ARTICLES
Archives of Veterinary Science
UFPR
https://www.ufpr.br/

Comparison of different ventilatory modalities and inspired oxygen fractions in the Common Rabbit, *Oryctolagus cuniculus* (Lynnaeus, 1758)

Submitted: *31/03/2025*
Accepted: *19/06/2025*

Aline Bertolini de Lauro[1], Gilberto Serighelli Júnior[*2], Vanessa Regina Olszewski[3], Felipe Comassetto[4], Nilson Oleskovicz[4]*

[1]Veterinary Doctor, Lages, State of Santa Catarina, Brazil. ORCID:0009-0007-1435-7085.
[2]Department of Veterinary Medicine, Federal University of Paraná (UFPR), Curitiba, Brazil, State of Paraná, Brazil. ORCID:0000-0002-0979-7939.
[3]Canine Nutrition Studies Laboratory, Department of Veterinary Medicine, Federal University of Paraná, Curitiba, Brazil, State of Paraná, Brazil. ORCID: 0000-0003-2035-8803.
[4]Department of Veterinary Medicine, Center for Agro-Veterinary Sciences (CAV), Santa Catarina State University (UDESC), Lages, State of Santa Catarina, Brazil. ORCID: 0000-0002-7355-6064; ORCID: 0000-0002-7596-730X.

[*]Correspondence email: gilberto.serighelli@ufpr.br

Abstract: This randomized clinical trial aimed to evaluate the effects of two inspired oxygen fractions (FiO_2: 40% and 60%) combined with different ventilatory modalities—spontaneous ventilation (SV, T0), volume-controlled ventilation (VCV, T1), and pressure-controlled ventilation (PCV, T2) on cardiopulmonary and metabolic parameters in anesthetized rabbits. Sixteen healthy male New Zealand White rabbits were randomly assigned to two groups (G0.4 and G0.6). Animals were premedicated with ketamine (15 mg/kg), midazolam (2 mg/kg), and morphine (2 mg/kg), and general anesthesia was induced via face mask with isoflurane at 4.0%, diluted in oxygen at either 40 or 60%, according to group allocation. Physiological variables, blood gas analyses, and indirect calorimetry data were collected at multiple time points. Regarding blood gas variables, arterial oxygen partial pressure (PaO_2) increased progressively with higher inspired oxygen fractions, showing statistically significant differences between groups at T0, T1, and T2 (p =0.003, 0.001, and 0.001, respectively), while oxygenation indices remained within clinically acceptable ranges. Controlled ventilation was associated with improved oxygenation efficiency in both FiO_2 groups. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) progressively declined throughout anesthesia, whereas the respiratory quotient (RQ) range of approximately 0.8-0.9, indicating reduced metabolic demand and preserved substrate utilization. No clinically relevant differences were observed in heart rate, mean arterial pressure, or arterial carbon dioxide pressure ($PaCO_2$) between groups. These findings indicate that both 40 and 60% FiO_2 are safe and effective when combined with appropriate ventilatory strategies in healthy anesthetized rabbits, supporting individualized oxygen titration based on ventilation mode and physiological monitoring.
Keywords: *Oryctolagus cuniculus*, fraction of inspired oxygen, mechanical ventilation, indirect calorimetry.

1. Introduction

Oxygen (O_2) is essential for cellular metabolism, and its adequate delivery depends on the integrated function of the cardiovascular and respiratory systems (Xu et al. 2016; Hor et al., 2019). During anesthesia, maintaining proper oxygenation is crucial to prevent hypoxia and its associated complications (Xu et al., 2016; Bellini et al., 2023). Although high fractions of inspired oxygen (FiO_2) are commonly employed to optimize oxygen delivery, prolonged exposure or excessively high concentrations may contribute to pulmonary complications, including atelectasis and lung injury, which are associated with several adverse outcomes such as pneumonia, empyema, sepsis, and acute lung injury (Xu et al., 2016; Hor et al., 2019). Despite these risks, the routine administration of 100% oxygen remains common in veterinary anesthesia (Hor et al., 2019).

