Effects of pneumoperitoneum and of an alveolar recruitment maneuver followed by positive end-expiratory pressure on cardiopulmonary function in sheep anesthetized with isoflurane–fentanyl


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Abstract

Objective To investigate the effects of pneumoperitoneum alone or combined with an alveolar recruitment maneuver (ARM) followed by positive end-expiratory pressure (PEEP) on cardiopulmonary function in sheep.

Study design Prospective, randomized, crossover study.

Animals A total of nine adult sheep (36–52 kg).

Methods Sheep were administered three treatments (≥10-day intervals) during isoflurane–fentanyl anesthesia and volume-controlled ventilation (tidal volume: 12 mL kg⁻¹) with oxygen: CONTROL (no intervention); PNEUMO (120 minutes of CO₂ pneumoperitoneum); PNEUMO_ARM/PEEP (PNEUMO protocol with an ARM instituted after 60 minutes of pneumoperitoneum). The ARM (5 cmH₂O increases in PEEP of 1 minute duration until 20 cmH₂O of PEEP) was followed by 10 cmH₂O of PEEP until the end of anesthesia. Cardiopulmonary data were recorded until 30 minutes after abdominal deflation.

Results PaO₂ was decreased from 435–462 mmHg (58.0–61.6 kPa) in CONTROL to 377–397 mmHg (50.3–52.9 kPa) in PNEUMO (p < 0.05). Quasistatic compliance (Cₚqst, mL cmH₂O⁻¹ kg⁻¹) was decreased from 0.85–0.92 in CONTROL to 0.52–0.58 in PNEUMO. PaO₂ increased from 383–385 mmHg (51.1–51.3 kPa) in PNEUMO to 429–444 mmHg (57.2–59.2 kPa) in PNEUMO_ARM/PEEP (p < 0.05) and Cₚqst increased from 0.52–0.53 in PNEUMO to 0.70–0.74 in PNEUMO_ARM/PEEP. Abdominal deflation in PNEUMO did not restore PaO₂ and Cₚqst to control values. Cardiac index (L minute⁻¹ m²) decreased from 4.80–4.70 in CONTROL to 3.45–3.74 in PNEUMO and 3.63–3.76 in PNEUMO_ARM/PEEP. Compared with controls, ARM/PEEP with pneumoperitoneum decreased mean arterial pressure from 81 to 68 mmHg and increased mean pulmonary artery pressure from 10 to 16 mmHg.

Conclusions and clinical relevance Abdominal deflation did not reverse the pulmonary function impairment associated with pneumoperitoneum. The ARM/PEEP improved respiratory compliance and reversed the oxygenation impairment induced by pneumoperitoneum with acceptable hemodynamic changes in healthy sheep.

Keywords alveolar recruitment maneuver, pneumoperitoneum, sheep.

Introduction

Anesthetized sheep may develop oxygenation impairment and increased alveolar-to-arterial oxygen gradient [P(A-a)O₂] owing to the presence of large areas of atelectasis in dependent lung regions.
Changes in lung function in anesthetized sheep are similar with those observed in anesthetized humans because the development of atelectatic areas on computed tomography show a strong linear correlation with the degree of oxygenation impairment and intrapulmonary shunt fraction \( (Q_s/Q_t) \) in both species (Wolf et al. 2015).

Pneumoperitoneum for laparoscopic surgery is commonly used in humans and in dogs. Inflation of the abdomen in humans with carbon dioxide \((CO_2)\) for laparoscopic procedures results in decreased total respiratory compliance owing to a cranial displacement of the diaphragm, although arterial oxygenation may not be significantly impaired (Andersson et al. 2002; Sprung et al. 2002; Nguyen et al. 2004). However, lung function in anesthetized sheep and other ruminants may be more severely affected by pneumoperitoneum because progressive gas trapping within the rumen may further displace the diaphragm cranially and result in oxygenation impairment because of atelectasis (Fujimoto & Leneham 1985; Hedenstierna et al. 1989).

An alveolar recruitment maneuver (ARM) is used during mechanical ventilation to open collapsed alveoli by temporary administration of a high inspiratory pressure (Lachmann 1992; Tusman et al. 1999). This procedure is followed by long-term application of positive end-expiratory pressure (PEEP) that is adjusted to maintain these alveoli open throughout the entire respiratory cycle. This strategy may increase arterial oxygen partial pressure \( (PaO_2) \) and respiratory system compliance during pneumoperitoneum for laparoscopic gastric banding in obese human patients (Almarakbi et al. 2009).

The hypothesis for the present study was that \( CO_2 \) pneumoperitoneum up to an intra-abdominal pressure of 15 mmHg would cause a negative effect on respiratory system compliance \( [\text{decreased quasistatic compliance (C}_{\text{qst}})\] \) and decrease \( PaO_2 \) by increasing \( Q_s/Q_t \). In this scenario, an ARM consisting of stepwise increases in PEEP until 20 cmH\(_2\)O followed by 10 cmH\(_2\)O of PEEP during volume-controlled ventilation would reverse the oxygenation impairment and decreased \( C_{qst} \) without causing excessive cardio-vascular depression.

