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A comparison of the effects of carbon dioxide and medical air for abdominal insufflation on respiratory parameters in xylazine-sedated sheep undergoing laparoscopic artificial insemination

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Abstract

AIMS: To determine if abdominal insufflation with medical air will improve oxygenation and ventilation parameters when compared to insufflation with CO2 in xylazine-sedated sheep undergoing laparoscopic artificial insemination (AI).

METHODS: Forty-seven sheep underwent oestrus synchronisation and were fasted for 24 hours prior to laparoscopic AI. Each animal was randomised to receive either CO2 or medical air for abdominal insufflation. An auricular arterial catheter was placed and utilised for serial blood sampling. Respiratory rates (RR) and arterial blood samples were collected at baseline, after xylazine (0.1 mg/kg I/V) sedation, 2 minutes after Trendelenburg positioning, 5 minutes after abdominal insufflation, and 10 minutes after being returned to a standing position. Blood samples were collected in heparinised syringes, stored on ice, and analysed for arterial pH, partial pressure of arterial O2 (PaO2), and CO2 (PaCO2). The number of ewes conceiving to AI was also determined.

RESULTS: Repeated measures ANOVA demonstrated temporal effects on RR, PaO2, PaCO2 and arterial pH during the laparoscopic AI procedure (p0.01). No sheep experienced hypercapnia (PaCO2>50 mmHg) or acidaemia (pH

CONCLUSIONS AND CLINICAL RELEVANCE: There were no statistical or clinical differences in RR, PaO2, PaCO2, pH, or conception to AI when comparing the effects of CO2 and medical air as abdominal insufflation gases. None of the sheep experienced hypercapnia or acidaemic, yet 42% (19/45) of sheep developed clinical hypoxaemia, with a higher percentage of ewes in the CO2 group developing hypoxaemia than in the medical air group. Based on the overall analysis, medical air could be utilised as a comparable alternative for abdominal insufflation during laparoscopic AI procedures.

Keywords

Laparoscopic artificial insemination, sheep, carbon dioxide, medical air, arterial blood gas analysis

Disciplines

Large or Food Animal and Equine Medicine | Veterinary Physiology

Comments

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Scientific Article

A comparison of the effects of carbon dioxide and medical air for abdominal insufflation on respiratory parameters in xylazine sedated sheep undergoing laparoscopic artificial insemination

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Abstract

AIMS: To determine if abdominal insufflation with medical air will improve oxygenation and ventilation parameters when compared to insufflation with carbon dioxide (CO_2) in xylazine sedated sheep undergoing laparoscopic artificial insemination (LAI).

METHODS: Forty-seven sheep underwent oestrus synchronisation and were fasted for 24 hours prior to LAI procedure. Each animal was randomised to one treatment group: CO_2 or medical air for abdominal insufflation. An auricular arterial catheter was placed and utilised for serial blood sampling. Respiratory rates (RR) and arterial blood samples were collected at baseline (T0), post xylazine (0.1mg/kg IV) sedation (T1), two minutes after Trendelenburg positioning (T2), five minutes post-abdominal insufflation (T3), and 10 minutes after being returned to a standing position (T4). Blood samples were collected in heparinised syringes, stored on ice, and batch run on a Nova Biomedical blood gas analyser for arterial pH, partial pressure of oxygen (PaO_2), and partial pressure of CO_2 ($PaCO_2$) values within two hours. Conception rates were later collected for evaluation of any effects on successful outcome of LAI procedure.

RESULTS: Repeated measures ANOVA demonstrated a significant temporal effect with respect to all the dependent variables (all $p<0.001$) with no significant difference between insufflation groups (all $p>0.01$). Based on predetermined clinical criteria, no sheep experienced hypercapnia ($PaCO_2>50$ mmHg) or acidaemia (pH<7.35). Overall, 42% (19/45) of the sheep became hypoxaemic ($PaO_2<70$ mmHg) during the procedure, 64% (14/22) in the CO_2 group and 35% (8/23) in medical air group. Chi-square analysis demonstrated no significant relationship between insufflation gas type and development of hypoxaemia. All sheep were normoxaemic by recovery. Overall, conception rates were 73.81% (31/42), 75% (15/20) in the CO_2 group and 72.7% (16/22) in the medical air group. Chi-square analysis showed no significant relationship between insufflation gas type and conception rates.