The selection of an appropriate FiO_2 is critical, as supraphysiological oxygen levels have been associated with cellular oxidative stress, inflammatory responses, and pulmonary injury (Xu et al. 2016; Dias-Freitas, 2016). Hyperoxia can induce the overproduction of reactive oxygen species, leading to lipid peroxidation, protein oxidation, and DNA damage, which contribute to cellular dysfunction and apoptosis (Helmerhorst et al., 2015). The pulmonary system is particularly vulnerable to oxygen toxicity, with acute lung injury being a well-documented consequence, characterized by diffuse alveolar damage, increased alveolar-capillary permeability, interstitial and alveolar edema, and an influx of inflammatory cells (Matthay et al., 2019). Furthermore, excessive oxygen administration can potentiate mechanical ventilation-induced lung injury, further impairing pulmonary function and gas exchange efficiency (Xu et al., 2016; Dias-Freitas et al., 2016; Bellini et al., 2023). Given these risks, determining an optimal FiO_2 that prevents hypoxia while minimizing oxygen-induced toxicity is essential for anesthetic management, particularly in species with unique respiratory physiology, such as rabbits.

Mechanical ventilation is widely employed to maintain respiratory stability during anesthesia, utilizing volume-controlled and pressure-controlled modes (Hor et al., 2019). However, it may contribute to lung injury even in healthy individuals, a phenomenon known as ventilator-induced lung injury (VILI), which results from cyclic alveolar overdistension, repetitive alveolar collapse and reopening, and shear stress on pulmonary structures (Xu et al., 2016; Walesa et al., 2018; Bellini et al., 2023). In rabbits (*Oryctolagus cuniculus*), the effects of different ventilatory strategies, particularly concerning variations in inspired oxygen fractions (FiO_2), remain poorly characterized. The limited availability of data regarding FiO_2 levels lower than those routinely applied in veterinary anesthesia raises concerns about the optimal oxygen concentration needed to ensure adequate oxygenation while minimizing the risk of oxygen-induced pulmonary injury (Xu et al., 2016; Hor et al., 2019).

This study aimed to evaluate the effects of FiO_2 at 40 and 60% on hemodynamic, metabolic, and blood gas parameters in rabbits undergoing different ventilatory modalities. By identifying safe anesthetic approaches for this species, this research seeks to optimize oxygen administration strategies while minimizing potential complications.

https://doi.org/10.5380/avs.v30i2.99093

1.1. Materials and Methods

1.2. Animals

A total of 16 male New Zealand White rabbits (*Oryctolagus cuniculus*), aged 6 ± 2 months and with a mean body weight of 3.2 ± 0.6 kg, were used in this block-randomized, blinded, prospective clinical experimental study. The animals were allocated into two groups with a 1:1 ratio (40% and 60% FiO_2) using an online randomization tool (www.randomization.org). The sample size was determined through power analysis using OpenEpi software (version 3.0; OpenEpi, TN, USA). Based on an alpha of 5% (two-sided), a beta of 20% (80% power), and the assumption that a 20% reduction in PaO_2 would be clinically significant, the required sample size was calculated. Assuming a standard deviation (SD), it was determined that eight animals per group were necessary to detect this difference.

Only animals classified as American Society of Anesthesiologists (ASA) physical status I were included after undergoing a health screening, which consisted of a physical examination, hematology, and biochemistry analyses. Rabbits presenting any abnormalities in respiratory function or other health parameters were excluded from the study. This study followed the ARRIVE guidelines for the care and use of laboratory animals and was approved by the Animal Experimentation Ethics Committee – CEUA/UDESC under protocol 7667170616.

1.3. Anaesthesia and instrumentation

The animals underwent a four-hour pre-procedural fasting period without water deprivation. Before administering pre-anesthetic medication, arterial cannulation was performed for blood sample collection and invasive blood pressure monitoring. A 24-gauge catheter (Safelet-ETFE; Nipro, Sao Paulo, Brazil) was inserted into the marginal ear artery and connected to a pre-calibrated disposable pressure transducer (Utah/Biotrans, GE Healthcare, Chicago, USA) linked to a multiparameter monitor (B650 monitor; Datex-Ohmeda, GE Healthcare, Chicago, USA). Calibration accuracy was ensured using a dynamic response test (square wave test). This system was connected via a low-compliance pressure tube to a pressurized heparinized saline solution (10 IU/mL), infused at a rate of 1 mL/h to prevent clot formation.

Following arterial cannulation, intramuscular premedication was administered, consisting of ketamine (15 mg/kg) (Cetamin 100 mg/mL, Syntec Ltda., Barueri, Brazil), midazolam (2 mg/kg) (Dormire 5 mg/mL, Cristália Ltda., Itapira, Brazil), and morphine (2 mg/kg) (Dimorf 10 mg/mL, Cristália Ltda., Itapira, Brazil). A 15-minute interval was allowed for drug onset. To ensure precise dosing, all volumes were standardized and diluted in 0.9% saline solution to a final volume of 1 mL, following the ARRIVE guidelines for laboratory animal research. Venous access was established in the contralateral marginal ear vein for intravenous fluid therapy with 0.9% NaCl solution at a rate of 3 mL/kg/h. Both arterial and venous accesses were secured before anesthesia induction.