### Materials and methods

### Animals and study design

This study was approved by the Animal Care Committee of the São Paulo State University (no. 171/2015). A total of nine Santa Inês sheep (four females and five males). 14–18 months old and weighing 36–52 kg were selected for the study. Animals were healthy determined by physical examination, complete blood cell count and fecal egg count within normal ranges before each experiment.

The animals were administered three treatments at intervals of 10 days in a prospective, randomized (www.randomizer.org) crossover design: 1) CONTROL treatment, anesthesia maintained with conventional volume-controlled ventilation [tidal volume \( (V_t) \) 12 mL kg\(^{-1}\)] and zero end-expiratory pressure (ZEEP) with no intervention; 2) PNEUMO treatment, \( CO_2 \) pneumoperitoneum with a 15 mmHg intra-abdominal pressure for 120 minutes, with the same ventilation protocol used in CONTROL; and 3) PNEUMO\(_{ARM/PEEP}\) treatment, \( CO_2 \) pneumoperitoneum with a 15 mmHg intra-abdominal pressure, ventilation protocol used in CONTROL for 60 minutes of pneumoperitoneum, followed by an ARM and 10 cmH\(_2\)O PEEP for another 60 minutes of pneumoperitoneum.

### Instrumentation and variables monitored

Food and water were withheld for 24 and 12 hours before anesthesia, respectively. A 20 gauge catheter was inserted into a cephalic vein for drug administration and infusion of lactated Ringer’s solution (2 mL kg\(^{-1}\) hour\(^{-1}\)), JP Indústria Farmacêutica, SP, Brazil during anesthesia. Ceftriaxone (1.1 mg kg\(^{-1}\)), Minoxel Plus, São Paulo, SP, Brazil was administered intramuscularly (IM) before anesthesia and at 24 and 48 hours after anesthesia. Animals were administered flunixin meglumine (1.1 mg kg\(^{-1}\)); Banamine, MSD, SP, Brazil) intravenously (IV) and fentanyl (5 \( \mu g \) kg\(^{-1}\), Fentanest; Cristália Produtos Químicos e Farmacêuticos Ltda, SP, Brazil) IV followed 15 minutes later by induction of anesthesia with IV propofol (5.6 ± 0.9 mg kg\(^{-1}\); Propovan; Cristália Produtos Químicos e Farmacêuticos Ltda) titrated until orotracheal intubation could be performed. Animals were positioned in dorsal recumbency and anesthesia was maintained with isoflurane (Isorfin; Cristália Produtos Químicos e Farmacêuticos Ltda) in oxygen and an infusion of fentanyl (5 \( \mu g \) kg\(^{-1}\) hour\(^{-1}\)) IV using a syringe pump (Pump 11 Elite; Harvard Apparatus, MA, USA). Inspired \( O_2 \) fraction \( (FI_{O_2}) \) was monitored by a paramagnetic cell, and end-expired isoflurane fraction \( (FE_{ISO}) \) and end-expired carbon dioxide partial pressure \( (FE_{CO_2}) \) were monitored by an infrared gas analyzer incorporated into the anesthesia apparatus (Dräger Primus; Drägerwerk AG & Co. Germany). \( FE_{ISO} \) and \( FI_{O_2} \) were maintained at 1.5% and \( \geq 90\%.\)
respectively, throughout anesthesia. Accuracy of the gas analyzer was verified before and at the end of the study using standard gas mixture containing 1.4 vol.% of isoflurane (White Martins, Gases Especiais, SP, Brazil).

Animals were allowed to breathe spontaneously during the initial 60 minutes of anesthesia. After this period, volume-controlled ventilation with ZEEP (Dräger Primus; Drägerwerk AG & Co) was initiated under conditions of neuromuscular blockade induced by atracurium (0.3 mg kg$^{-1}$ bolus, followed by 0.5 mg kg$^{-1}$ hour$^{-1}$; Tracur; Cristália Produtos Quimicos e Farmacêuticos Ltda) administered IV by a second infusion pump (Digipump SR 8X; Digicare Biomedical Technology, FL, USA). The expired VT and the inspiration:expiration ratio were held constant throughout anesthesia (12 mL kg$^{-1}$ and 1:2, respectively), whereas the respiratory rate ($f_R$) was adjusted as necessary to maintain the arterial partial pressure of carbon dioxide (PaCO$_2$) close to 45 mmHg (6 kPa). The ventilator was adjusted to produce a plateau pressure ($P_{plat}$) during inspiration by adding an inspiratory pause (zero flow after the preset VT was achieved) that corresponded to 30% of the inspiratory time. Accuracy of VT and of airway pressures measured by the ventilator was verified by a spirometry tester (Spirometry tester 844202; Datex-Ohmeda, Finland) before and after completion of the study (Videos S1 and S2).

The level of neuromuscular blockade was evaluated every 15 minutes by visual assessment of pelvic limb contractions in response to a supramaximal electrical stimulus (Neuroestimulador E2107; BGE Médica, SP, Brazil) applied to the tibial nerve.