CONCLUSIONS: The main findings of the study emphasised no statistical or clinical differences in RR, PaO_2, $PaCO_2$, pH, or successful procedural outcome when comparing the effects of CO_2 versus medical air as abdominal insufflation gases. None of the sheep experienced hypercapnia nor acidosis, yet 42% (19/45) of sheep developed clinical hypoxaemia with no difference noted between insufflation groups. Further studies would be necessary to evaluate long-term effects of hypoxaemia within this sheep population as well as clinical efficacy of oxygen supplementation in the prevention of hypoxaemia. Based on the overall analysis, medical air could be utilised as a comparable alternative for abdominal insufflation during LAI procedures.

KEY WORDS: *Laparoscopic artificial insemination, sheep, carbon dioxide, medical air, arterial blood gas analysis*

CO_2	Carbon dioxide
IAP	Intra-abdominal Pressure
ISU	Iowa State University
LAI	Laparoscopic artificial insemination
$PaCO_2$	Partial Pressure of arterial carbon dioxide
PaO_2	Partial pressure of arterial oxygen
HR	Heart rate
RR	Respiratory rate

Introduction

Killen and Caffrey (1982) first described LAI in sheep in 1982. A laparoscopic technique for artificial insemination is less invasive than the traditional surgical approach (Anel *et al.* 2006; Evans *et al.* 2004), provides higher conception rates and more efficiently utilises semen compared to transcervical or other surgical techniques (Shipley *et al.* 2007; Fantinati *et al.* 2005; Stefani *et al.* 1990). LAI can be performed under sedation, which is more economical than general anesthesia. This procedure can be performed both in a hospital environment and on the farm, which makes it more readily available than surgical techniques.

Laparoscopic procedures require abdominal insufflation with gas to improve visualisation. The most commonly utilised gas is CO_2 because it is inexpensive, extremely soluble in blood, easily eliminated by the patient's lungs, and suppresses combustion. It is also associated with minimal side effects such as gas embolism when compared to helium, air, and nitrous oxide in humans (Ho *et al.* 1992). The main disadvantages of a CO_2 pneumoperitoneum include increased partial pressure of arterial CO_2, decreased partial pressure of arterial oxygen, which may lead to respiratory acidosis and hypoxaemia (Griffenhagen *et al.* 2015; Hunter *et al.* 1995; Ho *et al.* 1992). Insufflated CO_2 can also react with the moist peritoneal surface and convert into carbonic acid, which leads to peritoneal irritation and has been reported to be associated with postoperative discomfort in humans (Holthausen *et al.* 1999).

There are numerous studies, in human and animal models, assessing alternative laparoscopic insufflation gases that would minimise these side effects. Menes and Spivak (2000) reviewed various human studies evaluating alternative insufflation gases. Helium, nitrous oxide, and air, when compared to CO_2 have less haemodynamic and acid-base effects but do carry more risk of gas embolism in humans. Kuntz *et al.* (2000) performed a study in rats comparing the pH effect of abdominal insufflation with CO_2 versus air. They demonstrated that insufflation with air led to less pH changes in the blood, subcutaneous tissues and abdomen. Filtered ambient air, as an insufflation gas was determined to be a safe, inexpensive, and a practical alternative for field laparoscopy in cattle (Janowitz 1998.) ISU's Theriogenology service utilises both CO_2 and filtered air for abdominal insufflation during LAI procedures and their clinical impression is that sheep do better with filtered ambient air pneumoperitoneum.

The aim of this study was to determine if abdominal insufflation with medical air would improve oxygenation and ventilation parameters when compared to insufflation with $CO_{2.}$ Thus medical air or filtered air could serve as a convenient economical alternative. To the author's knowledge, there are no published studies comparing the effects of abdominal insufflation gases in sheep undergoing xylazine sedated laparoscopic artificial insemination.

Materials and methods

This was a collaborative project between ISU's Anesthesia and Theriogenology services. It was a prospective, blinded, randomised, clinical study. The study protocol was approved by the Institutional Animal Care and Use Committee of Iowa State University (Ames, Iowa, USA; 6-16-8289-O).

