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Monitoring comparison of anaesthetised healthy sheep submitted to four different intraabdominal pressures

Comparação dos parâmetros de monitoramento de ovelhas saudáveis anestesiadas submetidas a quatro diferentes pressões intra-abdominais

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Abstract: The sheep model is used for a variety of laparoscopy studies, as well as in routine procedures such as oocyte aspiration and artificial insemination. However, little information is published about the monitoring parameters and ventilatory mechanics changes and when they begin to occur during these procedures with pneumoperitoneum. Ten adult, healthy, non-pregnant Santa Inês sheep were anesthetized and mechanically ventilated (VT, 15 ml kg^{-1}; positive end-expiratory pressure, 3 cmH_2O; FiO_2, 1.0), randomized in a crossover design for four different intra-abdominal pressures (IAP) as treatments: 0 mmHg (G1), 10 mmHg (G2), 12 mmHg (G3) and 15 mmHg (G4), for 60 min, with data collected every 10 min (HR, SpO_2, body temperature, $ETCO_2$, SAP, DAP, MAP, MACinsp, MACexp, $Ppeak_{RS}$, VT and Cdyn). End-expiratory CO_2 tension ($ETCO_2$) increased over time in all groups, except in G1. Peak airway pressure (Ppeak) and dynamic compliance (Cdyn) increased over time in all groups but were significantly higher in G3 and G4 from 20 min onwards. The minimum alveolar concentration (MAC) during inspiration and expiration increased over time in all groups, except G1. Within 20 minutes of the procedure, it was possible to notice that in the groups with higher IAP (G3 and G4) there was an increase in Ppeak and Cdyn, compatible with other findings of clinical importance such as an increase in $ETCO_2$, a decrease in VT and SpO_2. Therefore, 20 minutes after the procedure, monitoring must be more careful, especially in animals with previous health conditions.

Key-words: laparoscopy; airway peak pressure; pneumoperitoneum; dynamic compliance.

Resumo: O modelo ovino é usado para uma variedade de estudos em laparoscopia, bem como em procedimentos de rotina, como aspiração de oócitos e inseminação artificial. No entanto, pouca informação é publicada sobre os parâmetros de monitoramento, alterações da mecânica ventilatória e quando estas começam a ocorrer durante esses procedimentos com pneumoperitônio. Dez ovelhas Santa Inês adultas, saudáveis e não prenhes foram anestesiadas e ventiladas mecanicamente (VT, 15 ml.kg^{-1}; pressão expiratória final positiva, 3 cmH_2O; FiO_2, 1,0), randomizadas em um delineamento cruzado para quatro diferentes pressões intra-abdominais (PIA) como tratamentos: 0 mmHg (G1), 10 mmHg (G2), 12 mmHg (G3) e 15 mmHg (G4), por 60 min, com dados coletados a cada 10 min (FC, SpO_2, temperatura corporal, $ETCO_2$, PAS, PAD, PAM,

CAMinsp, CAMexp, $Ppico_{,rs}$, VT e Cdyn). A tensão expiratória final de CO_2 ($ETCO_2$) aumentou ao longo do tempo em todos os grupos, exceto no G1. A pressão de pico nas vias aéreas (Ppico,rs) e a complacência dinâmica (Cdyn) aumentaram ao longo do tempo em todos os grupos, mas foram significativamente maiores em G3 e G4 a partir dos 20min. A concentração alveolar mínima (CAM) durante a inspiração e expiração aumentou ao longo do tempo em todos os grupos, exceto no G1. Em 20 minutos do procedimento, foi possível verificar que nos grupos com maior PIA (G3 e G4) houve aumento da Pico,rs e Cdyn, compatível com outros achados de importância clínica, como aumento do $ETCO_2$ e diminuição do VT e SpO_2. Portanto, 20 minutos após o procedimento, a monitorização deve ser mais criteriosa, principalmente em animais com condições de saúde prévias.

Palavras-chave: laparoscopia; pico de pressão nas vias aéreas; pneumoperitônio; complacência dinâmica.

1. Introduction

The sheep model is used widely for a variety of studies such as extracorporeal membrane oxygenation (ECMO), transfusion-related to acute lung injury (TRALI), proteogenomic studies to understand selective susceptibility to endotoxin, CRASH-Sepsis and CRASH-Hemorrhage studies, studies on acute smoke inhalation lung injury, ovine brain stem death (BSD), BiVACOR and BiVAD artificial heart and the ovine left ventricular assist device (LVAD) and studies to assess cerebral microcirculation[1]. The sheep model is also of particular interest for studies of trauma by laparotomy or laparoscopy in sheep and humans, because of an abdominal cavity of similar size, and although the anatomical relationships are quite different, several experimental techniques can be tested initially in sheep [2]. However, in some reports, little information is published about the monitoring and the animal model, which there are significant information gaps, therefore limiting the understanding of the reproducibility or translatability of those studies [1].