An 18 gauge catheter (Inyte; Becton Dickinson, MG, Brazil) was inserted percutaneously into a femoral artery for monitoring mean arterial pressure (MAP) via a fluid-filled pressure transducer system (Tru Wave PX 260; Edward Lifesciences Corp., CA, USA). A 7.5 Fr balloon-tipped thermocatheter (131HF7; Edwards Lifesciences Corp.) was inserted into the jugular vein through an 8 Fr introducer sheath and advanced until its tip was positioned in the pulmonary artery (PA) guided by characteristic pressure waveforms (AS/3; Datex Ëngstrom, Finland). The proximal and distal ports of the catheter were connected to two pressure transducers to monitor central venous pressure (CVP) and mean PA pressure (MPAP). PA occlusion pressure (PAOP) was measured at predefined time points by inflating the balloon of the PA catheter with 0.7 mL of air. Transducers were zeroed level with the scapulo-humeral joint.

Heart rate (HR) was calculated from the arterial pressure waveform. Cardiac output (CO) was measured by injecting 5 mL of ice-cold (3–5 °C) physiologic saline solution into the CVP port. For each data sampling time, CO was averaged from three serial measurements. Body surface area, cardiac index (CI), pulmonary vascular resistance index (PVRI) and systemic vascular resistance index (SVRI) were calculated using standard formulas (Appendix S1).

Peak inspiratory pressure ($P_{peak}$), $P_{plat}$, and $C_{spi}$ [calculated as $VT/(P_{plat} −$ end-expiratory pressure)], and minute volume ($V_{E} = VT \times f_R$) were recorded from the mechanical ventilator. The driving pressure ($\Delta P$) was calculated as the gradient between $P_{plat}$ and end-expiratory pressure. Arterial and mixed-venous blood samples were collected from the femoral artery and PA catheters, respectively, and immediately analyzed for temperature-corrected blood gases (RAPIDLab 348; Siemens Healthcare, UK). The PaO$_2$/FiO$_2$ ratio and the arterial-to-end-expired CO$_2$ gradient (PaCO$_2$−Pe′CO$_2$) were calculated at each data sampling time. Hemoglobin concentration, PaO$_2$, PaCO$_2$ and partial pressures of mixed-venous oxygen (PvO$_2$) and carbon dioxide (PvCO$_2$) were used to calculate $Q_s/Q_t$ (Appendix S1).

**Experimental protocol**

After completion of instrumented procedure during the initial 60-minute period of spontaneous ventilation, volume-controlled ventilation with ZEEP was initiated as previously described and baseline (BL) cardiopulmonary variables were recorded after 30 minutes of conventional mechanical ventilation in all treatments (Fig. 1). After BL data collection was performed in the PNEUMO and PNEUMO ARM/PEEP treatments, pneumoperitoneum was induced by inflating the abdomen with a CO$_2$ insufflator (Surgical CO$_2$ insufflator; Olympus, Japan) through a 14 gauge catheter inserted percutaneously into the abdominal cavity. 5–10 cm cranial to the udder and 15 cm to the right of the midline. The CO$_2$ influx into the abdomen, initially set at 1 L minute$^{-1}$, was automatically adjusted to maintain an intra-abdominal pressure of 15 mmHg during the 120 minutes of pneumoperitoneum. Cardiopulmonary data were collected at 15, 30, 60, 90, and 120 minutes of intra-abdominal insufflation in the PNEUMO and PNEUMO ARM/PEEP treatments (or no intervention in controls). After this time, the abdomen was
Figure 1 Experimental protocol of treatments CONTROL, PNEUMO, and PNEUMO\textsubscript{ARM/PEEP}.

VCV, volume controlled ventilation; ARM, alveolar recruitment maneuver; PEEP, positive end-expiratory pressure.
deflated, and the last set of cardiopulmonary data was recorded 30 minutes later (150 minutes after induction of pneumoperitoneum in the PNEUMO and PNEUMOARM/PEEP treatments or no intervention in the CONTROL treatment).

After 60 minutes of pneumoperitoneum under volume-controlled ventilation and ZEEP in the PNEUMOARM/PEEP treatment, an ARM was initiated by adding 5 cmH2O of PEEP, on a minute-by-minute basis, until 20 cmH2O of PEEP was achieved and maintained for another minute. After this time, PEEP was decreased to 10 cmH2O and maintained at this level until the end of the observation period.

In addition, the immediate cardiovascular effects of the ARM were evaluated by comparing HR and MAP measured immediately before the ARM at ZEEP and at the end of the 1 minute of 20 cmH2O PEEP.