Animals

Forty-seven healthy mixed breed ewes of varying ages and weights were selected using simple randomisation from over 250 sheep that presented for LAI through the ISU Theriogenology service during the 2016 and early 2017 breeding seasons. Initial criterion for study enrollment was owner consent. Between 4-20 sheep presented for LAI procedures on a given day. These sheep were herded into individual pens. A complete history and physical examination was performed to assess basic health of the potential participants. Exclusion criteria included any history of cardiovascular or pulmonary disease, current treatment for these illnesses, signs of overt disease or health issues based on physical exam findings (nasal discharge, coughing, crackles or wheezes on thoracic auscultation, cardiac murmurs, open mouth breathing, etc). The number of sheep selected per day was based on personnel and time available to collect samples without slowing down the clinical procedure. Overall, 47 participants were enrolled from 10 different farms spanning 14 different LAI procedure days throughout the 2016 and 2017 breeding seasons.

Blinding and Randomisation

Sheep were assigned a participation number (1-47) based on when they were recruited. The participation numbers were randomly assigned a treatment group by an online randomisation program (http://www.randomization.com/thirdgeneration). One non-blinded person documented the subject's participation number and was responsible for changing the gas tank prior to each procedure. The CO_2/Air tanks and hoses were not visible to the surgeons or the persons collecting the data.

Experimental Design

Each animal underwent oestrus synchronisation for timed artificial insemination two weeks prior to the AI date, and fasted from food and water for 24 hours prior to the procedure. Sheep were randomly selected and enrolled in the study with owner permission. Each animal was assigned a participation number, which was randomised to one of two treatment groups: CO_2 or medical air. The ear was clipped and sterilely cleaned with chlorhexadine and alcohol gause. EMLA cream (AstraZeneca LP, Wilmington, DE, USA) was then applied to aid in placement of a 20 gauge arterial catheter (Surflo, Terumo Corporation, Laguna, Philippine) in the auricular artery. This catheter was secured with tape and utilised for serial blood sampling.

Temperature, RR, and HR were measured, and arterial samples were collected for blood gas analysis while the patient was standing and prior to any sedation (time point 0 (T0)). Xylazine (0.1mg/kg) sedation (MWI, Boise, ID, USA) was administered intravenously via a jugular vein. Blood samples were collected and RR measured 2 minutes post sedation, (time point 1 (T1)). The ewes were positioned in dorsal recumbency in the laparoscopic AI prepping cradle. The patient was blindfolded, secured with leg ties, and the abdomen clipped and prepped for the procedure. The clipped area started about four inches cranial to the mammary glands and extended laterally up to the lateral fold of the flank. A lidocaine (APP Pharmaceuticals LLC, Lake Zurich, IL, USA) local block (40 mg/site) was utilised to provide analgesia at the two portal sites. The patient was then transferred to the surgical suite and the cradle raised to Trendelenburg positioning where the head was tilted below the hind feet at a 45-degree angle. Two minutes after positioning was established, blood samples were collected and RR was measured (time point 2 (T2)). An incision (through skin, subcutaneous tissue, and fascia) was made over each proposed portal site and the abdomen was insufflated with a previously assigned gas via a teat cannula. The pressure was measured and regulated with a Karl Storz insufflator (Storz, Tuttlingen, Germany) in order to ensure that all patients were insufflated to the same intra-abdominal pressure (IAP) (10 cm H_2O) and rate (6 L/min). Trocars were then placed through both portal sites. The laparoscope was inserted through the laparoscope portal and the caudal abdomen inspected. A LAI gun was inserted into the instrument portal and the animal was bred by injecting semen directly into the uterine horns. All procedures took about 5 minutes or less from the start of insufflation. Blood samples were collected and RR measured after 5 minutes of insufflation (time point 3 (T3)) in order to keep the variable of time standard in both treatment groups. At the end of the procedure the laparoscope and LAI gun were withdrawn and the insufflation gas was removed through the sleeves by opening the valves and applying manual abdominal compression. The trocars were then removed and the patient was returned to a supine position. The incisions were closed with either suture or staples and the patient was returned to sternal or standing position in the recovery pen. Blood samples were collected and temperature, RR, and HR were measured 10 minutes after being returned to the recovery stall (time point 4 (T4)).

