S1: Temporal changes in airway pressure (P<sub>aw</sub>, left axis) and arterial partial pressure of oxygen measured by a fibroptic sensor (PaO<sub>2Sensor</sub>, right axis) during an alveolar recruitment manoeuvre of a recruitable horse (Horse 2).

Figure S2: Typical temporal changes in Airway Pressure (P<sub>aw</sub>, left axis) and arterial partial pressure of O<sub>2</sub> measured by a fibroptic sensor (PaO<sub>2Sensor</sub>, right axis) during a slow inflation/deflation manoeuvre in Horses undergoing general anaesthesia. The graph below is from Horse 5 mechanically ventilated with no positive end-expiratory pressure (ZEEP).

Figure S3: Temporal changes in arterial blood pressure (ABP) and PaO<sub>2</sub> continuously measured by a fibroptic sensor (PaO<sub>2Sensor</sub>) after changes in the intravenous dobutamine infusion rate (P = 0) in horses anaesthetised with isoflurane and mechanically ventilated in dorsal recumbency. In the top graph, an increase in ABP and PaO<sub>2Sensor</sub> was observed in Horse 1 after starting dobutamine at 1 mcg/kg/min during ventilation with positive end-expiratory pressure (PEEP) of 20 cmH<sub>2</sub>O. In the bottom graph, a decrease in ABP and PaO<sub>2Sensor</sub> was observed after the dobutamine infusion rate of 1 mcg/kg/min was stopped during ventilation of horse 2 during PEEP of 15 cmH<sub>2</sub>O.

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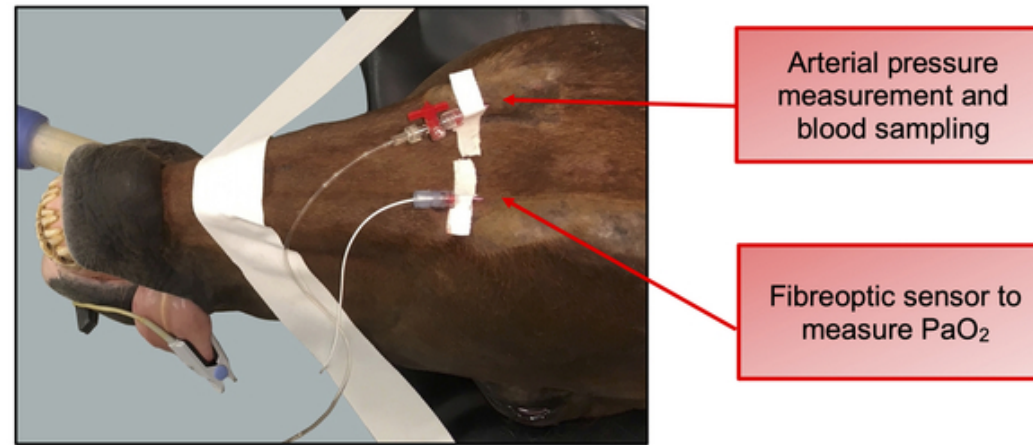


Figure 1: Illustration of the two arterial catheters positioned in each facial artery: one for the continuous measurement of partial pressure of oxygen (PaO<sub>2</sub>) and the other for blood pressure measurement and sampling for blood gas analysis.

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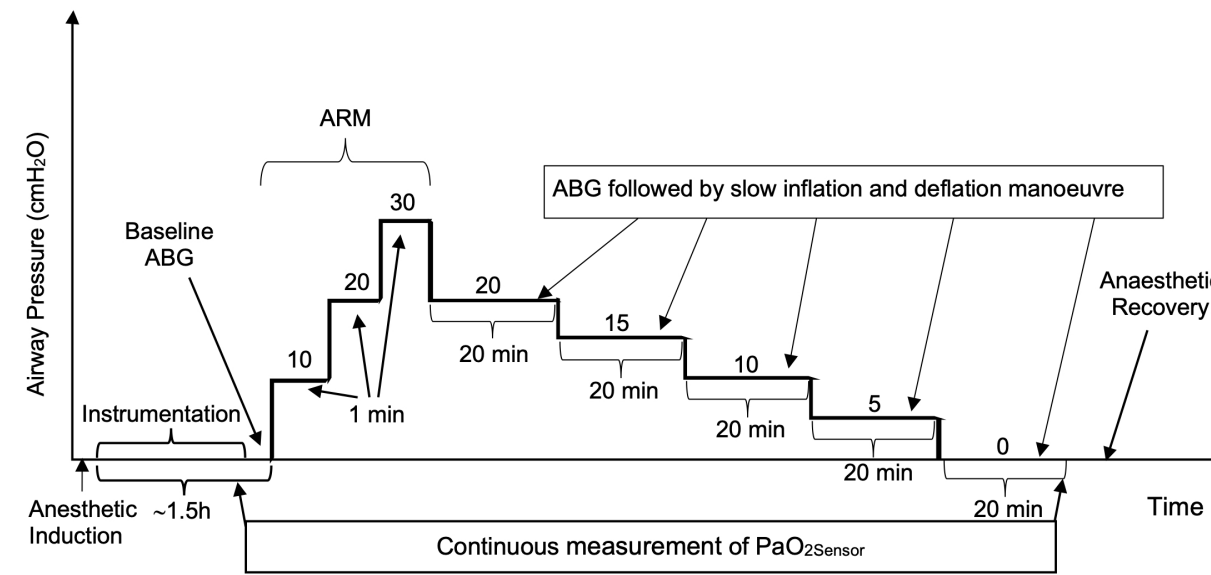