Apart from improving animal welfare standards, anesthesia and analgesia are essential to make the procedures easier and improve both animal and personnel safety [3]. It is also a requirement during interventions in animal research to minimize or eliminate the experimental induction of pain. Complex experiments using ovine models therefore necessitate access to a well-equipped operating theatre with advanced organ system monitoring and point-of-care technology that allows real-time tailoring of therapy and standardization of anesthesia and critical care practices [1].

In sheep, a laparoscopic approach with pneumoperitoneum, as seen in oocyte aspiration and artificial insemination [4,5], causes harmful side effects, such as acidosis, hypercapnia, reduced cardiac output, decreased lung compliance, hypothermia and postoperative pain which have been associated with an established pneumoperitoneum with CO_2 insufflation during laparoscopy. Although some authors claim that the physiological changes seen with CO_2 insufflation in intra-abdominal pressures (IAP) routinely used in humans and animal models are mild and transient in duration [6], research is needed with different anesthetic protocol, ventilation, decubitus and IAP in sheep.

To date, however, there are limited studies that describe in detail sheep anesthesia and monitoring practices in biomedical research establishments with modern large animal intensive care units (ICUs) [1]. However, alterations of ventilatory mechanics and echocardiography at endpoint of laparoscopy

with four different IAP were already described[7], and therefore this study aims to find out at what moment these changes began to occur by gathering and comparing monitoring data of parameters in the transoperative period in these different IAP, which can be especially important for animals with underlying health conditions submitted to these procedures and future research using sheep as animal models.

2. Material and methods

2.1 Ethics

Ethical approval for this study (Ethical Committee N° 2115100418) was provided by the Ethical Committee on the Use of Animals of Fluminense Federal University, Niterói, Brazil. In addition to the ethical guidelines established by the University's Committee, the experiment followed the ARRIVE guidelines described by Percie du Sert [8].

2.2 Animal preparation

Ten female non-pregnant healthy adult Santa Ines ewes (Age: 3.8 ± 1.2 yo, 48.8 ± 5.6 Kg) were used. They were clinically examined and were kept in collective stalls, fed with chopped Napier grass (*Pennisetum purpureum*), commercial feed (300g/day/animal; 12% protein), water and mineral salt ad libitum. Red blood cells, total proteins, fibrinogen, parasite control tests were done, and 72 hours prior to experimental procedure, all animals received half of the food; 24 hours before procedure they underwent food fasting and, the water supply was suspended 6 hours before [9].

2.3 Experimental design and protocol for measurements

All animals were submitted to the following pneumoperitoneum pressures: 0mmHg (G1), 10mmHg (G2), 12mmHg (G3) and 15mmHg (G4) in a crossover design[7], considering G1 is the CONTROL group. Thus, at each trial, one IAP was randomly selected, and no animal was induced to the same IAP twice. The interval between rounds was 15 days, which is the average period used in clinical practice for patient discharge, and was enough to reduce the fibrinogen concentration to baseline condition [10]. The IAP and sample collection times were chosen based on previous laparoscopy procedures in humans [11,12]. The timeline of the procedures is depicted in Figure 1.

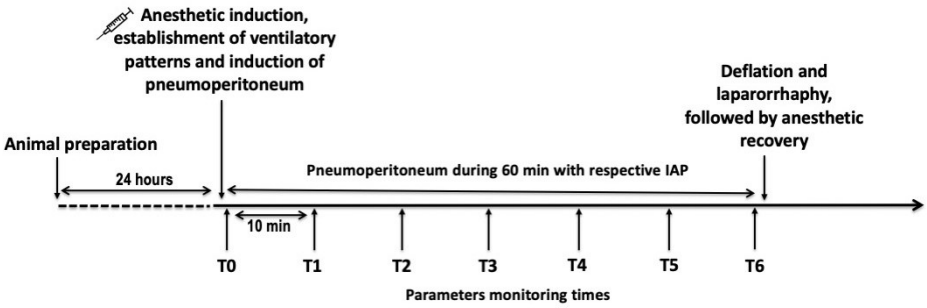

Figure 1. Experimental design.