**Recovery from anesthesia**

After the last data collection, atracurium and fentanyl infusions were discontinued. Upon the return of three twitches in response to the train-of-four stimulation, neostigmine (0.03 mg kg\(^{-1}\); Normastig, Uniao Quimica, MG, Brazil) and atropine (0.03 mg kg\(^{-1}\); Pasmodex, Isofarma, CE, Brazil) were administered IV. After the return of four twitches of a similar strength (based on visual assessment) in response to the train-of-four stimulation, mechanical ventilation and isoflurane administration were interrupted, and animals were disconnected from the breathing circuit once \(P_t/CO_2\) values were <45 mmHg (6 kPa) during spontaneous breathing. Pulse oximetry was monitored when animals started breathing room air to ensure that oxygen supplementation was no longer necessary (hemoglobin saturation values ≥95%). Animals were maintained in sternal recumbency, and the orotracheal tube was removed with the cuff inflated after they were able to maintain their heads in an upright position without assistance. The total duration of anesthesia and the time elapsed from the end of anesthesia until the animals were able to remain in standing position were recorded. The animal’s overall status, appetite and rectal temperature were monitored once daily during the first 5 days of the postanesthetic period to monitor for the risk of aspiration pneumonia associated with regurgitation of gastric contents during anesthesia.

**Statistical analysis**

Based on \(P_{O_2}\) values recorded in anesthetized sheep during volume-controlled ventilation and ZEEP in our laboratory [mean ± standard deviation (SD), 446 ± 67 mmHg (59.3 ± 8.9 kPa)] (Lagos-Carvajal et al. 2015), a sample of nine animals was estimated to detect a reduction of 25% in mean \(P_{O_2}\) after pneumoperitoneum, assuming the same coefficient of variation (15%) and a statistical power of 90% with an alpha error of 5% (G*Power for Windows Version 3.1.6; Heinrich Heine Universität, Germany). Data were analyzed with a commercial statistical software (Prism Version 6.02; GraphPad, CA, USA). Normality of data distribution and the presence of outliers were verified by a Shapiro–Wilk and a Grubb’s test, respectively. In the case of significant outliers (\(p < 0.05\)) on the main outcome variables, data from that individual were not included in the statistical analysis. The immediate effects of the ARM on \(P_{peak}\). HR and MAP were compared by a paired Student t test. Comparisons between treatments were performed by a two-way analysis of variance (ANOVA) for repeated measures by the two factors (time and treatment). Post hoc comparisons among treatments were performed by a Tukey’s test. A Dunnet’s post hoc test assessed time-related changes within each treatment in relation to BL. Values are presented as mean ± SD, and the statistical significance was set at \(p < 0.05\).

**Results**

**Immediate effects of ARM on HR and MAP**

The \(P_{plat}\) values (mean ± SD) recorded at each step-wise increase in PEEP performed during the ARM are shown in Figure 2. HR did not change significantly (\(p = 0.19\)) between values recorded during ZEEP (113 ± 39 beats minute\(^{-1}\)) and values recorded during the highest PEEP level (124 ± 44 beats minute\(^{-1}\)), whereas MAP was significantly decreased from 79 ± 16 mmHg during ZEEP to 65 ± 19 mmHg during 20 cmH2O of PEEP (\(p = 0.0008\)).

**Effects of pneumoperitoneum alone or combined with an ARM followed by PEEP on cardiopulmonary function**

The \(P_{O_2}, P_{O_2}/FiO_2\) ratio and \(Q_{s}/Q_{t}\) recorded after pneumoperitoneum alone were asymmetrically distributed because one animal was detected as a significant outlier (\(p < 0.05\)). Oxygenation data (\(P_{O_2}, P_{O_2}/FiO_2\) ratio, \(P_{TtO_2}, S_{vO_2}\) and \(Q_{s}/Q_{t}\)) from this animal were removed from the statistical analyses, and these results are presented and discussed in supporting information (Table S1, Discussion S1).
Airway pressures recorded during alveolar recruitment maneuver (ARM) in sheep with CO2 pneumoperitoneum. During stepwise increases in positive end-expiratory pressure (PEEP) in volume-controlled mode, the ventilator maintained the inspiratory flow constant, and consequently the tidal volume adjusted in volume control mode remained constant (12 mL kg⁻¹). The driving pressure (ΔP), measured as the gradient between plateau pressure (Pplat) and end-expiratory pressure (PEEP), resulted in Pplat values of approximately 40 mmHg at the highest PEEP level (20 cmH₂O). Mean ΔP values decreased after the ARM to approximately 15–16 cmH₂O.
In PNEUMO, PaO₂ and PaO₂/FIO₂ ratio were significantly decreased from BL and from values recorded in CONTROL throughout the period of intra-abdominal insufflation (Figs 3a & b). In PNEUMOARM/PEEP, PaO₂ and PaO₂/FIO₂ ratio were significantly decreased from BL and from values recorded in CONTROL only during the period of pneumoperitoneum preceding the ARM (15–60 minutes). In PNEUMO, the decrease in PaO₂ and PaO₂/FIO₂ ratio from BL and from corresponding values recorded in CONTROL persisted after abdominal deflation (150 minutes). After the ARM was initiated in PNEUMOARM/PEEP, PaO₂ and PaO₂/FIO₂ ratio values did not differ from BL. The PaO₂ and PaO₂/FIO₂ ratio values in PNEUMOARM/PEEP were significantly higher than values recorded in PNEUMO after the ARM was instituted (90 and 120 minutes) and did not differ significantly from PaO₂ and PaO₂/FIO₂ ratio values recorded in CONTROL until 30 minutes after abdominal deflation (90–150 minutes).