Figure 2: Timeline of the study. ABG = arterial blood gas, ARM = alveolar recruitment manoeuvre, arterial partial pressure of oxygen measured by the fibreoptic sensor (PaO<sub>2</sub>Sensor).

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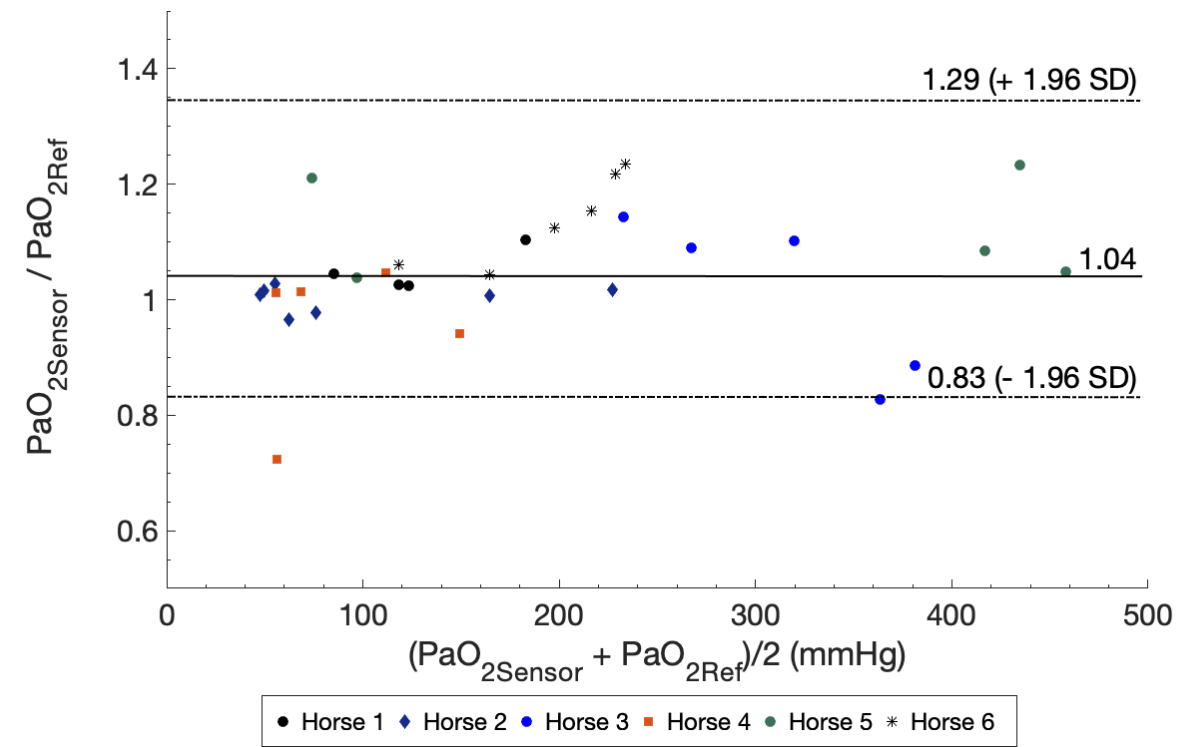


Figure 3: Bland-Altman plot showing the relative bias (bold line) and the 95% limits of agreement (dashed lines) between the arterial partial pressure of oxygen measured by the fiberoptic sensor ( $\text{PaO}_{2\text{Sensor}}$ ) and with standard blood gas analysis ( $\text{PaO}_{2\text{Ref}}$ ). SD = standard deviation.