General anesthesia was induced using isoflurane at 4.0% via face mask. The animals were then randomly assigned to two groups: G0.6, which received isoflurane diluted in an inspired oxygen fraction (FiO_2) of 60%, and G0.4, which received isoflurane diluted in an FiO_2 of 40%. Endotracheal intubation was performed using an appropriately sized endotracheal tube, and the animals were connected to a partial rebreathing circular system with an oxygen flow rate of 5 mL/kg/min. Anesthetic maintenance was achieved with isoflurane at 2.1%, with adjustments based on anesthetic depth, differing only in the inspired oxygen fraction between groups.

1.4. Ventilation Protocol and Experimental Design

After induction and stabilization, the animals were subjected to randomized allocation of different ventilatory modalities, remaining in each assigned mode for 20 minutes before data collection. The first phase (T0) consisted of spontaneous ventilation, followed by mechanical ventilation in two distinct modalities. The volume-controlled ventilation phase (T1) was performed with a tidal volume of 6 mL/kg, an inspiratory-to-expiratory (I:E) ratio of 1:2, and a respiratory rate adjusted to maintain normocapnia. The pressure-controlled ventilation phase (T2) was conducted with an inspiratory pressure of 12 cmH_2O, an I:E ratio of 1:2, and a respiratory rate adjusted to achieve normocapnia. All animals remained in sternal recumbency throughout the procedure.

Physiological parameters were recorded at different time points to evaluate cardiovascular and respiratory function. Heart rate (HR), respiratory rate (RR), rectal temperature (RT), mean arterial pressure (MAP), and peripheral oxygen saturation (SpO_2) were continuously monitored using a multiparameter monitor. In addition to these parameters, end-tidal carbon dioxide ($EtCO_2$), oxygen consumption indexed by body surface area (VO_2), carbon dioxide production indexed by body surface area (VCO_2), and respiratory quotient (RQ) were assessed at all time points. Blood samples were collected at T-15 (baseline after arterial cannulation), M5 (5 minutes after induction), T0 (spontaneous ventilation phase, SV), T1 (volume-controlled ventilation phase, VCV), and T2 (pressure-controlled ventilation phase, PCV). Blood gas analysis was performed to determine pH, arterial oxygen pressure (PaO_2), arterial carbon dioxide pressure ($PaCO_2$), bicarbonate concentration (HCO_3^-), and arterial oxygen saturation (SaO_2). The oxygenation index (OI) was calculated using the formula $OI = PaO_2/FiO_2$, expressed in mmHg.

After completion of the ventilation protocol, all animals underwent elective castration. The same surgeon performed the procedure and began with trichotomy and antisepsis of the scrotal region, followed by a local anesthetic block with intratesticular and incisional administration of 2 mg/kg of 2% lidocaine without vasoconstrictor (Xylestesin 20 mg/mL, Cristália Ltda., Itapira, Brazil) using the stylet of a 24G catheter. Five minutes after the block, orchiectomy was performed.

Statistical analysis was conducted using GraphPad Prism 9.3.0 software. A paired t-test was applied to compare differences between groups, and one-way ANOVA, followed by Dunnett's test, was used to detect differences between time points. Statistical significance was set at $p \leq 0.05$. Parametric results were expressed as mean and standard deviation.

2. Results

Heart rate remained stable across all time points in both groups, with no statistically significant differences observed ($p > 0.05$). A slight increase was noted during the mechanical ventilation phases in both treatments. Respiratory rate showed a marked reduction following anesthetic induction, particularly at the initiation of controlled ventilation, with a more pronounced decrease in G0.4.