In PNEUMO, intra-abdominal insufflation significantly increased Qs/Ql from values recorded in BL (except for Qs/Ql at 30 minutes) and from values recorded in CONTROL (except for Qs/Ql at 15 minutes) (Fig. 3c). During the period of intra-abdominal insufflation that preceded the ARM (15–60 minutes) in PNEUMOARM/PEEP, the Qs/Ql was significantly increased from BL. During the period of pneumoperitoneum alone in PNEUMOARM/PEEP, Qs/Ql was significantly higher (at 30 minutes) in comparison with CONTROL. After the ARM/PEEP was established, Qs/Ql was significantly lower than corresponding values recorded in PNEUMO (90–150 minutes) and did not differ from CONTROL (90–150 minutes).

Compared with BL, Cqst was significantly decreased at 90–150 minutes, whereas ΔP was significantly increased at 120 and 150 minutes in CONTROL (Figs 3d & e). Compared with BL, Cqst was significantly decreased and ΔP was significantly increased throughout the 120 minutes of pneumoperitoneum and after abdominal deflation at 150 minutes in PNEUMO and PNEUMOARM/PEEP. In PNEUMO, Cqst was significantly lower and ΔP was significantly higher than CONTROL during pneumoperitoneum (15–120 minutes). In PNEUMOARM/PEEP, intra-abdominal insufflation significantly decreased Cqst and significantly increased ΔP in relation to CONTROL before the ARM/PEEP was initiated (15–60 minutes). After institution of the ARM/PEEP (90–150 minutes), Cqst values in PNEUMOARM/PEEP were significantly higher than values recorded at the same time points in PNEUMO. However, Cqst values recorded after the ARM/PEEP (90–120 minutes) were significantly lower than corresponding values recorded in CONTROL. Institution of the ARM/PEEP decreased ΔP to values that were similar to CONTROL.

The PaCO₂–PvCO₂ gradient significantly increased from BL throughout the observation period in all treatments, except at 60 and 150 minutes in CONTROL (Fig. 3i). PaCO₂–PvCO₂ gradients were significantly higher during intra-abdominal insufflation (15–120 minutes) in PNEUMO and PNEUMOARM/PEEP in comparison with CONTROL (except at 30 minutes in PNEUMO). After abdominal deflation, only PaCO₂–PvCO₂ values recorded in PNEUMO remained significantly higher than CONTROL. Ppeak, Pplat, arterial pH, PaCO₂, V̇E and base excess data are presented in supporting information (Results S1).

CVP increased significantly from BL during intra-abdominal insufflation in PNEUMO and PNEUMOARM/PEEP (Fig. 4a). Significant increases in PAOP from BL were recorded throughout the period of intra-abdominal insufflation in PNEUMO and after the ARM was initiated in PNEUMOARM/PEEP (Fig. 4b). After abdominal deflation, CVP and PAOP did not differ significantly from BL in PNEUMO, but remained higher than BL in PNEUMOARM/PEEP. CVP was higher in PNEUMO and PNEUMOARM/PEEP than in CONTROL during pneumoperitoneum, except at 15 minutes in PNEUMO. After the ARM/PEEP was instituted in PNEUMOARM/PEEP, CVP and PAOP were significantly higher when compared with PNEUMO and CONTROL (90–150 minutes).

MPAP significantly increased from BL at 60 minutes of intra-abdominal insufflation in PNEUMO (Fig. 4c). During ARM/PEEP with pneumoperitoneum (90 and 120 minutes), MPAP was significantly higher in comparison with BL and in comparison with the other treatments; these changes persisted after abdominal deflation. MAP was significantly increased from BL at 90 and 120 minutes in CONTROL and, at these time points, MAP recorded in PNEUMOARM/PEEP was significantly lower in comparison with CONTROL (Fig. 4d).