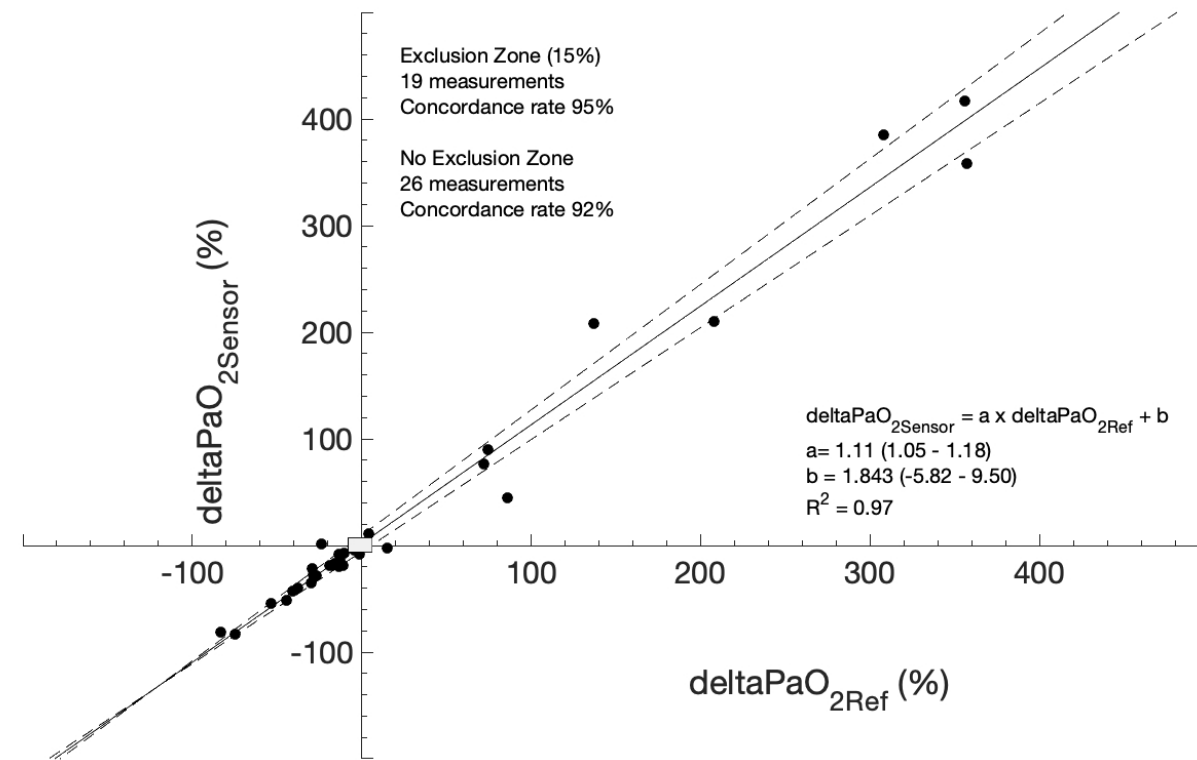


Figure 4: Evaluation of the trending ability of the fiberoptic sensor by the four-quadrant plot showing the relation between the relative changes in arterial partial pressure of oxygen measured by standard blood gas analysis ( $\text{deltaPaO}_{2\text{Ref}}$ ) and the fiberoptic sensor ( $\text{deltaPaO}_{2\text{Sensor}}$ ). The bold line is the regression line, the dashed lines the 95% confidence intervals for the linear regression and the square in the centre is the exclusion zone of 15%. Values for a and b within parenthesis are the 95% confidence intervals.