Arterial blood samples obtained at T0, T1, T2, T3, and T4 were collected in heparinised syringes (Pro-Vent Arterial Blood Sampling Kit, Smith Medical ASD, Keene, NH, USA), stored on ice, and batch run within one to two hours. All blood ~~gas~~ samples were analysed on a *Stat Profile pHOx Ultra* blood gas analyser (NOVA Biomedical, Waltham, MA, USA) that was calibrated each morning. Each blood sample was evaluated for pH, PaO_2, and $PaCO_2$.

Owners were later contacted via phone to gather data for conception rates.

Statistical Analysis

All data was collected, input into a Microsoft Office Excel (2013) spreadsheet, double-checked for errors, and analysed with IBM SPSS statistical software for window v. 24 (IBM Corporation, Software Group, Armonk, NY, USA).

A power analysis was performed and a sample size of 23 sheep per group was determined sufficient to achieve a power 0.8 with an alpha of 0.05%. SPSS was utilised to evaluate data for normality. An independent samples t-test evaluated the raw means of each dependent parameter (RR, HR, $PaCO_2$, PaO_2, and pH) as well as demographics data (age, weight) to ensure equality of the two randomised groups (CO_2, air) at baseline (T0). A repeated measures analysis of variance (ANOVA) was used to evaluate the outcomes of the dependent variables (RR, $PaCO_2$, PaO_2, and pH) over time and between the condition groups (CO_2 and medical air insufflation). Pearson's correlation coefficients were calculated to determine any relationships between variables, establishing any significant correlations to outcome so that they could be controlled for in the ANOVA analysis. Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, and therefore the Greenhouse-Geisser correction was used for comparisons within the ANOVA analysis and thus reported. There were a few outliers in various time points. Statistical analysis of the data with and without the outliers had the same outcomes, thus it was elected that keep the outliers in the data set which was more reflective of a clinical scenario. Since multiple analyses were performed, a Bonferroni correction was applied by dividing the critical p value by the number of tests performed (0.05/16 = 0.003). Based on the correction, a $p \leq 0.003$ was considered statistically significant.

Clinically concerning parameters for the dependent variables (PaO_2, $PaCO_2$, and pH) were established during the study design. These were defined as arterial blood gas values that may warrant intervention in a clinical setting: hypercapnia ($PaCO_2$ > 50 mmHg), hypoxaemia (PaO_2 < 70 mmHg), or acidaemia (pH < 7.35). These physiologic parameters were assessed for percentage occurrence at each time point during the LAI procedure. Pearson's chi-square analysis was utilised to evaluate relationship of clinically concerning parameter and condition (insufflation gas type).

Conception rates were tallied on a percentage per sheep basis. This data was evaluated with a pearson's chi-square analysis comparing relationship between conception rates and a hypoxaemic state as well as conception rates and insufflation gas type.

Results

Forty-seven sheep with a mean age of 2.64 (SD 1.58) years (range 1-6) and mean weight 76 (SD 9.5) kg (range 59-91) were enrolled in the study. Logistical challenges with arterial catheters clotting or not being adequately secured allowed for some missing data points in the initial days of data collection. Complete exclusion of sheep #3 due to multiple erroneous blood gas values associated with air bubbles in sample syringes and sheep #23 for multiple missing data points due to catheter complications, resulted in 45 sheep included in the final data analysis. Baseline physiologic parameters (temperature, RR, HR) and arterial blood ~~gas~~ samples (PaO_2, $PaCO_2$, and pH) were collected prior to any treatments or procedures. Data was assessed for outliers and found to have a normal distribution. An independent samples t-test found no significant difference when comparing raw mean values for each parameter (RR, HR, $PaCO_2$, PaO_2, and pH) as well as demographic data (age, weight) between the two randomised treatment groups (CO_2 and medical air) at baseline (T0) (all p >0.1).

Cardiopulmonary variables

HR at recovery was unchanged when compared to baseline ($F = 3.117$, $p = 0.085$) and did not differ between insufflation groups ($F = 1.808$, $p = 0.186$). There was a significant effect of time on RR during the LAI procedure ($F = 43.457$, $p<0.001$) (Table 1) with no significant difference between insufflation gas types ($F = 87.113$, $p=0.530$) (Table 2). All time points were significantly lower than baseline.