After weighting, the animal was sent to the operating room. Anesthetic procedure was according to Rodrigues *et al*.[13], pre-anesthetic medication was administered consisting of midazolam (0.3 $mg.kg^{-1}$, Dormire®, Cristália, Butantã, Brazil) and meperidine (3.0 $mg.kg^{-1}$, União Química, Embu-Guaçu, Brazil) in the same syringe intramuscularly (IM), dipyrone (20 $mg.kg^{-1}$, Ibasa, Porto Alegre, Brazil) intravenously (IV). Pharmacological induction consisted of 4 $mg.kg^{-1}$ of IV propofol (Midfarma, Mandaguaçu, Brazil) and, once the state of unconsciousness of the animal was clinically observed, the animal was placed in sternal recumbency and an endotracheal tube (Surgivet® FN7 – ID: 7mm; OD: 10mm; length: 55cm) was inserted to maintain airway flow and connected to the circular anesthesia system with capnograph tube (Dameca® Siesta Breasy, Rødovre, Denmark) connected to the endotracheal tube. For anesthetic maintenance, the inhaled anesthetic agent isoflurane (Cristália, Itapira, Brasil) was used, with a vaporized fraction between 1.5 and 2.5 volume percent (V%)[14]. Subsequently, continuous citrate fentanyl IV was started at a rate of 5 $\mu g.kg^{-1}.h^{-1}$ with a syringe infusion pump (Digicare®, Florida, USA).

Tidal volume (VT) was adjusted to 15 $mL.kg^{-1}$, positive end-expiratory pressure (PEEP) was set at 3 cmH_2O, mean respiratory rate (RR) adjusted to 8 breath per minute (bpm), since ideal is ranging between 6 and 10 bpm [9]. Femoral artery catheterization was proceeded for continuous monitoring of blood pressure, using a 20G catheter (Safelet NIPRO®, São Paulo, Brazil). Five minutes before peritoneal cavity insufflation with CO_2, 0.3 mg.kg-1 of atracurium besylate (Tracur®, Cristália, Butantã, Brazil) was administered IV, for neuromuscular blockade. After cardiovascular and ventilatory parameters were acquired, animals were subjected to the following IAPs according to groups: G1 (0 mmHg), G2 (10 mmHg), G3 (12 mmHg) and G4 (15 mmHg).

The pneumoperitoneum was induced and maintained by the positioning of Hasson's trocar-cannula attached to an insufflator (Eletronic Endoflator 264305 20, Karl Storz, Germany). Thereafter, the insufflation of the peritoneal cavity was started, gradually with a CO_2 influx of 5L/min(4), toward the IAP for each group. After achieving the target IAP, all animals were rigorously submitted to 60 minutes of pneumoperitoneum. After this time, passive deflation occurred. In G1, the device was also connected at all times, and the pressure was kept at 0 mmHg.

Postoperative management consisted of analgesia with dipyrone 50% (Dipirona Ibasa 50%, Laboratório Ibasa Ltda, Porto Alegre, Brasil) 20 $mg.kg^{-1}$ IV every 24 hours, for five days and enrofloxacin 10% (Zelotril, Agener União, Embu-Guaçu, Brasil) 2.5 $mg.kg^{-1}$ IV, every 24 hours, for seven days. The animals were evaluated for pain through physical examinations and blood tests postoperatively[15].

2.4 Cardiovascular and ventilatory monitoring

The cardiovascular and ventilatory parameters (Digicare® Multi-Parameter Physiologic Monitor Lifewindow 6000, USA) were continuously registered before peritoneal insufflation (T0) and at each 10 minutes (T1, T2, T3, T4, T5 and T6) throughout the experimental procedure that lasted 60 minutes (Fig. 1). Specifically, continuous electrocardiogram at the DII derivation, heart rate (HR), peripheral oxygen saturation (SpO_2), esophageal temperature, CO_2 tension at the end of expiration ($ETCO_2$), RR, invasive systolic (SAP), diastolic (DAP), and mean arterial pressures (MAP), and anesthetic agent minimum inspiratory and expiratory alveolar concentration (MAC insp and MAC exp, respectively) were continuously recorded.

The system of anesthesia and mechanical controlled ventilation (Dameca® Siesta Beasy, Rødovre, Denmark) allowed to monitor PEEP, RR, peak airway pressure (Ppeak,RS) and VT. Therefore, we were able to calculate dynamic compliance (Cdyn) by the following formula(16):

$$Cdyn = VT\ (mL) / (Ppeak,RS - PEEP)$$

2.5 Statistical analysis

We did not perform a formal sample size calculation a priori, but instead we used all sheep available (10 animals). The primary outcome assessed in a previous study[7] was $\Delta P_{,RS}$, which difference taking into account at initial and final time of procedure, the calculated effect size was 1.50. In such way, we did a post-hoc analysis to calculate the achieved statistical power (1-β err prob = 0.88), which was adequate. Each variable was tested for normal distribution using Shapiro-Wilk test. Data were presented as mean ± SD. The results obtained were assessed using Two Way Analysis of Variance (Two-way ANOVA) for repeated measures (RM), followed by post-hoc Holm-Šídák's multiple comparisons test for interactions among groups and sampling times. Significance for all tests was assumed when $p<0.05$. All the described statistical analysis was performed using Microsoft Excel and GraphPad Prism version 9.2.0 (La Jolla, CA, USA).