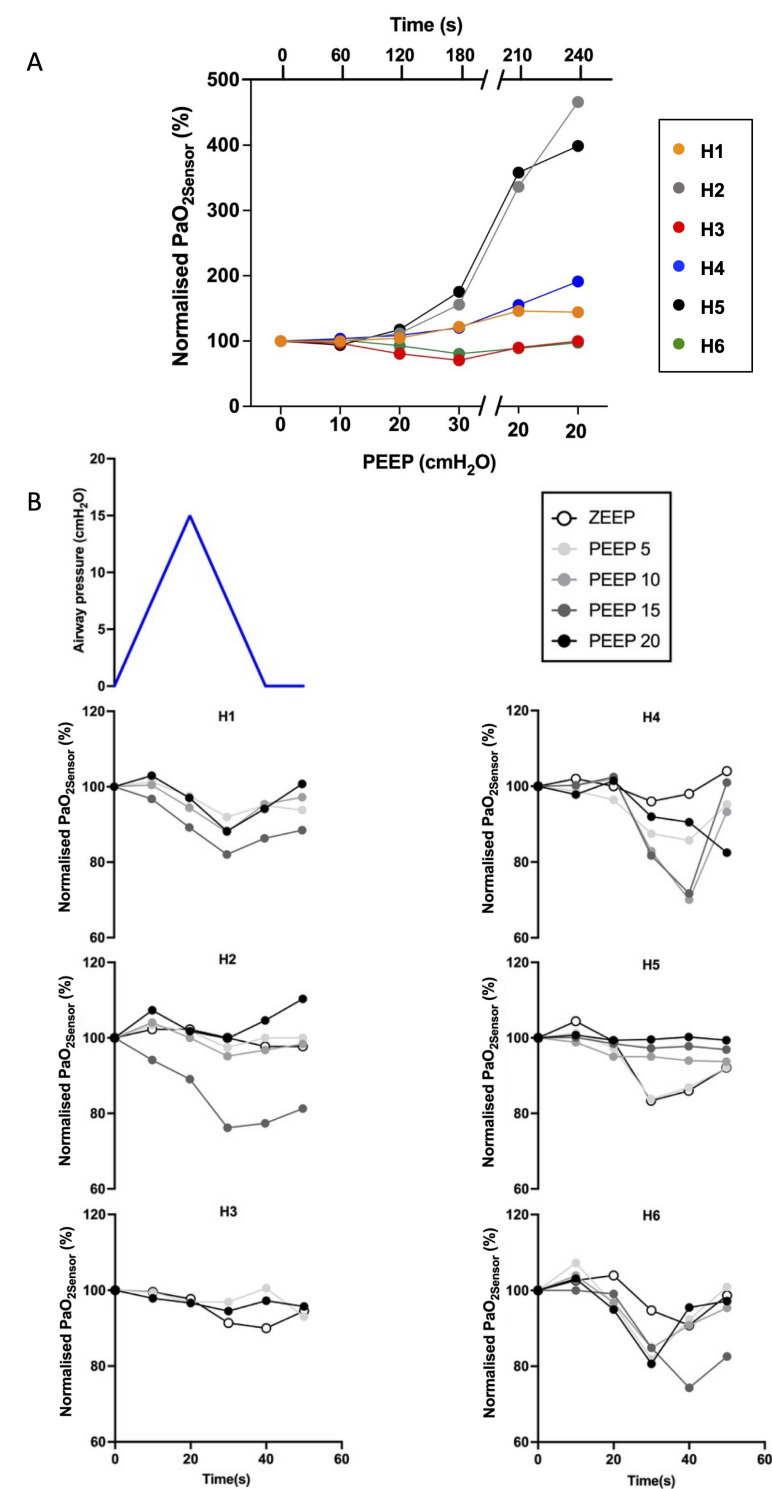


Figure 5: Panel A: Relative changes in arterial partial pressure of oxygen measured with the fiberoptic sensor ( $\text{PaO}_{2\text{Sensor}}$ ) in response to the alveolar recruitment manoeuvre (ARM). The relative  $\text{PaO}_{2\text{Sensor}}$  changes (normalised  $\text{PaO}_{2\text{Sensor}}$ ) were calculated for the three steps of positive end-expiratory pressure (PEEP) used during the ARM (10, 20 and 30 cmH<sub>2</sub>O) and the first minute of PEEP of 20 cmH<sub>2</sub>O, using as a reference (100%) the  $\text{PaO}_{2\text{Sensor}}$  recorded immediately before the ARM in the absence of PEEP. Data are from six horses, where each horse is indicated with a different colour. Panel B: Relative changes in  $\text{PaO}_{2\text{Sensor}}$  (normalised  $\text{PaO}_{2\text{Sensor}}$ ) during a slow pulmonary inflation and deflation manoeuvre performed in the absence (PEEP 0) and in four levels of PEEP (5, 10, 15, and 20 cmH<sub>2</sub>O). The relative changes in  $\text{PaO}_{2\text{Sensor}}$  were calculated in 10-second intervals during the manoeuvre and at the first 10 seconds of tidal breathing following the manoeuvre, using as the reference (100%) the  $\text{PaO}_{2\text{Sensor}}$  recorded immediately before the manoeuvre. The top left panel shows the variation in airway pressure during the manoeuvre (blue line). The other graphs of the panel illustrate the relative changes in  $\text{PaO}_{2\text{Sensor}}$  in all horses [Horses 1-6 (H1-6)].