		Time Points				
Variable	**Groups**	**T-15**	**Tbasal**	**T0**	**T1**	**T2**
HR (bpm)	G 0.4	211 ± 21	247 ± 40	244 ± 39	242 ± 46	240 ± 46
	G 0.6	222 ± 38	254 ± 32	236 ± 28	268 ± 30	250 ± 30
RR (mpm)	G 0.4	147 ± 59	45 ± 22	17 ± 11A	17 ± 11	25 ± 9
	G 0.6	173 ± 77	37 ± 15	15 ± 7A	22 ± 8	21 ± 9
SpO_2 (%)	G 0.4	92 ± 7	84 ± 9	93 ± 6	94 ± 7A	94 ± 5a
	G 0.6	93 ± 4	82 ± 5	93 ± 6A	97 ± 3A	97 ± 2Ab
MAP (mmHg)	G 0.4	79 ± 10	76 ± 19	68 ± 8A	61 ± 7A	62 ± 11A
	G 0.6	88 ± 15	77 ± 20	67 ± 21A	67 ± 15A	67 ± 14
$EtCO_2$ (mmHg)	G 0.4			50 ± 11	44 ± 8	47 ± 9
	G 0.6			49 ± 23	38 ± 13	40 ± 14
VO_2 (mL/kg/m²)	G 0.4			600 ± 536a	321 ± 498	366 ± 308
	G 0.6			990 ± 343b	266 ± 181	230 ± 155
VCO_2 (mL/kg/m²)	G 0.4			394 ± 343	378 ± 265	261 ± 275
	G 0.6			416 ± 401	269 ± 193	250 ± 141
RQ (Respiratory quotient)	G 0.4			0.75 ± 0.1	0.9 ± 0.17	0.83 ± 0.05
	G 0.6			0.79 ± 0.11	0.87 ± 0.08	0.83 ± 0.05
ETISO (V%)	G 0.4			0.82 ± 0.5	1.08 ± 0.42	1.3 ± 0.5
	G 0.6			0.91 ± 0.24	0.9 ± 0.54	0.89 ± 0.3

Table 1 - Mean values and standard deviation of trans-anesthetic physiological parameters of heart rate (HR), respiratory rate (RR), peripheral oxygen saturation – SpO_2; mean arterial pressure – MAP; end-tidal carbon dioxide – $EtCO_2$; oxygen consumption – VO_2; carbon dioxide production – VCO_2; respiratory quotient – RQ and end-tidal isoflurane concentration – ETISO) in rabbits subjected to spontaneous ventilation, volume-controlled ventilation, and pressure-controlled ventilation under isoflurane anesthesia, with inspired oxygen fractions of 40% (G0.4) or 60% (G0.6). Uppercase letters in the same row indicate significant differences compared to baseline. Different lowercase letters in the same column indicate significant differences between groups.

Peripheral oxygen saturation remained within physiological limits throughout the experiment in both groups. However, during the induction period, values dropped below 90% in both groups before recovering shortly thereafter.

Mean arterial pressure decreased following anesthetic induction in both groups. Although controlled ventilation appeared to induce a greater reduction in mean arterial pressure compared to spontaneous ventilation, no statistically significant differences were observed between the administered oxygen fractions ($p = 0.389$). End-tidal carbon dioxide remained relatively stable throughout the different phases of the experiment, without significant variations across time points or between groups.

Oxygen consumption, indexed by body surface area, showed a consistent reduction throughout the procedure in both groups. In G0.4, oxygen consumption decreased by approximately 39%, whereas in G0.6, the reduction reached nearly 77% from baseline to the final time point. Carbon dioxide production followed a similar trend, with an overall decrease of about 34% in G0.4 and 64% in G0.6. The respiratory quotient increased progressively after anesthetic induction, reaching a peak during the first stage of mechanical ventilation. This increase was approximately 20% in G0.4 and 10% in G0.6, followed by a slight decline at the final stage, although values remained above baseline levels.

Regarding hemogasometric variables, arterial oxygen partial pressure (PaO_2) increased progressively with higher inspired oxygen fractions, showing statistically significant differences at T0, T1, and T2 (p = 0.003, 0.001, and 0.001, respectively). All values remained within expected physiological ranges for each FiO_2 level, with reference values for PaO_2 ranging from 100–137 mmHg for 40% FiO_2 and 140–169 mmHg for 60% FiO_2 (Egi et al., 2007). Predicted PaO_2 values aligned with expectations of being approximately two to three times the FiO_2 administered. None of the ventilatory modalities or oxygen fractions resulted in values consistent with hypoxemia (PaO_2 < 60 mmHg and SaO_2 < 90%).

Arterial carbon dioxide partial pressure ($PaCO_2$) remained within normal limits or showed mild elevations depending on FiO_2 and ventilatory strategy. Values exceeding 45 mmHg were more frequently observed in groups exposed to 60% FiO_2, although no statistically significant differences were detected. The highest $PaCO_2$ values were observed in the group receiving sevoflurane with 60% oxygen.