3. Results

Results are exposed in table 1 and in figure 2 with the main points reported below. In summary, the variables that showed significant values between groups were Peak airway pressure (Ppeak) and Dynamic compliance (Cdyn). Ppeak increased overtime in all groups, with significant higher levels in G3 and G4 from T2 onward. Cdyn decreased overtime in all groups, showing significant lower levels in G3 and G4 from T2 onward. Also, MAC insp and exp presented punctual significant differences, but none overtime between groups, even though it was noticeable that G1 presented the lowest values in T2, T3 and T4 for both. Some variables showed no significant differences between groups, but their behavior increasing or decreasing according to the pneumoperitoneal pressure is noticeable. For instance, ETCO2 levels were greater in the groups with sustained pneumoperitoneum (G2, G3 and G4) at each time, and increased levels can be noticed form T2 onward, respectively. HR and Temperature decreased overtime and SAP, DAP and MAP increased overtime in all groups with no significant difference. VT and SpO2 presented no significant differences between groups or times.

Table 1. Descriptive statistics (Mean±SD) of monitored variables for comparison of the same group at different times (row) and different groups at the same time (column).

Variables	Groups	T0	T1	T2	T3	T4	T5	T6	Time effect	Group effect	Interaction
									p<0.0001	*p*<0.0001	*p*=0.895
HR (bpm)	G1 (0 mmHg)	128±16.7	108±17.3	98.8±20.9 [a]	94.2±18.4 [a]	93.4±17.9 [a]	95.7±20.6 [a]	102±18.3 [a]	[a]vs T0		
	G2 (10 mmHg)	141±16.3	120±17.3 [a]	118±19.0 [a]	113±13.4 [a]	109±11.7 [a]	109±11.8 [a]	106±15.3 [a]			
	G3 (12 mmHg)	128±32.0	117±20.7	119±20.8	119±21.6	118±23.7	115±25.1	119±26.5			
	G4 (15 mmHg)	128±18.0	113±16.2 [a]	117±15.4	116±19.7	112±24.1	112±24.5	112±24.3			
									p=0.144	p=0.020	p=0.341
SpO_2 (%)	G1 (0 mmHg)	96.3±2.5	95.4±2.8	96.8±1.7	96.7±1.8	96.4±1.5	96.6±1.4	96.6±2.1			