SUPPLEMENTARY DATA S1

1. Details of the Methods

1.1. Animal preparation and anaesthesia:

The demographic data of the six horses used in this study are presented in Table 1S.

The horses were fasted for 12 hours before the experiments, and water was available *ad libitum*.

After infiltration with 2 ml of 2% lidocaine (VET ONE, USA) a 14-gauge intravenous catheter (BD Infusion Therapy Systems, USA) was placed into the left jugular vein for the administration of drugs and fluid therapy. Anaesthesia was induced with intravenous (IV) ketamine (2.2 mg/kg, VET ONE, USA) and propofol (3 mg/kg, Sagent Pharmaceuticals, USA). Following endotracheal intubation with a 30 ID cuffed endotracheal tube (Jorvet, USA) horses were connected to a circle breathing system primed with (2.5 %) isoflurane in 70% oxygen. Then they were hoisted onto a movable padded surgical table and placed into dorsal recumbency.

Anaesthesia was maintained during the instrumentation and the study protocol with a target end-tidal concentration of isoflurane (FE'ISO) between 1.8 and 2.2% [approximately 1.5 minimum alveolar concentration<sup>1</sup> and mechanical ventilation was performed as described in the manuscript. Hartmann's solution (Dechra Veterinary Products US) was intravenously administered at 10 ml/kg/h through a jugular catheter. During the experiments, a base-apex electrocardiogram was used to monitor heart rate (HR) and rhythm, and pulse oximetry (SpO<sub>2</sub>) was measured with the sensor at the tongue of the horses.

Two 9 Fr sheath introducers (Arrow, USA) were placed in the right jugular vein for the placement of central venous (PE 240, 110 cm, Becton Dickinson and Company, USA) and a thermodilution pulmonary artery catheter (7-F, 110 cm, Arrow, USA) for the measurement of central pressures and cardiac output (CO) unrelated to the aim of this study. All arterial and central catheters were maintained heparinized and connected to calibrated pressure transducers. Thermodilution CO was measured by the fast injection (4s) of 35 ml of cold saline (0 – 2°C) into the right atrium.<sup>2</sup> Mean CO was calculated from three measurements within 10% difference. Cardiac index was calculated as CO to the 0.67 power.<sup>3</sup>

Heating lamps and blankets were applied to maintain body temperature between 35.5 and 37.5°C, which was measured by the thermistor of the thermodilution catheter. A urinary catheter was aseptically placed and connected to a sterile collecting bag.

1.2. Anaesthetic recovery

After the data collection at the last PEEP step, all catheters and introducer sheaths were removed, and compression haemostasis applied to all puncture sites until no evident bleeding was noted. Isoflurane was discontinued and horses transferred to the recovery stall for anaesthetic recovery. A padded helmet was placed on the horse's head, the endotracheal tube taped to the mandible, and 3 ml of phenylephrine (1 mg/ml, Sagent Pharmaceuticals, India) administered into each nasal cavity. Once horses started breathing spontaneously, 0.2 mg/kg of xylazine (Xylamed, VET ONE, USA) was given intravenously and oxygen insufflation (15 l/min) was provided by the endotracheal tube throughout the recovery period. Anaesthesia recovery was closely monitored from outside of the recovery stall until the horse was standing and stable. After approximately 20 minutes from standing up, the horse was walked back to his stall. On the day after the experiments, the horses were returned to the institutional equine research herd.

2. Changes in PaO<sub>2</sub> caused by changes dobutamine infusion rate

The dobutamine infusion rates used during the study were annotated in real time in the data acquisition software files for the offline identification of the resulted changes in PaO<sub>2</sub> (ΔPaO<sub>2</sub>). After any change in dobutamine infusion rate (ΔDobutamine), ΔPaO<sub>2</sub> was calculated as the percentage difference between PaO<sub>2Sensor</sub> measured five minutes after and its respective values immediately before the change in dobutamine rate.

Changes in PaO<sub>2Sensor</sub> were observed in many cases where the intravenous administration of dobutamine was started or its dose was increased or decreased. Fourteen points of ΔPaO<sub>2</sub> associated with changes in dobutamine rate of administration were analysed and in 71% of the cases, ΔPaO<sub>2</sub> presented the same direction of variation as ΔDobutamine (Table S2).