		Time Points				
Variable	**Groups**	**T-15**	**Tbasal**	**T0**	**T1**	**T2**
pH	G 0.4	7.43±0.06	7.40±0.08	7.27±0.06A	7.31±0.09A	7.26±0.09A
	G 0.6	7.4±0.08	7.4±0.08	7.2±0.1A	7.32±0.13	7.26±0.08A
SaO_2 (%)	G 0.4	93±1	83±8.5	94.2±9A	98±1.7A	98±1.1A
	G 0.6	94±1.6	85±4	96.3±7A	97.5±4.6A	99±1.3A
HCO_3 (mmol L^{-1})	G 0.4	18.9±3.65	22.4±5.2	26.4±3.1A	24.3±2.6	25.7±4.1
	G 0.6	19.7±5.0	22.7±5.5	29.4±50A	27.6±4.9A	28.3±6.3A
PaO_2 (mmHg)	G 0.4	73.5±6	54±12	119±33Aa	158±31Aa	144±25Aa
	G 0.6	75±9	58±11.5	180±53Ab	215±68Ab	204±50Ab
$PaCO_2$ (mmHg)	G 0.4	29±2.7	37±8	49±13A	49±11A	50±17
	G 0.6	28.5±4.4	36±4.5	55±20A	56±17A	53±14A
OI (PaO_2/FiO_2)	G 0.4	350±28	257±59	297±83	394±77A	360±63A
	G 0.6	356±44	278±55	299±88	359±113	341±83

Table 2 – Mean values and standard deviations of blood gas parameters and oxygenation index (OI) in rabbits subjected to spontaneous ventilation, volume-controlled ventilation, and pressure-controlled ventilation under isoflurane anesthesia, with inspired oxygen fractions of 40% (G0.4) or 60% (G0.6). Different lowercase letters in the same column indicate significant differences between groups.
pH – hydrogen ion potential; SO_2 – arterial oxygen saturation; HCO_3^- – bicarbonate; PaO_2 – arterial oxygen partial pressure; $PaCO_2$ – arterial carbon dioxide partial pressure; OI – oxygenation index; T-15 – 15 minutes before baseline; Tbasal – baseline moment. Obs: Uppercase letters in the same row indicate significant differences compared to baseline.

Oxygen saturation (SaO_2) remained above 94% across all FiO_2 groups and ventilatory modalities, except during the induction period—particularly after orotracheal intubation—when animals were breathing room air (21% oxygen). At that point, transient decreases were observed.

The oxygenation index (PaO_2/FiO_2) varied according to the inspired oxygen fraction and ventilation mode. Although no significant differences were found between groups, higher FiO_2 levels were associated with increased PaO_2/FiO_2 ratios, indicating improved arterial oxygenation. The highest ratios were observed during pressure-controlled ventilation, whereas the lowest occurred during spontaneous ventilation with 40% FiO_2, in which some animals exhibited values near 200 mmHg. In both groups, PaO_2/FiO_2 values progressively improved following the initiation of controlled ventilation. No animal presented a PaO_2/FiO_2 value below 150 mmHg, suggesting that although mild reductions in oxygenation efficiency occurred, criteria for moderate or severe hypoxemia were not met.

3. Discussion

The results of this study indicate that both inspired oxygen fractions (FiO_2 40% and 60%) were suitable for use in rabbits undergoing general anesthesia, regardless of whether they were under spontaneous ventilation or pressure- and volume-controlled mechanical ventilation. Heart rate remained stable throughout the experimental protocol, with no statistically significant changes between time points or FiO_2 groups. This cardiovascular stability suggests that the anesthetic protocol comprising isoflurane combined with opioid, benzodiazepine, and ketamine premedication was effective in enabling mechanical ventilation and endotracheal intubation without compromising hemodynamic function. A similar study in rabbits receiving 15 mg/kg of ketamine

and 2 mg/kg of midazolam, combined with 2 mg/kg of morphine, reported comparable results, with no evidence of bradycardia at any time point (Júnior et al., 2025).

Mean arterial pressure decreased following anesthetic induction in both groups, likely due to the vasodilatory properties of isoflurane, which induces dose-dependent reductions in blood pressure by decreasing systemic vascular resistance (Barter & Epstein, 2013; Uccello et al., 2020). After the initiation of controlled ventilation, mean arterial pressure declined further, probably due to reduced venous return secondary to positive pressure ventilation, which exacerbates arterial pressure drops (Barter & Epstein, 2013). Despite some values approaching or falling below reference limits, tactile stimulation such as applying pressure to a limb was sufficient to restore blood pressure, indicating preserved cardiovascular responsiveness. Additionally, the lowest pressure values were recorded in the absence of surgical stimulation, underscoring the role of nociceptive input in maintaining sympathetic tone during anesthesia.