HR and CI were significantly increased from BL in all treatments at some time points (Figs 4e & f). CI was significantly lower than in CONTROL during pneumoperitoneum alone in PNEUMO (90 and 120 minutes) and after the ARM/PEEP was instituted in PNEUMOARM/PEEP (90 and 120 minutes). SVRI and PVRI are presented in supporting information (Results S1).
Figure 3 Arterial oxygen tension (PaO₂, n = 8), ratio between PaO₂ and inspired O₂ fraction (PaO₂/FIO₂ ratio, n = 8), intrapulmonary shunt fraction (Q_s/Q_t, n = 8), quasistatic compliance (C_qst, n = 9), driving pressure (ΔP, n = 9) and arterial-to-end-expired CO₂ gradient (PaCO₂e−Pe′CO₂, n = 9) recorded in sheep anesthetized in dorsal recumbency with isoflurane and fentanyl under volume-controlled mechanical ventilation (V₁ = 12 mL kg⁻¹). Treatments were no intervention (CONTROL), 120 minutes of pneumoperitoneum (PNEUMO), or 120 minutes pneumoperitoneum and an alveolar recruitment maneuver (ARM) at 60 minutes followed by 10 cmH₂O positive-end-expiratory pressure (PEEP) (PNEUMOARM/PEEP). The ARM was stepwise increases in PEEP of 5 cmH₂O each minute until PEEP was 20 cmH₂O for another minute. BL, baseline. *Significant difference from baseline (p < 0.05). a,b,cSignificant differences between treatments indicated by different superscript letters (p < 0.05).
Figure 4 Central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), mean pulmonary artery pressure (MPAP), mean arterial pressure (MAP), heart rate (HR) and cardiac index (CI) recorded in sheep (n = 9) anesthetized in dorsal recumbency with isoflurane and fentanyl under volume-controlled mechanical ventilation (Vt = 12 mL kg\(^{-1}\)). Treatments were no intervention (CONTROL), 120 minutes of pneumoperitoneum (PNEUMO), or 120 minutes pneumoperitoneum and an alveolar recruitment maneuver (ARM) at 60 minutes followed by 10 cmH\(_2\)O positive-end-expiratory pressure (PEEP) (PNEUMO ARM/PEEP). The ARM was stepwise increases in PEEP of 5 cmH\(_2\)O each minute until PEEP was 20 cmH\(_2\)O for another minute. BL, baseline. *Significant difference from baseline (p < 0.05). a,b,cSignificant differences between treatments indicated by different superscript letters (p < 0.05).
The total duration of anesthesia and the times until animals were in standing position did not differ between treatments [overall median (interquartile range): 272 (251–289) minutes and 29 (18–43) minutes, respectively]. No postanesthetic complications were observed.

Discussion

This study highlights two aspects: 1) in addition to a deterioration in total respiratory compliance, CO₂ pneumoperitoneum in sheep anesthetized with isoflurane–fentanyl caused a mild degree of oxygenation impairment as a result of increases in $Q_s/Q_t$, which was not reversed by abdominal deflation; 2) the ARM/PEEP proposed was able to reverse the oxygenation impairment and to minimize the decreases in $C_{qst}$ associated with pneumoperitoneum, while resulting in acceptable cardiovascular changes in healthy animals. These effects were evident when comparisons were performed with BL values and with CONTROL values. The CONTROL treatment was included in the study design to account for time-related changes in respiratory function—changes that may be particularly important in sheep because of the progressive gas trapping that occurs within the ruminal compartment during anesthesia.

Assessment of the respiratory system viscoelastic properties during mechanical ventilation, without the confounding effects of frictional component of the work done (airway resistance), requires that the ratio between $V_T$ and the change in airway pressure (end-inspiratory minus end-expiratory pressures) be recorded during zero flow, which can be obtained by pausing the inspiratory flow once the preset $V_T$ is achieved (inspiratory pause) (Henderson & Sheel 2012). In the present study, the period of inspiratory pause ranged from 0.4 to 1.0 second (30% of inspiratory time) as a function of changes in $f_R$ (inspiration/expiration ratio kept constant at 1:2). Because the length of inspiratory pause/$P_{plat}$ was shorter than required for the measurement of true static compliance (2–5 seconds), compliance measurements were defined as quasistatic (Barberis et al. 2003; Henderson & Sheel 2012). However, $C_{qst}$ recorded in the present study likely provided a reasonable approximation of static compliance and allowed accurate estimation of changes in the elastic properties of the respiratory system because the sheep had no signs of lung disease.

The CONTROL treatment revealed a significant decrease in $C_{qst}$ at the end of the observation period (120 and 150 minutes). Providing that $V_T$ remained constant throughout the study (12 mL kg$^{-1}$), the decrease in $C_{qst}$ was caused by significant increases in $\Delta P$ (because of increased $P_{plat}$) during the same period. Progressive bloating from gas accumulation within the rumen probably contributed to the decrease in $C_{qst}$ in the CONTROL treatment. Although decreases in total respiratory system compliance might also suggest that airway closure and/or the development of atelectasis are taking place (Tokics et al. 1996; Wolf et al. 2015), the stability of PaO₂ and $Q_s/Q_t$ values over time suggest that changes in lung parenchyma were not responsible for the decreases in $C_{qst}$ in control animals.