Hypotension is a frequently encountered negative cardiovascular effect of general anaesthesia, and dobutamine is a synthetic catecholamine used as a first line treatment for its management in anesthetized horses.<sup>4</sup> Dobutamine effects are primarily on α adrenergic receptors resulting in increased myocardial contractility, stroke volume and subsequent increase in cardiac output.<sup>5</sup> The effects of dobutamine is gas exchange and V̇/Q mismatch are controversial, with reports of increase<sup>6</sup> or no effect<sup>7</sup> in venous admixture and PaO<sub>2</sub> in anesthetized horses. However, dobutamine increased CO, venous admixture and PaO<sub>2</sub> in a study with human patients with one lung atelectasis.<sup>8</sup> In most times that the administration of dobutamine was initiated or changed, PaO<sub>2Sensor</sub> followed the same direction in 71% of the cases. Examples of an increase and decrease in PaO<sub>2Sensor</sub> are presented in Figure S3. It is beyond the scope of this study to elucidate the effects of dobutamine on PaO<sub>2</sub> and this topic deserves further investigation in future studies.

3. References

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Table S1: Baseline characteristics for six horses anesthetized with isoflurane and mechanically ventilated with tidal volume of 15 mL kg<sup>-1</sup>, no positive end-expiratory pressure, inspiratory to expiratory time ratio of 1:2, and respiratory rate adjusted to maintain end-tidal partial pressure of CO<sub>2</sub> between 30 and 40 mmHg. Data expressed as mean (SD) or median (quartile 1 – quartile 3).

Age (years)	9.8 (1.5)
Weight (kg)	581 (47)
Heart Rate (beats/minute)	44 (4)
MAP (mmHg)	74 (15)
Cardiac output (L/minute)	36.5 (8.6)
Cardiac index (L/min/kg <sup>0.67</sup> )	0.50 (0.11)
MPAP (mmHg)	11 (2)
Hb (g/dL)	12.7 (1.9)
FIO <sub>2</sub>	0.91 (0.89 – 0.91)
pH	7.372 (0.044)
PaO <sub>2Ref</sub> (mmHg)	66.0 (53.2 – 88.2)
PaCO <sub>2</sub> (mmHg)	48.6 (4.7)
PaO <sub>2</sub> :FIO <sub>2</sub> (mmHg)	73 (72 – 97)
VT <sub>BW</sub> (mL/kg)	14.5 (0.5)
RR (breaths/minute)	7 (2)
P <sub>peak</sub> (cmH <sub>2</sub> O)	28 (5)
FE'ISO (%)	2.0 (0.2)
Body temperature (°C)	36.1 (0.5)
MAP = mean arterial pressure, MPAP = mean pulmonary artery pressure, Hb = haemoglobin concentration, FIO <sub>2</sub> = fraction of inspired O <sub>2</sub> , PaO <sub>2Ref</sub> = arterial O <sub>2</sub> partial pressure measured by an automated blood gas machine, PaCO <sub>2</sub> = arterial CO <sub>2</sub> partial pressure, VT <sub>BW</sub> = tidal volume indexed to body weight, RR = respiratory rate, PEEP = positive end-expiratory pressure, P <sub>peak</sub> = peak inspiratory pressure, FE'ISO = expired fraction of isoflurane	



Table S2. Variations in intravenous dobutamine dosage ( $\Delta$ Dobutamine) and the oxygen arterial partial pressure ( $\Delta$ PaO<sub>2Sensor</sub>) measured by the fiberoptic sensor (PaO<sub>2Sensor</sub>) positioned in the facial artery of anaesthetized horses in dorsal recumbency (n = 6). After any change in dobutamine infusion rate,  $\Delta$ PaO<sub>2Sensor</sub> was calculated as the percentage difference between PaO<sub>2Sensor</sub> measured five minutes after and its values immediately before the change in dobutamine rate. EEP = end-expiratory pressure

Horse	EEP (cmH <sub>2</sub> O)	$\Delta$ Dobutamine (mcg/kg/min)	$\Delta$ PaO <sub>2Sensor</sub> (%)
1	20	2	93
2	15	2	53
2	0	0.25	-6
2	15	-1	-24
3	0	3	35
3	20	3	4
3	15	-3	2
4	0	0.5	-8
5	0	0.5	21
5	15	-1	-1
6	20	1	127
6	10	1	38
6	15	-1	-21
6	5	-0.5	28