	G2 (10 mmHg)	96.6±2.3	96.6±1.8	96.0±1.8	95.8±1.8	95.4±2.6	95.8±1.9	95.8±1.2			
	G3 (12 mmHg)	94.8±3.4	96.4±2.9	95.7±3.8	96.5±4.1	95.6±3.9	94.8±4.2	94.4±4.2			
	G4 (15 mmHg)	97.8±6.2	96.6±3.9	94.8±1.9	95.5±1.9	93.7±3.7	92.5±4.7	92.0±7.2			
									p<0.0001	p=0.381	p=0.976
Temp. (°C)	G1 (0 mmHg)	37.5±0.6[c]	37.0±0.6[c]	36.7±0.6[c]	36.4±0.6 [c]	36.2±0.5 [c]	36.0±0.6 [c]	35.9±0.5 [c]	[b] vs T0, T1, T2, T3, T4; [c] vs T1, T2, T3, T4, T5, T6		
	G2 (10 mmHg)	37.0±0.8[c]	36.6±0.6[c]	36.4±0.7[c]	36.1±0.6 [c]	35.9±0.6 [c]	35.7±0.6 [b]	35.6±0.6 [b]			
	G3 (12 mmHg)	37.2±0.5[c]	36.8±0.6[c]	36.4±0.7[c]	36.3±0.6 [c]	36.0±0.6 [c]	35.8±0.7 [b]	35.7±0.2 [b]			
	G4 (15 mmHg)	37.5±0.9[c]	37.1±0.9[c]	36.9±0.8[c]	36.7±0.8 [c]	36.4±0.8 [c]	36.2±0.8 [c]	36.1±0.7 [c]			
									p=0.0002	p=0.899	p=0.002
$ETCO_2$ (mmHg)	G1 (0 mmHg)	49.0±5.6	45.4±5.7	43.6±6.2	42.3±6.5	42.2±6.7	42.9±6.3	45.5±8.1	[a]vs T0		
	G2 (10 mmHg)	45.8±5.6	42.7±6.3	43.5±6.3	44.7±7.0	45.8±7.1	45.9±6.5	46.8±7.3			
	G3 (12 mmHg)	44.8±7.7	40.8±8.4	44.0±6.5	46.2±6.7	46.9±7.2	47.6±6.7	48.7±8.2			
	G4 (15 mmHg)	47.5±6.1	43.9±5.6 [a]	43.5±6.4	46.2±6.6	46.4±7.2	47.8±7.4	50.4±7.8			
									p<0.0001	p=0.622	p=0.015
SAP (mmHg)	G1 (0 mmHg)	67.0±8.1	81.3±21.7	80.9±11.3	87.7±14.4	90.6±19.6	94.6±19.6	99.9±19.8 [a]	[a] vs T0		
	G2 (10 mmHg)	83.2±17.4	86.9±10.1	85±12.7	88.4±12.3	88.6±14.5	94.4±13.5	89.8±12.0	[c] vs T1, T2, T3, T4, T5, T6		
	G3 (12 mmHg)	74.3±11.1[c]	92.0±12.2	89.8±10.2	87.2±11.5	87.3±11.5	90.9±12.2	95.7±6.3			
	G4 (15 mmHg)	86.0±24.7	84.8±6.5	88.9±9.9	89.2±19.7	93.3±19.1	97.4±16.4	100.0±14.3			
									p<0.0001	p=0.409	p=0.160
DAP (mmHg)	G1 (0 mmHg)	44.6±5.1	55.9±21.3	53.7±8.5	60.2±11.0	60.4±12.0	63.0±13.4	66.0±13.8		[A] vs G1	
	G2 (10 mmHg)	56.0±12.4	56.2±6.3	61.6±7.2	61.8±9.2	63.4±10.0	66.4±10.2	64.8±9.3			
	G3 (12 mmHg)	50.6±13.0	66.4±16.0	65.6±8.6 [A]	63.2±10.8	63.8±8.42	66.8±8.0	68.3±5.5			
	G4 (15 mmHg)	55.5±9.7 [A]	57.0±8.9	59.0±4.8	56.6±6.1	62.4±12.6	68.1±11.0	70.4±11.6			
									p<0.0001	p=0.476	p=0.036