The impaired oxygenation and increased $Q_s/Q_t$ observed during pneumoperitoneum in the sheep of the present study were likely the result of an increase in atelectatic lung areas (Hedenstierna et al. 1989; Wolf et al. 2015). Although it seems plausible that $Q_s/Q_t$ will increase during pneumoperitoneum because intra-abdominal insufflation may cause a cephalad shift of the diaphragm, the effects of pneumoperitoneum on PaO₂ are controversial in human patients. In morbidly obese humans anesthetized with isoflurane and fentanyl, laparoscopic gastric bypass surgery performed under CO₂ pneumoperitoneum (15 mmHg of intra-abdominal pressure) did not decrease PaO₂ in comparison with patients that underwent open gastric bypass surgery (Nguyen et al. 2004). CO₂ pneumoperitoneum (20 mmHg of intra-abdominal pressure) did not change P(A-a)O₂ from values recorded before intra-abdominal insufflation in both obese and normal human subjects anesthetized with propofol—alfentanil (Sprung et al. 2002). Other studies in humans have shown that CO₂ pneumoperitoneum (11–13 mmHg of intra-abdominal pressure) during propofol—fentanyl anesthesia may be paradoxically associated with a small increase in PaO₂ from values recorded before intra-abdominal insufflation in both obese and normal human subjects anesthetized with propofol—alfentanil (Sprung et al. 2002). Abdominal insufflation significantly decreased PaO₂ from 435–462 mmHg (CONTROL) to 377–397 mmHg in the PNEUMO treatment (range of mean values), whereas $Q_s/Q_t$ fraction was significantly increased from 9–11% (CONTROL) to 14–16% during abdominal insufflation (PNEUMO). The decrease in PaO₂ and increase in $Q_s/Q_t$ associated with pneumoperitoneum were mild and do not characterize hypoxic (type I) respiratory failure because the PaO₂/FIO₂ ratio remained >300 mmHg (40 kPa). Contrasting with the results of the study...
reported here, substantially higher $\dot{Q}_e/\dot{Q}_l$ and lower PaO$_2$ [median values, 39% and 240 mmHg (32 kPa), respectively] were reported in healthy anesthetized sheep undergoing volume-controlled ventilation and ZEEP with oxygen (Wolf et al. 2008). However, this discrepancy may not be real because the oxygenation deficit and high $\dot{Q}_e/\dot{Q}_l$ secondary to alveolar collapse may have been favored by the use of ventilation with low $V_T$ (4–6 mL kg$^{-1}$), which is in contrast with the $V_T$ (12 mL kg$^{-1}$) used in the present study.

Halogenated anesthetic agents, but not IV anesthetics (propofol), inhibit hypoxic pulmonary vasoconstriction (HPV), which is an important physiological mechanism by which $\dot{Q}_e/\dot{Q}_l$ is minimized or prevented as the blood flow is diverted from nonventilated (or atelectatic) to ventilated alveoli (Benuñof 1979; Abe et al. 1998). Inhibition of HPV by isoflurane in the presence of atelectasis might explain why pneumoperitoneum increased $\dot{Q}_e/\dot{Q}_l$ and caused oxygenation impairment in the sheep of the present report. As demonstrated by Wolf et al. (2015), sheep demonstrate weak HPV even when injectable anesthetics are used. Corroborating the current findings, CO$_2$ pneumoperitoneum (12 mmHg of intra-abdominal pressure) in rats anesthetized with nitrous oxide—isoflurane also resulted in significantly lower PaO$_2$ than in anesthetized animals that did not undergo intra-abdominal insufflation (Hazbroek et al. 2002).

The ARM followed by 10 cmH$_2$O of PEEP in sheep that underwent pneumoperitoneum ameliorated C$_{pqt}$ and decreased $\Delta P$ when compared with animals that underwent pneumoperitoneum alone. The present study was insufficiently instrumented to differentiate changes in lung compliance from the assessment of respiratory system compliance provided by C$_{pqt}$. However, the increase in C$_{pqt}$ and the decrease in $\Delta P$ induced by the ARM/PEEP was paralleled by an increase in PaO$_2$ and decreased $\dot{Q}_e/\dot{Q}_l$, suggesting that C$_{pqt}$ and $\Delta P$ changes reflected an improvement in lung compliance from a decrease in atelectatic areas. For practical purposes, when C$_{pqt}$ measurements are not available, a decrease in $\Delta P$ during volume-controlled ventilation should be evident if nonventilated lung areas are effectively recruited by the ARM/PEEP.

The ARM followed by PEEP returned $\Delta P$ to values recorded in the CONTROL treatment, but it was not enough to return C$_{pqt}$ to control values. Although the $V_T$ was preset at 12 mL kg$^{-1}$, decreases in expired $V_T$ used for C$_{pqt}$ calculations explain why compliance did not return to values that were similar to values recorded in CONTROL after the normalization of $\Delta P$ values by the ARM/PEEP.