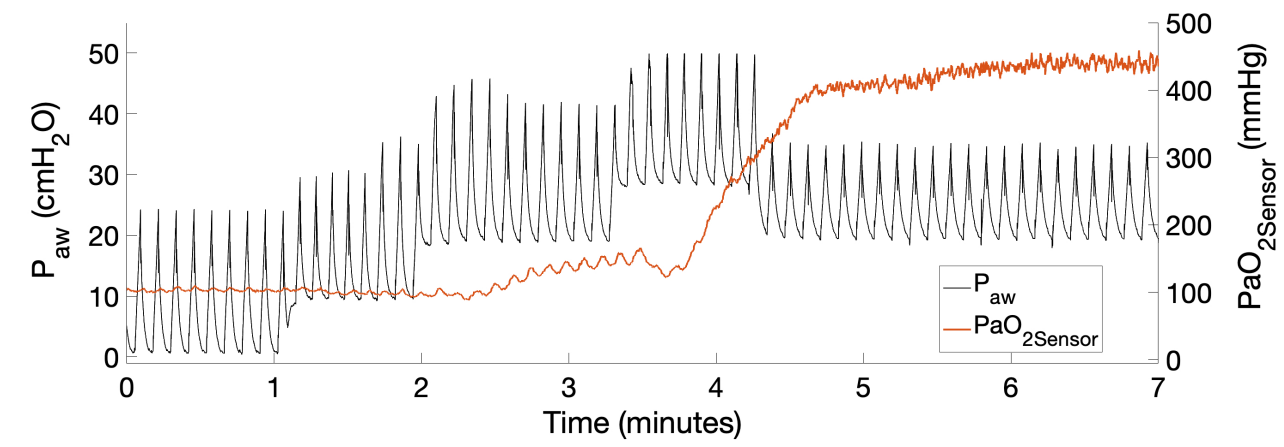


Figure S1

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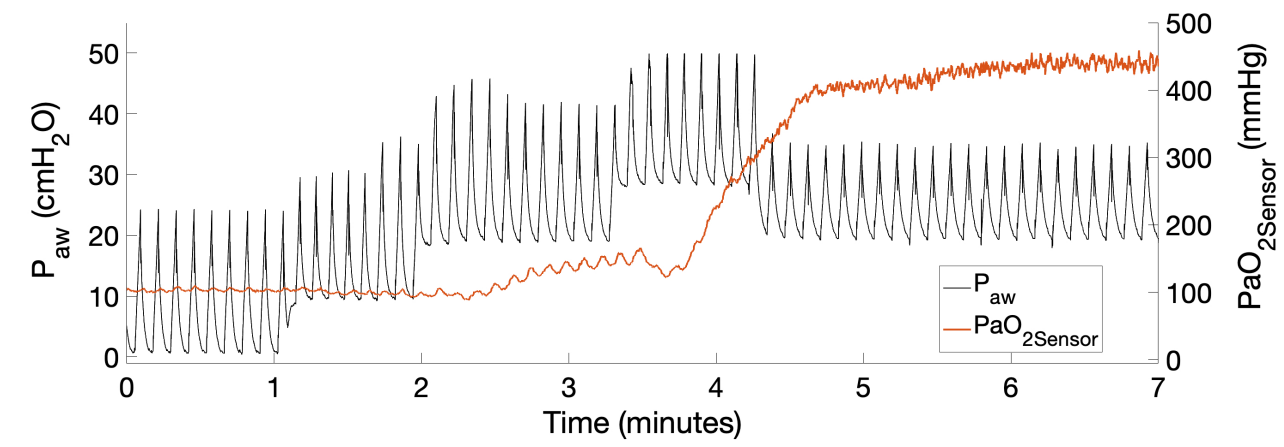


Figure S1

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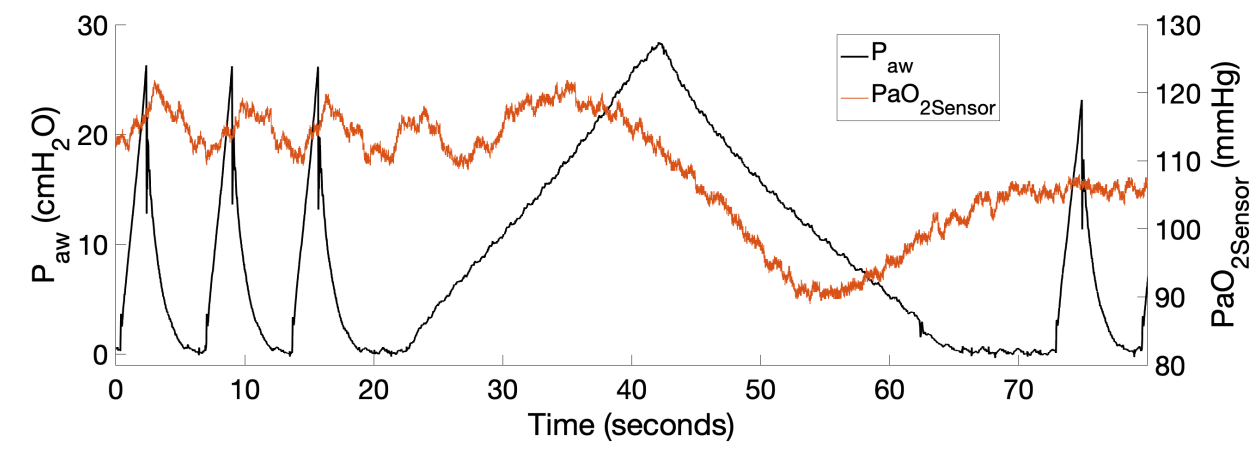