Blood gas variables

With respect to arterial blood gas parameters, there was a significant effect of time on arterial blood pH ($F = 28.737$, $p<0.001$) but no significant difference between insufflation groups ($F = 3.45$, $p = 0.017$). All values of pH were lower when compared to baseline, but only T2 was statistically significant ($F = 62.77$, $p<0.001$). A significant effect of time on $PaCO_2$ was present ($F = 15.232$, $p<0.001$) with no significant difference between insufflation groups ($F = 1.918$, $p = 0.123$). $PaCO_2$ values were increased in times points T1, T2, T3 when compared to baseline but only T2 and T3 were significant (all $p<0.001$) while T4 was significantly decreased ($p = 0.002$). Based on predetermined clinical criteria, none of the sheep experienced hypercapnia or acidaemia at any time point and remained within clinically normal reference ranges.

The analysis of partial pressure of arterial oxygen levels demonstrated a significant temporal effect on PaO_2 values ($F = 58.051$, $p<0.001$) with no significant differences between insufflation groups ($F = 1.048$, $p = 0.377$). In comparison to baseline, PaO_2 was significantly lower at times points T1, T2, T3 (all $p <0.001$) and returned to baseline at T4. Based on predetermined clinical criteria, 18% (8/45), 24% (11/45), 42% (19/45) of sheep became clinically hypoxaemic at T1, T2, T3 respectively, but all sheep were normoxaemic by recovery. Hypoxaemia was present in 63.6% (14/22) of the sheep insufflated with CO_2 versus 34.7% (8/23) of the sheep insufflated with medical air. Based on a chi-square analysis there is no significant relationship between hypoxaemia and insufflation gas type, $X^2(1, N = 45) = 3.746$, $p = .053$.

Conception rates

Conception rates were collected and evaluated for the study participants of the 2016/2017 breeding season. Unfortunately, three sheep were lost in follow up, two in the CO_2 group and one in the medical air group. The overall conception rate of the study participants was 73.81% (31/42). Among the sheep insufflated with CO_2, 75% (15/20) had a successful procedural outcome and 25% (4/20) did not. For the sheep insufflated with medical air, 72.73% (16/22) reported a successful procedural outcome and 27.27% (6/22) did not. Chi-square analysis was performed to examine the relationship between insufflation group and conception rate, $X^2(1, N = 42) = .028$, $p = .867$ as well as hypoxaemia and conception rate, $X^2(1, N = 42) = .123$, $p = .726$. Neither condition significantly affected conception rates.

Discussion

LAI procedures are very commonly performed around the world in many types of environments. This study was designed to evaluate how the LAI procedure affected the sheep and more specifically to compare the effects of two different abdominal insufflation gases. Based on previous studies assessing cardiopulmonary effects of laparoscopy in various species (Fukushima *et al.* 2011; Aksakal *et al.* 2014; Griffenhagen *et al.* 2015) we chose to evaluate oxygenation and ventilation parameters by utilising arterial blood gas analysis and monitoring RR. We know from the literature that intravenous sedation with xylazine (Doherty 1986; Nolan 1986; Celly *et al*. 1997), trendelenburg positioning (Grupullo 1996; Duke *et al.* 2002; Griffenhagen *et al.* 2015), and abdominal insufflation (Griffenhagen *et al.* 2015) cause significant blood gas changes, mainly decreases in PaO_2, decreases in pH, and an increase in $PaCO_2$. Five time points (baseline, sedation, trendelenberg positioning, abdominal insufflation, and recovery) were selected to assess changes in oxygenation and ventilation parameters with respect to these uncontrollable procedural variables. The main finding of the study was that there were no statistical or clinical differences between the insufflation gases with respect to blood gas parameters, respiratory rate changes, and conception rates.

At baseline, none of the sheep exhibited signs of cardiovascular or pulmonary disease detectable on physical examination. Baseline physiologic parameters such as heart rate and body temperature were within pre-established normal ranges (Pugh and Baird 2001) and all pretreatment (T0) arterial blood gas values were within previously documented normal ranges (Sobiech 2005; McDonnell and Kerr 2015). Respiratory rates were mildly elevated in some sheep, which may have been associated with excitement from a long trailer ride or introduction to a new environment. During the LAI procedure, there was a significant time effect on RR with an overall decrease in RR at all time points when compared to baseline. Xylazine, an alpha-adrenergic agonist, has been well documented to cause cardiopulmonary changes. Various doses, ranging from 0.05 mg/kg IV (Waterman *et al.* 1987) to 0.15 mg/kg IV (Doherty 1986; Celly *et al.* 1997) have been reported in the literature to cause a decrease in respiratory rate in sheep. In our study we found no difference between the CO_2 group and the medical air group over time, suggesting that the decrease in RR was more associated with the sedation and less affected by abdominal insufflation gas type.