Peripheral oxygen saturation remained within physiological limits throughout the study, though transient decreases were observed during induction in both groups. These short-term reductions were likely related to airway instrumentation and the transition from ambient air to oxygen supplementation. Similar fluctuations have been documented during anesthetic transitions, particularly when animals breathe room air or lower FiO_2 levels before securing the airway (Horr et al., 2019).

End-tidal carbon dioxide ($EtCO_2$) represents the partial pressure of CO_2 at the end of exhalation, reflecting alveolar ventilation. It functions as an indirect indicator of arterial carbon dioxide ($PaCO_2$), with $EtCO_2$ values typically measuring 2 to 5 mmHg lower than $PaCO_2$, whose physiological reference range in awake mammals is between 35 and 45 mmHg (Allweiler, 2016). In the present study, during the spontaneous breathing phase (T0), $EtCO_2$ values were elevated beyond the normal range, likely associated with transient hypoventilation following anesthetic induction. However, after the initiation of controlled mechanical ventilation (T1 and T2), $EtCO_2$ values promptly returned to within the expected physiological range (35–45 mmHg) across both inspired oxygen fractions evaluated. This finding highlights the effectiveness of controlled ventilation in rapidly correcting hypercapnia secondary to hypoventilation, frequently observed during the transition from spontaneous to mechanical ventilation under general anesthesia (Allweiler, 2016).

Oxygen consumption (VO_2), assessed via indirect calorimetry, progressively declined throughout the procedure in both groups. This reduction was likely associated with the transition to controlled ventilation, which minimizes the animal's respiratory effort and decreases metabolic oxygen demand (Gehrcke et al., 2017; Júnior et al., 2024). Additionally, anesthetic-induced suppression of cellular metabolism and attenuation of sympathetic activity may have contributed to the observed decline in VO_2 (Bailey, 2003). Carbon dioxide production (VCO_2) followed a similar pattern, decreasing progressively in both groups. These findings align with previous studies showing reductions in metabolic rate in dogs under general anesthesia, illustrating the physiological downregulation of oxidative metabolism in response to decreased tissue energy demands (Gehrcke et al., 2017; Júnior et al., 2024).

The respiratory quotient (RQ), defined as the VCO_2/VO_2 ratio, increased progressively after anesthetic induction, reaching a peak during early mechanical ventilation. This elevation suggests a metabolic shift favoring carbohydrate oxidation, as RQ values between 0.8 and 0.9 typically indicate glucose as the predominant energy source (Gehrcke et al., 2017; Júnior et al., 2024). The maintenance of RQ within this range suggests limited protein catabolism, which is beneficial in perioperative settings, as preserving protein stores supports immune function, tissue repair, and metabolic stability (Queau et al., 2011; Júnior et al., 2024). These findings highlight a stable metabolic profile under the anesthetic protocol used, with controlled ventilation contributing to reduced energy expenditure and efficient substrate utilization (Gehrcke et al., 2017; Júnior et al., 2024).

Arterial oxygen partial pressure (PaO_2) increased proportionally with FiO_2, with statistically significant differences observed between groups at multiple time points. All values remained within the physiological range previously described for rabbits breathing 40% or 60% oxygen (Egi et al., 2007), confirming the expected linear relationship between FiO_2 and PaO_2 in healthy animals under general anesthesia. These findings demonstrate that even modest FiO_2 increases can enhance oxygenation. Importantly, no cases of hypoxemia were detected in either group, indicating that both oxygen fractions were sufficient to maintain adequate tissue oxygenation under the ventilatory conditions applied.

Arterial carbon dioxide partial pressure ($PaCO_2$) remained within normal limits, though mild elevations were more frequent in animals receiving 60% FiO_2. While these increases were not statistically significant, they may reflect subtle reductions in minute ventilation or early changes in pulmonary compliance due to higher FiO_2, which is known to promote atelectasis (Xu et al., 2016; Walesa et al., 2018). Nonetheless, the maintenance of normocapnia highlights the efficacy of the employed ventilatory strategy and supports the safety of both FiO_2 levels in preserving acid–base balance. Oxygen saturation (SaO_2) remained consistently above 94% in both groups, except for brief reductions during induction, particularly in animals breathing ambient air. Although these transient declines were of no clinical consequence, they emphasize the need for prompt airway control and supplemental oxygen at induction to prevent hypoxia and ventilation–perfusion (V/Q) mismatch. Similar peri-induction desaturation has been described in dogs, where transient falls in SaO_2 attributable to V/Q disturbances resolve quickly once airway patency and oxygenation are restored (Ambros et al., 2018).