MAP (mmHg)	G1 (0 mmHg)	53.6±6.4	63.2±17.0	63.2±8.5	70.7±12.2	71.3±13.0	74.4±15.7	79.2±15.2 [a]	[a] vs T0	[A] vs G1	
	G2 (10 mmHg)	67.4±13.7	69.5±8.2	67.6±10.6	71.3±9.8	73.5±10.1	77.6±10.7	74.7±9.2			
	G3 (12 mmHg)	60.3±12.3	75.4±16.0	74.1±8.1 [a]	72.1±9.7	72.3±7.9 [a]	75.8±7.3 [a]	80.1±3.8 [a]	[d] vs T3		
	G4 (15 mmHg)	65.6±10.5[A]	66.7±7.8	72.2±10.7	69.2±10.9	72.5±13.0	78.5±10.9	82.5±10.7[d]			
									p=0.006	p=0.267	p=0.006
MAC insp	G1 (0 mmHg)	1.1±0.15	1.1±0.21	0.94±0.18 [e]	0.96±0.17 [e]	0.99±0.17 [e]	1.1±0.22[e]	1.2±0.19	[e] vs T6	[A] vs G1	
	G2 (10 mmHg)	1.1±0.18	1.1±0.17	1.2±0.14[A]	1.1±0.15	1.1±0.17	1.1±0.16	1.2±0.15			
	G3 (12 mmHg)	1.0±0.14	1.0±0.13	1.1±0.14	1.0±0.08	1.0±0.09	1.1±0.12	1.1±0.11			
	G4 (15 mmHg)	1.1±0.25	1.0±0.05	1.1±0.14	1.1±0.13	1.0±0.18	1.0±0.16	1.1±0.18			
									p<0.0001	p=0.241	p=0.002
MAC exp	G1 (0 mmHg)	0.9±0.2	0.9±0.1	0.8±0.0 [e]	0.8±0.07	0.8±0.07	0.9±0.1	1.0±0.2	[e] vs T6 [f] vs T5	[A] vs G1	
	G2 (10 mmHg)	0.8±0.2	0.9±0.1	1.0±0.1[A]	1.0±0.1[A]	1.0±0.2	1.0±0.1	1.0±0.1			
	G3 (12 mmHg)	0.8±0.08 [e]	0.9±0.1	0.9±0.07[A]	0.9±0.07	0.9±0.09	0.9±0.09	1.0±0.08			
	G4 (15 mmHg)	0.8±0.2	0.8±0.0 [f]	1.0±0.1[A]	0.9±0.1	0.9±0.1	0.9±0.0	1.0±0.1			
									p<0.0001	p=0.002	p<0.0001
Ppeak,$_{RS}$ (cmH_2O)	G1 (0 mmHg)	14.0±2.4	13.7±2.8	13.0±1.9	13.6±2.5	13.4±2.3	14.6±3.9	14.3±2.7	[g] vs T0, T1 [d] vs T3	[A] vs G1 [B] vs G1, G2	
	G2 (10 mmHg)	13.8±2.4	13.8±2.5	14.7±1.9	15.2±2.0	15.2±2.1	15.7±2.1	15.9±2.6			
	G3 (12 mmHg)	13.8±2.5	14.5±2.8	17.6±3.4[Ag]	18.2±2.5 [Bg]	18.3±2.5[B g]	18.6±2.5 [g]	18.9±2.6[Adg]			
	G4 (15 mmHg)	13.2±1.3	14.2±2.8	19.0±3.6 [Bg]	18.8±3.3 [Bg]	19.2±3.3[Bg]	19.1±3.1 [g]	19.9±3.9[Ag]			
									p=0.055	p=0.433	p=0.628
V_T (mL/Kg)	G1 (0 mmHg)	15.3±0.5	15.7±1.0	15.7±0.9	15.7±0.9	15.7±0.9	15.6±1.0	15.6±1.0			
	G2 (10 mmHg)	15.7±1.8	16.0±2.4	16.1±2.7	16.1±2.7	15.9±2.5	16.0±2.5	16.1±3.0			
	G3 (12 mmHg)	15.3±0.6	15.5±0.9	15.5±0.9	15.6±1.0	15.5±1.1	15.4±1.2	15.3±1.2			
	G4 (15 mmHg)	14.6±1.6	15.4±0.8	15.3±0.9	14.7±1.9	14.7±1.9	14.5±2.0	14.5±2.0			
Cdyn (ml/cmH_2O)	G1 (0 mmHg)	65.5±8.1	70.7±10.5	74.4±8.5[e]	71.3±11.8 [e]	71.7±11.2[e]	67.0±15.1	65.5±12.0	[e] vs T6 [h] vsT2, T3, T4, T5, T6	[A] vs G1 [B] vs G1, G2	
	G2 (10 mmHg)	64.7±11.2 [h]	66.2±14.2 [h]	60.2±9.9 [A]	57.6±8.6 [eA]	57.5±12.5	55.1±10.2	55.3±11.8			
	G3 (12 mmHg)	65.7±11.1 [h]	62.6±11.8 [h]	45.8±4.8[B]	46.6±7.0[B]	45.9±6.3[A]	44.7±6.5[A]	43.8±6.4[A]			
	G4 (15 mmHg)	66.7±9.1	66.4±13.4	46.4±11.7[B]	41.6±7.8[B]	43.9±12.4[A]	43.5±12.7[A]	38.3±8.8[A]			