The major findings of the blood gas analysis were a significant decrease in PaO_2 over time with no significant differences between insufflation groups. When assessing for clinically concerning parameters, hypoxaemia was present in 42% (19/45) of the sheep undergoing LAI procedure. The presence of hypoxaemia is most likely multifactorial. It has been shown in previous studies that xylazine sedation (Doherty 1986; Celly *et al.* 1997; Waterman *et al.* 1987), trendelenberg positioning (Galuppo *et al.* 1996; Duke *et al.* 2002), and the creation of a pneumoperitonium (Griffenhagen *et al.* 2015) can all contribute to the development of hypoxaemia. Although not statistically significant, more sheep were hypoxaemic when insufflated with CO_2 (14/22, 64%) than with medical air (8/23, 35%). This data is supported by previous studies that assess the cardiopulmonary and blood gas changes when evaluating CO_2 versus medical air (Menes and Spivak 2000). One suggestion for the percentage difference that was not statistically significant may be highlighted in the Kunz *et al.* (2000) paper. This paper demonstrated that duration of procedure and variable IAPs can have an effect on blood gas parameter changes over time when comparing different insufflation gases. Our study had both a shorter insufflation times and lower IAPs then previously reported laparoscopic studies. Nonetheless, a decrease in PaO_2 can be life threatening. It is strongly recommended that oxygen supplementation be provided either via a tight fitted mask or nasal oxygen lines during this procedure whenever available. Oxygen supplementation has been proven to improve hypoxaemia in other species during field anesthesia/sedation events (Reed *et al.* 2001; Lian 2014; Coutu *et al.* 2015).

Regarding the other blood gas analysis parameters, the data showed statistical but not clinically significant changes in $PaCO_2$ and pH over time with no significant differences between insufflation groups. None of the sheep experienced hypercapnia or acidaemia, and all $PaCO_2$ and pH values remained within normal reference range. This contradicts prior studies (Griffenhagen *et al.* 2015, Güzel *et al.* 2012) that looked at the effects of laparoscopic procedures on these blood gas values. The main differences between those studies and our study were that our sheep were sedated and not under general anesthesia, insufflated at lower IAPs (~8 mmHg versus 15-20 mmHg), and the duration of the pneumoperitonium was much shorter (5 minutes versus 30-60 minutes). Kuntz *et al* (2000) suggested that variable IAPs and duration of pneumoperitonium were major contributing factors to the development of abdominal, blood, and subcutaneous pH changes when assessing the effects of a single gas type or comparing insufflation gases (helium, air, CO_2.) During parts of their study they did not observe a significant difference in blood gas parameters until after 30 minutes of insufflation. The brevity of the LAI procedure may explain why we did not observe statistically significant blood gas parameters changes when comparing two insufflation gases.

Conception rates are considered a measure of successful outcome of the LAI procedure. The overall conception rate of this procedure during the study was 73.81% (31/42), which is consistent or even slightly higher than conception rates of previous LAI studies. Based on the data collected, there was no significant relationship between insufflation group and conception rates. Chi-square analysis also demonstrated no significant relationship between hypoxaemia and conception rate.

The main limitations of this study were the number of physiologic parameters collected for each study participant. Previous published studies assessing the effects of laparoscopy on cardiopulmonary parameters included instrumentation devices such as blood pressure, pulse oximetry, capnography, and spirometry. Our study was designed to fit into a clinical setting. The LAI procedure averaged about 15-20 minutes from sedation to recovery and sheep were lined up in an assembly line fashion to successfully AI up to 30 sheep per day. The brevity and efficiency of this procedure did not allow for aggressive instrumentation. Also, measurements such as spirometry and capnography were unavailable because the sheep were not under general anesthesia thus not intubated. Body temperature, HR, and RR were recorded at the very beginning and in recovery to acquire a baseline and ensure that the sheep returned to normal physiologic parameters. It would have been desirable to measure these physiologic parameters throughout the procedure, but due to time constraints between data points the main focus was spent on arterial blood sample collection.