Figure S2

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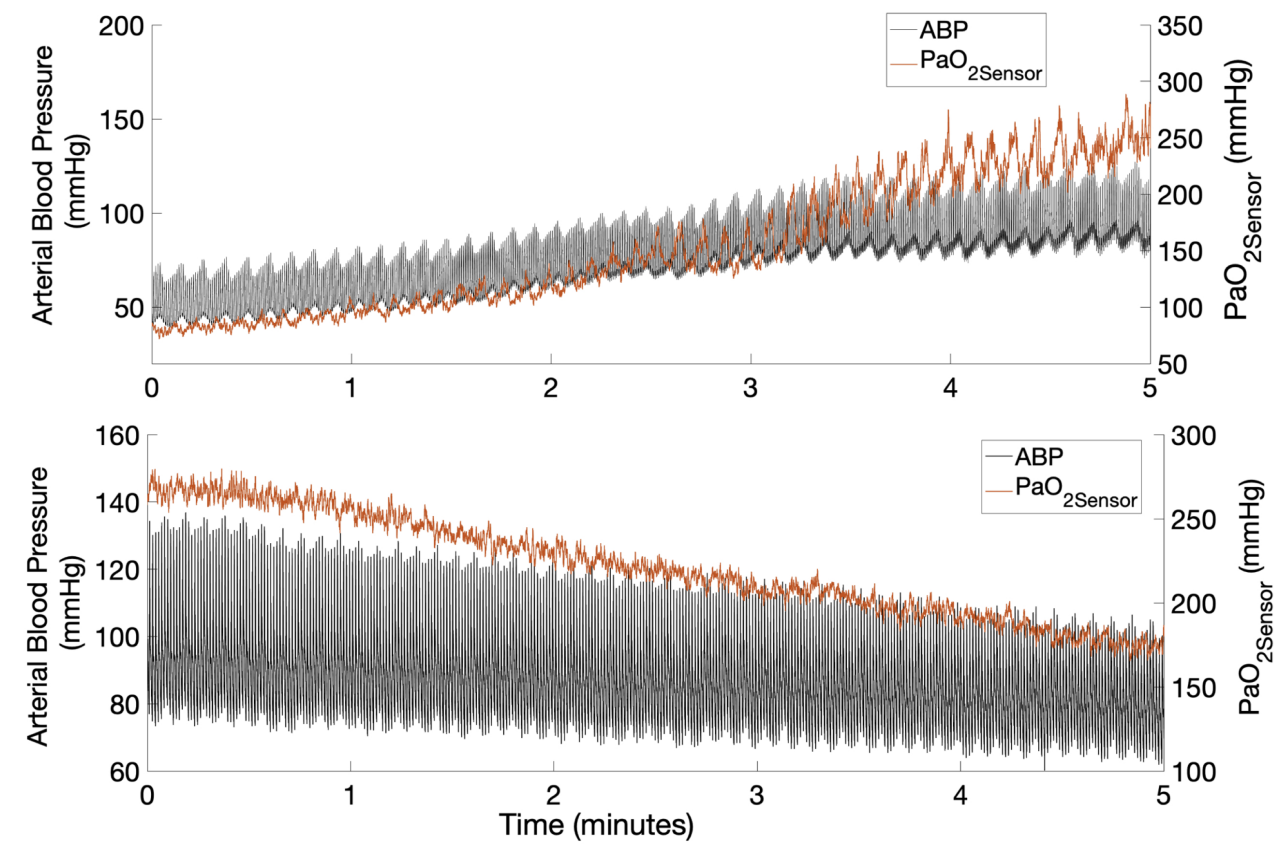


Figure S3

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