Monitoring variables obtained at T0(0min), T1(10min), T2(20min), T3(30min), T4(40min), T5(50min) and T6(60min) during procedures, expressed as means ± standard deviation (SD) of 10 animals in each group, distributed in a cross-over model. Data were assessed with Two-way ANOVA RM and post hoc Holm–Sidák multiple comparisons test ($p < 0.05$). Legend: Heart rate (HR), pulse oximetry (SpO_2), temperature (Temp.), end-tidal carbon dioxide ($ETCO_2$), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), minimum alveolar concentration in inspiration (MAC insp); minimum alveolar concentration in expiration (MAC exp), peak pressure (Ppeak,$_{RS}$), tidal volume (V_T) and dynamic compliance (Cdyn). [a] vs T0; [b] vs T0, T1, T2, T3, T4; [c] vs T0, T1, T2, T3, T4, T5, T6; [d] vs T3; [e] vs T6; [f] vs T5; [g] vs T0, T1; [h] vs T2, T3, T4, T5, T6. ; [A] vs G1; [B] vs G1, G2.

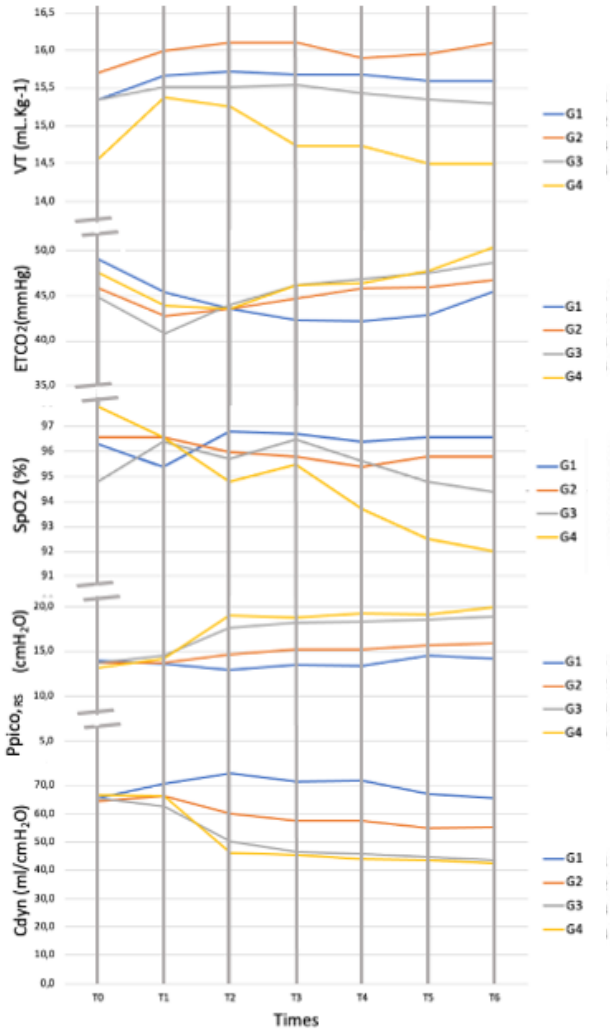

Figure 2. Linear graph of mean values of the VT, SpO_2, $ETCO_2$, $Ppeak_{rs}$ and Cdyn overtime.

4. Discussion

Studies like this are important since validated ovine models may, to some extent, be comparable to other animal models such as swine that can generate robust mechanistic data that lend themselves to translational research [1]. In fact, there is a stronger correlation between atelectasis and oxygenation or shunt, respectively, in humans or sheep breathing pure oxygen, comparing to pigs, since interspecies differences in hypoxic pulmonary vasoconstriction (HPV) are closer between humans and sheep, than other species frequently used as animal models [17–19]. Therefore, the increase of sheep as an experimental model requires knowledge of monitoring changes in these conditions of pneumoperitoneum to improve care during and after these procedures.

In this study, G4 presented the VT lowest values at all times, and from 30min onward, ETCO2 increased noticeably in groups with pneumoperitoneum (G2, G3 and G4), until reached the highest values at 60 min. In all groups there was a decrease of ETCO2 at T1 (10min), however, at T2 (20min), G2, G3 and G4 started rising again until T6 (60min), while the opposite occurred to G1, which increased only at final time (T6). Corroborating these findings, there was a significant difference between the PaCO2 of G3 and G4 at final time (T6) (70.3 ± 7.1 and 70.6 ± 10.6, respectively) when compared to G1 (57.1 ± 8.3)[7].

In sequence, it is noted that from T4 onward, even though SpO_2 levels presented no significant difference, its values in G4 are remarkably lower than other groups, especially when compared to G1 and G2. Although no significant difference was observed, G4 had higher values than all other groups. In a study with anesthetized sheep without pneumoperitoneum, but with different recumbency positions under mechanical ventilation with pressure-controlled ventilation (12 cmH_2O peak inspiratory pressure), VT and $ETCO_2$ remained constant in animals in dorsal recumbency even after 60 and 120 min [20]. Therefore, these findings may be due to CO_2 peritoneal insufflation, but also by the basal lung regions, compressed by diaphragmatic elevation due to higher IAP, and consequent atelectasis that increase dead space and unbalance the ventilation/perfusion ratio, causing shunt and increasing arterial pressure of CO_2 ($PaCO_2$) [21,22].

The absorption of CO_2 from pneumoperitoneum is not linear, it is larger in the first 30 minutes, tending to stabilize overtime [22]. However, in this study, there's a drop of $ETCO_2$ levels after 10 min (T1), with a gradual rise especially at 30 min (T3) in G3 and G4, which can also be noticed by the inversion of values of G1 and G4 and T0 (49.0±5.6; 47.5±6.1) and T6 (45.5±8.1; 50.4±7.8), respectively. This is followed by a SpO_2 decrease that started at T3, corroborating the fact that atelectasis, dead space, and unbalanced ventilation/perfusion ratio occurred and may have contributed to it.

In a study with humans, $ETCO_2$ rose steadily and significantly from 29.5 to 36 mmHg after 10 min of IAP at 15 mmHg ($p<0.05$) without any further changes and stayed at 35 mmHg 5 min after deflation in supine position [23]. In swine, one of the species used for videolaparoscopic studies, after one hour of pneumoperitoneum with CO_2 at 16 mmHg, there was a clear tendency to hypercapnia and acidosis[24].