The oxygenation index (PaO_2/FiO_2) confirmed that both FiO_2 levels supported adequate oxygenation, with no significant differences in overall gas exchange efficiency. While the highest indices were recorded in animals ventilated with 60% oxygen, this did not confer a substantial advantage over 40% FiO_2 in healthy individuals under stable anesthesia. Conversely, the lowest values were observed during spontaneous ventilation with 40% FiO_2, where some animals reached pressures of 200 mmHg. Although this does not meet the threshold for moderate hypoxemia ($PaO_2/FiO_2 < 200$ mmHg) (Hor et al., 2019), it indicates reduced oxygenation efficiency in these conditions. This highlights the limitations of lower FiO_2 during spontaneous breathing. The progressive

improvement in PaO_2/FiO_2 following the initiation of mechanical ventilation reinforces the benefits of ventilatory support in optimizing pulmonary gas exchange.

These findings underscore the importance of individualized FiO_2 titration, based on anesthetic depth, ventilation modality, and continuous physiological monitoring, to ensure both safety and efficacy during anesthesia in small mammals. A limitation of this study is the relatively small sample size, which may have reduced statistical power to detect subtle differences between groups. Additionally, as only clinically healthy animals were included, the results may not fully reflect physiological responses in individuals with pre-existing respiratory dysfunction. Further studies involving larger sample sizes and animals with respiratory compromise are warranted to validate these findings and explore their clinical applicability.

4. Conclusion

Both inspired oxygen fractions evaluated in this study, 40% and 60%, effectively maintained cardiopulmonary stability and adequate gas exchange in healthy rabbits anesthetized with isoflurane under different ventilatory modalities. Although 60% FiO_2 resulted in higher arterial oxygen partial pressures and oxygenation indices, 40% FiO_2 was also sufficient to prevent hypoxemia under the experimental conditions. Controlled ventilation improved oxygenation efficiency in both groups. Furthermore, the progressive reduction in oxygen consumption and carbon dioxide production during anesthesia reflects a decrease in metabolic demand, indicating a preserved metabolic balance throughout the anesthetic period.

Acknowledgments: The authors wish to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and State University of Santa Catarina (UDESC), Lages, Santa Catarina, for providing the facilities for conducting the study.

Informative Notes: The study was approved by the Ethics Committee on Animal Use (CEUA) of the Animal Experimentation Ethics Committee – CEUA/UDESC, protocol: 7667170616.

5. References

Allweiler, S.I. How to Improve Anesthesia and Analgesia in Small Mammals. Veterinary Clinics of North America: Exotic Animal Practice. v.19, n.2, p. 361–377, 2016. https://doi.org/10.1016/j.cvex.2016.01.012

Ambros B., Carrozzo MV, Jones T. Desaturation times between dogs preoxygenated via face mask or flow-by technique before induction of anesthesia. Vet Anaesth Analg. 2018 Jul;45(4):452-458. doi: 10.1016/j.vaa.2018.03.004. Epub 2018 Apr 10. PMID: 29739649

Bailey, T.C., Martin, E.L., Zhao, L., & Veldhuizen, R.A. High oxygen concentrations predispose mouse lungs to the deleterious effects of high stretch ventilation. Journal of Applied Physiology, v. 94, p. 975–982, 2003. https://doi.org/10.1152/japplphysiol.00619.2002.

Barter, L.S., & Epstein, S.E. Cardiopulmonary effects of three concentrations of isoflurane with or without mechanical ventilation and supramaximal noxious stimulation in New Zealand white rabbits. American Journal of Veterinary Research, v. 74, n. 10, p. 1274-1280, 2013. https://doi.org/10.2460/ajvr.74.10.1274.

Bellini, L., Schrank, M., Veladiano, I.A., Contiero, B., Mollo, A. Retrospective comparison of the effects of laryngeal mask and endotracheal tube on some cardio-respiratory variables in pet rabbits undergoing anaesthesia for elective gonadectomy. Acta Vet Scand.. v.65, n.1, p.10, 2023. https://doi.org/10.1186/s13028-023-00673-2

Dias-Freitas, F., Metelo-Coimbra, C., Roncon-Albuquerque, R. Jr. Molecular mechanisms underlying hyperoxia acute lung injury. Respiratory Medicine, v. 119, p. 23–28, 2016. https://doi.org/10.1016/j.rmed.2016.08.010.