The high alveolar pressures are responsible for the opening of the alveolar units during the ARM (Tusman et al. 2004). However, after the lung is opened by the ARM, the emphasis is on adequate post-recruitment PEEP to maintain the alveolar units open (Maisch et al. 2008). In the present study, the improvement in lung function (PaO$_2$ and C$_{pqt}$) after a single ARM followed by 10 cmH$_2$O PEEP lasted for approximately 60 minutes of pneumoperitoneum, suggesting that the post-recruitment PEEP prevented the alveoli from collapsing again during this time. However, a single PEEP level may not be applicable to all individuals, and PEEP titration to a minimum level may be necessary to prevent alveolar collapse (Maisch et al. 2008). In horses that underwent colic surgery, repeated ARMs ($P_{peak} = 60–80$ cmH$_2$O) were necessary to maintain PaO$_2 >$400 mmHg (53.2 kPa), despite using a 10–15 cmH$_2$O post-recruitment PEEP (Hopster et al. 2011). In obese humans undergoing laparoscopic gastric banding, the use of a single ARM (inspiratory pressure of 40 cmH$_2$O applied for 15 seconds) followed by 10 cmH$_2$O of PEEP resulted in a transient increase in PaO$_2$ and compliance (Almarakbi et al. 2009). Furthermore, when the same ARM protocol was repeated every 10 minutes in combination with the same PEEP level, the improvement in PaO$_2$ and compliance were sustained (Almarakbi et al. 2009). Taken together, results from these studies suggest that the post-recruitment PEEP was not high enough to prevent the collapse of alveolar units after those units were opened by the ARMs. However, in addition to significant adverse cardiovascular effects, higher PEEP could also be associated with alveolar overdistension and ventilator-induced lung injury (Beck-Schimmer & Schimmer 2010). Therefore, repeated ARMs might be preferred over the use of high PEEP to improve lung function (Almarakbi et al. 2009).

Pneumoperitoneum alone induced mild increases in CVP and PAOP while the ARM followed by PEEP caused major increases in CVP and PAOP, which persisted until abdominal deflation. CVP and PAOP are dependent not only on the venous return to the heart but also on other factors, such as HR, vascular tone and intrapleural pressure. The increase in these variables may be attributable to the compression of thoracic vessels (vena cava for CVP and pulmonary vein for PAOP) secondary to the increase in intra-pleural pressure induced by pneumoperitoneum and by the ARM/PEEP (Bigatello & George 2002).
The ARM followed by PEEP caused sustained increases in MPAP. This effect was not caused by PA vasoconstriction (increased PVRI) because the driving pressure in the pulmonary circulation (MPAP – PAOP) was unaltered in the face of a relatively constant pulmonary flow (CI). Similar to the increases in CVP and PAOP, the increase in MPAP may be caused by compression of the PA from increases in intrathoracic pressures induced by the ARM/PEEP. Although the increases in MPAP induced by the ARM/PEEP did not reach values that characterize pulmonary hypertension in humans (MPAP ≥25 mmHg), these effects may be a reason for concern if this procedure is used to improve arterial oxygenation in individuals with a pre-existing pulmonary hypertension, such as in acute pulmonary thromboembolism (Lagos-Carvajal et al. 2015).

Pneumoperitoneum alone or combined with the ARM/PEEP decreased CI values by 20–28% in comparison with controls. During the period of abdominal insufflation combined with the ARM/PEEP, MAP was decreased by 16% in comparison with CONTROL because of the negative impact of high PEEP and high inspiratory pressures on cardiovascular function (Pinsky 1997). The effects of the ARM followed by PEEP on systemic hemodynamics were clinically acceptable because the decrease in CI was not associated with hypotension (MAP <60 mmHg).

The design of the present study was limited in that the relative contributions of the ARM and post-recruitment PEEP on the outcome variables were not differentiated. Administration of 10 cmH2O of PEEP, without previously recruiting the alveoli, has been reported to increase PaO2 and static compliance during volume-controlled ventilation in sheep (Staffieri et al. 2010), and it is possible that PEEP alone might have resulted in some improvement in lung function during pneumoperitoneum. However, in mechanically ventilated human patients with pneumoperitoneum, the institution of an ARM followed by PEEP, with repetitions of the ARM at regular intervals, results in better lung function than the use of 10 cmH2O PEEP alone throughout anesthesia (Almarakbi et al. 2009).

In summary, CO2 pneumoperitoneum in sheep decreased \( Q_{\text{O}} \), increased \( Q_{\text{V}}/Q_{\text{O}} \), and resulted in a mild decrease in PaO2. These changes were not ameliorated by abdominal deflation. The ARM/PEEP protocol used in this study may be implemented clinically in healthy anesthetized sheep because it reverses the \( Q_{\text{O}}/Q_{\text{V}} \) associated with pneumoperitoneum, increases respiratory system compliance and improves arterial oxygenation with acceptable cardiovascular changes. Future studies are necessary to evaluate if the increase in MPAP induced by ARM/PEEP procedures could adversely affect pre-existing pulmonary hypertensive states.

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**Authors’ contributions**

JCR: data collection and interpretation, refinement of study design, data analysis, manuscript preparation. FJT-N: conception and design of the study, manuscript preparation, data interpretation and analysis. SAC: data collection and interpretation, refinement of study design. NC-R: data collection and analysis, critical revision of manuscript. NAG: data collection and analysis, critical revision of manuscript. JGQ: data collection and interpretation, refinement of study design. TLAR: data collection and analysis, critical revision of manuscript.

**Conflict of interest statement**

Authors declared no conflict of interest.

**References**


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Supporting Information

Additional Supporting Information may be found in the online version of this article (Appendix S1, Tables S1 & S2, Results S1, Discussion S1, Figs S1 & S2, Videos describing pressure accuracy and tidal volume accuracy) at http://dx.doi.org/10.1016/j.vaa.2016.05.017.
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