Corroborating previous findings, respiratory mechanics worsened in animals with increased IAP (G3 and G4), presenting crescent values of Ppeak,RS starting at T1 onward. IAP can cause stiffening of the chest wall and their components, abdominal wall and diaphragm muscle, which in turn increases the transmural pressure and reflects the increase of airway pressure ($Ppeak_{,RS}$) during volume-controlled ventilation[25]. This characterizes a reduction of Cdyn, which in humans during laparoscopic procedures with elevation of IAP can be of about 50%, observed by increment in Ppeak,RS [23]. In this study, Cdyn decreased under 50% significantly from T2 onward in G3 and G4, following $Ppeak_{,RS}$ behavior. Studies have demonstrated a reduction in pulmonary compliance with a rise in peak and plateau airway pressures along with reduction in functional residual capacity, which can predispose to ventilation-perfusion mismatch, leading to hypoxemia [26]. In a study with sheep during spontaneous ventilation in lateral recumbency for magnetic resonance, after 10 minutes, the values of F-shunt calculated in this group of sheep suggested that atelectasis developed[27]. Other study determined that the shunt fraction (Qs/Qt) in sheep during mechanical ventilation was higher in sheep in right lateral recumbency with 60 min of procedure, than with sheep in left lateral or dorsal recumbency [20]. This information is important, as there are differences in recumbency positions, but even so, it proves that changes in pulmonary perfusion and compliance occur early in both situations.

Considering the decrease of HR, this can occur by activation of vagal tone with peritoneal distention, and can also lead to bradyarrhythmias and asystole, during carbon dioxide insufflation (or shortly thereafter), or with traction on pelvic structures [28,29]. However, in this study, G1 (0 mmHg) and G2 (10 mmHg) showed the most evident decrease over time and could not be correlated with the vagal effect of pneumoperitoneum, nor with increasing depth of anesthesia. For instance, monitoring of

CAM insp and exp showed very subtle differences, which is compatible with a stable anesthesia depth. Mean, diastolic and systolic arterial pressures increased in all groups overtime. It has been shown that pneumoperitoneum increases MAP and systemic vascular resistance (SVR) and may decrease cardiac output (CO), since it can cause abdominal aortic compression, allied to neuroendocrine effects that even at short term cannot be neglected [30–32]. In other study with sheep under inhalation anesthesia during 120 min and variation of recumbency, but without pneumoperitoneum, no alterations of HR and MAP were found in dorsal recumbency, reinforcing previous explanations for MAP findings, but not HR ones [20].

Plato airway pressure was not obtained overtime, as it could influence other ventilatory parameters when performing the tracheal tube clamping maneuver, and without this variable was not possible to assess the driving pressure and mechanical power values, this could only be obtained at initial and final times and were higher in groups with IAP settled at 12 and 15mmHg[7]. However, it was possible to obtain Ppeak and calculate Cdyn at all times, which was also an important finding.

5. Conclusion

Within 20 min of procedure was possible to notice that in groups with higher IAP (12 and 15 mmHg) there was a significant progressive impairment in respiratory mechanics, expressed by increase of Ppeak and decrease of Cdyn, and as consequence a gradual increase in the values of $ETCO_2$, which was more evident at 60 min. These early changes seen in healthy patients undergoing higher IAPs during laparoscopy are of considerable importance particularly for patients with previous debilitating respiratory conditions.

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: L. V. de Gouvêa, P. R. L. do Nascimento, T. R. S. Leite., D. A. B. Lessa and P. L. Silva. Data curation: L. V. de Gouvêa, P. R. L. do Nascimento and T. R. S. Leite. Formal analysis: L. V. de Gouvêa and P. L. Silva. Funding acquisition: D. A. B. Lessa, P. L. Silva and P. R. L. do Nascimento. Project management: L. V. de Gouvêa, P. R. L. do Nascimento, T. R. S. Leite, D. A. B. Lessa, P. L. Silva., A. L. de S. Teixeira, P. C. do A. R. da Silva, J. A. D. Ferreira Filho, M. J. S. A. Helayel. Methodology: D. A. B. Lessa, P. L. Silva, L. V. de Gouvêa, P. R. L. do Nascimento and T. R. S. Leite. Supervision: D. A. B. Lessa and P. L. Silva. Investigation: L. V. de Gouvêa, P. R. L. do Nascimento, T. R. S. Leite, A. L. de S. Teixeira, P. C. do A. R. da Silva, J. A. D. Ferreira Filho, M. J. S. A. Helayel, A. N. Júnior, M. G. Chenard, L. de A. Carvalho and D. A. B. Lessa. Visualization: L. V. de Gouvêa, P. R. L. do Nascimento e T. R. S. Leite. Writing (original draft): L. V. de Gouvêa, P. R. L. do Nascimento and T. R. S. Leite. Writing (proofreading and editing): L. V. de Gouvêa, P. R. L. do Nascimento, T. R. S. Leite, D. A. B. Lessa and P. L. Silva.

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