Egi, A., Kawamoto, M, Kurita, S, & Yuge, O. Systolic arterial pressure variability reflects circulating blood volume alterations in hemorrhagic shock in rabbits. Shock, v. 28, n. 6, p. 733-740, 2007. https://doi.org/10.1097/shk.0b013e318054dfe3.

Gehrcke, M.I., Regalin, D., Padilha, V.S., Comassetto, F, da Silva, G., Moraes, A.N. de, & Oleskovicz, N. Arterial, mixed venous or central venous hemogasometry and end tidal CO2 in dogs under different hemodynamic states. Acta Scientiae Veterinariae, v. 45, p. 1-8, 2017. https://doi.org/10.22456/1679-9216.80007.

Helmerhorst, H.J., Schultz, M.J., van der Voort, P.H., de Jonge, E., & van Westerloo, D.J. Bench-to-bedside review: the effects of hyperoxia during critical illness. Critical Care, v. 19, p. 284, 2015. https://doi.org/10.1186/s13054-015-0996-4

Horr, M., Nunes, N., Biteli, E.G.F., Lopes, P.C.F., Gering, A.P., Moro, J.V., Rocha, F.D.L. Efeitos de frações inspiradas de oxigênio em coelhos anestesiados com isoflurano ou sevoflurano, mantidos em ventilação espontânea. Arquivos Brasileiros de Medicina Veterinária e Zootecnia, v. 71, n. 3, p. 944-952, 2019. https://doi.org/10.1590/1678-4162-10472

Júnior, G.S., Comassetto, F., Conterno, G.B., Victor de Souza, J. de Souza Ferreira, W., Griebeler, L.B., & Oleskovicz, N. The effect of intravenous magnesium sulphate infusion on total intravenous anesthesia with propofol in adult dogs: A randomized, blinded trial. Veterinary Anaesthesia and Analgesia, v. 51, n. 6, p. 603–612, 2024. https://doi.org/10.1016/j.vaa.2024.07.007

Júnior, G.S., Riva, A.B., Cipriani, D.S., Fernandes, K.M., Conterno, G.B., Rocha, V.A., Ronchi, S.J. de Souza, M.C., Comassetto, F., de Moraes, A.N. Evaluation of antinociceptive and sedative effects of methadone or morphine combined with ketamine-midazolam in rabbits undergoing elective orchiectomy. Archives of Veterinary Science, v. 29, n.4, 2025. https://doi.org/10.5380/avs.v29i4.97168

Matthay, MA., Zemans, RL., Zimmerman, GA., Arabi, YM., Beitler, JR., Mercat, A, Herridge, M, Randolph, AG, Calfee, CS. Acute respiratory distress syndrome. Nature

Reviews Disease Primers, v. 5, p. 18, 2019. https://doi.org/10.1038/s41572-019-0069-0

Queau, Y., Larsen, J.A., Kass, P.H., Glucksman, G.S., & Fascetti, AJ. Factors associated with adverse outcomes during parenteral nutrition administration in dogs and cats. Journal Of Veterinary Internal Medicine, v. 25, n. 3, p. 446–452, 2011. https://doi.org/10.1111/j.1939-1676.2011.0714.x

Uccello, O., Sanchez, A., Valverde, A., & Beaufrère, H. Cardiovascular effects of increasing dosages of norepinephrine in healthy isoflurane-anesthetized New Zealand White rabbits. Veterinary Anaesthesia and Analgesia, 2020. https://doi.org/10.1016/j.vaa.2020.07.001

Walesa, M., Bayat, S., Albu, G., Baudat, A., Petak, F., Habre, W. Comparison between neurally-assisted, controlled, and physiologically variable ventilation in healthy rabbits. Br J Anaesth. v. 121, n.4, p.918-927, 2018. https://doi.org/10.1016/j.bja.2018.01.020

Xu, Z., Gu, L., Bian, Q., Li, P., Wang, L., Zhang, J., Qian, Y. Oxygenation, inflammatory response and lung injury during one lung ventilation in rabbits using inspired oxygen fraction of 0.6 vs. 1.0. J Biomed Res. v.31, n.1, p.56-64, 2016. https://doi.org/10.7555/JBR.31.20160108