In conclusion, the main findings of the study demonstrated no statistical or clinical differences in RR, PaO_2, $PaCO_2$, pH, or successful procedural outcome rates when comparing CO_2 to medical air as an insufflation gas in routine LAI procedures. None of the sheep that underwent this brief procedure experienced hypercapnia or acidaemia, but overall 42% (19/45) of sheep developed clinical hypoxaemia with no observable difference between insufflation groups. This was contradictory to previous studies. We concluded that this was most likely due to the brevity of the procedure and the lower IAPs utilised when compared to those previously published studies. Further studies would be necessary to evaluate the long-term effects of hypoxaemia in this sheep population as well as clinical efficacy of oxygen supplementation in preventing a hypoxaemia during this procedure. Study design limitations did not allow for the assessment of cardiopulmonary parameters such as cardiac output and tidal volume, nor afford the use of non-invasive monitoring modalities such as pulse oximetry, oscillametric blood pressure, or capnography. Based on the data collected, medical air may be a comparable cost-effective alternative to the gold standard CO_2 for abdominal insufflation during xylazine sedated LAI procedures in sheep.

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TABLE 1. Physiologic and blood gas variables (mean ± SEM) in sheep participants during the LAI procedure for the within-subjects Repeated Measures ANOVA analysis

Dependent Variable	Baseline (T0)	Sedation (T1)	Positioning (T2)	Insufflation[a] (T3)	Recovery[a] (T4)
RR (breath $minute^{-1}$)	69 ± 3	48 ± 3[x]	38 ± 2[x]	39 ± 2[x]	44 ± 2[x]
PaO_2 (mmHg)	96.6 ± 1.6	82.4 ± 2.4[x]	75.7 ± 2.1[x]	68.9 ± 2.0[x]	99.3 ± 2.0
$PaCO_2$ (mmHg)	29.3 ± 0.6	30.3 ± 0.6[y]	31.3 ± 0.5[y]	32.3 ± 0.6[y]	29.1 ± 0.4[x]
pH	7.492 ± 0.004	7.478 ± 0.005[x]	7.458 ± 0.004[x]	7.452 ± 0.005[x]	7.487 ± 0.004[x]

RR = respiratory rate, PaO_2 = partial pressure of arterial oxygen, $PaCO_2$ = partial pressure of arterial carbon dioxide, pH = arterial blood pH
a – condition applied to participants
x - Represents statistically significant decrease from baseline
y - Represents a statistically significant increase from baseline

TABLE 2. Physiologic and blood gas variables (mean ± SEM) in sheep participants for each insufflation group during the LAI procedure for the between-subject Repeated Measures ANOVA analysis

Dependent Variable	Condition	Baseline (T0)	Sedation (T1)	Positioning (T2)	Insufflation[a] (T3)	Recovery[a] (T4)
RR (breath $minute^{-1}$)	CO_2	67 ± 4	48 ± 4	38 ± 2	39 ± 2	42 ± 2
	Air	71 ± 4	48 ± 4	38 ± 2	38 ± 2	46 ± 2
PaO_2 (mmHg)	CO_2	96.9 ± 2.3	80.9 ± 3.5	72.1 ± 3.1	65.8 ± 2.9	99.4 ± 2.8
	Air	96.3 ± 2.3	83.9 ± 3.4	79.3 ± 3.0	72.0 ± 2.8	99.2 ± 2.8
$PaCO_2$ (mmHg)	CO_2	28.7 ± 0.8	29.9 ± 0.8	30.6 ± 0.7	31.1 ± 0.8	29.2 ± 0.6
	Air	29.9 ± 0.8	30.8 ± 0.8	32.0 ± 0.6	33.5 ± 0.8	28.9 ± 0.6
pH	CO_2	7.487 ± 0.006	7.476 ± 0.008	7.457 ± 0.006	7.457 ± 0.007	7.457 ± 0.006
	Air	7.496 ± 0.005	7.48 ± 0.007	7.460 ± 0.006	7.448 ± 0.007	7.500 ± 0.006

CO_2 = group designated to receive carbon dioxide insufflation gas; Air = group designated to receive medical air insufflation gas

a - condition applied to participants