Equine respiratory anatomy and physiology have unique features that contribute to the challenges of general anesthesia in horses. The prominent caudodorsal equine lung fields lie dorsal to a dome-shaped diaphragm in the standing horse. The ventilation of the equine lung has a vertical distribution that is paralleled by a perfusion gradient, and there are regional differences in pulmonary vascular mechanics that are controlled by local factors and mechanisms, such as hypoxic pulmonary vasoconstriction, to optimize ventilation and perfusion (V/Q) matching. Anesthetic agents cause respiratory depression. Additionally, due to the weight of the abdominal viscera on the thoracic cavity and cranial displacement of the diaphragm by the abdominal organs, recumbency, particularly dorsal recumbency, can cause compression of the lung (compression atelectasis). Furthermore, the V/Q matching observed in the standing horse can become uncoupled, leading to V/Q inequality or mismatching in the horse under general anesthesia. An area that is ventilated but is not perfused is known as alveolar dead space (high V/Q ratio). Shunt refers to contexts where blood passes through the pulmonary vasculature without, or with limited opportunity, for gas exchange in areas that are ventilated but not perfused. Adaptive strategies for rest or for exercise are impaired when the horse is placed under general anesthesia.

OBJECTIVE
To investigate the effects of Flow-controlled Expiration (FLEX) ventilation expiration time and speed on respiratory and pulmonary mechanics in anesthetized horses in dorsal recumbency.

ANIMALS
6 healthy adult research horses.

METHODS
In this randomized crossover experimental study, horses were anesthetized 3 times and were ventilated each time for 60 minutes using conventional volume-controlled ventilation (VCV), linear emptying of the lung over 50% of the expiratory time (FLEX50), or linear emptying of the lung over 100% of the expiratory time (FLEX100) in a randomized order. The primary outcome variables were dynamic compliance (Cdyn), hysteresis, and alveolar dead space. The data was analyzed using two-factor ANOVA. Significance was set to $P < .05$.

RESULTS
Horses ventilated using FLEX50 and FLEX100 showed significantly higher $C_{dyne}$ and significantly lower hysteresis values compared to horses ventilated using VCV. Horses ventilated using FLEX50 had significantly lower alveolar dead space compared to horses ventilated using FLEX100 or VCV. Horses ventilated using FLEX100 had significantly lower alveolar dead space compared to VCV horses.

CLINICAL RELEVANCE
Our results demonstrate improved $C_{dyne}$, hysteresis, and alveolar dead space in horses ventilated with either FLEX50 or FLEX100 relative to traditional VCV. The use of FLEX with a faster exhalation speed (FLEX50) offers additional respiratory advantages.

Keywords: equine, ventilation, lung compliance, airway pressure, hysteresis

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that are perfused but are not well ventilated (low V/Q ratio). These factors can all lead to the impairment of gas exchange under general anesthesia, with the ultimate result of hypoxemia. Compromised oxygen delivery to tissues is an important source of equine anesthetic morbidity, particularly for critical cases, with a recent report documenting hypoxemia in 3.8% of elective cases and 23% of horses undergoing emergency exploratory laparotomy.8

Multiple strategies have been explored to combat hypoxemia during general anesthesia and to improve ventilation.2,12 Most of these techniques rely on manipulation of the inspiratory (stretching or inflation) phase of the respiratory cycle and require high inspiratory pressures to recruit collapsed alveoli.2,3 Manipulations of the expiratory (relaxation or deflation) phase of the respiratory cycle using positive end-expiratory pressure (PEEP) have shown promising results.10–14 However, the maintenance of positive airway pressures throughout the respiratory cycle can have cardiovascular sequelae and can actually worsen V/Q mismatching.2,3 Additionally, during the expiratory phase in conventional mechanical ventilation, airflow can be rapid and inhomogeneous, particularly in contexts of low pulmonary compliance or high body mass, which can lead to increased shear stresses, air trapping, and atelectasis.15–18

Flow-controlled EXpiration (FLEX) ventilation is a recent technique that modifies the expiratory phase by linearizing airflow throughout exhalation and homogenizing lung emptying (relaxation or deflation). This ventilatory strategy has shown promising results in people, pigs, and horses, and its implementation has documented improvements in arterial oxygenation, with limited cardiovascular compromise.12,17,19–24 It is unknown, however, how the application of FLEX ventilation alters respiratory and pulmonary mechanics, particularly when it is applied to the entire exhalation phase versus only a portion. Thus, the objective of this study was to investigate the effects of FLEX ventilation expiration time and speed on respiratory and pulmonary mechanics in healthy, anesthetized horses in dorsal recumbency. Parameters from linear emptying of the lung over 50% of the expiratory time (FLEX50 [faster exhalation speed]) or linear emptying of the lung over 100% of the expiratory time (FLEX100 [slower exhalation speed]) were compared with those from conventional volume-controlled ventilation (VCV), with a focus on hysteresis and compliance variables. We hypothesized that FLEX50 and FLEX100 would demonstrate increased compliance, lower hysteresis, and less alveolar dead space compared with VCV.

Methods

Animals

The study was approved by the IACUC of the University of Pennsylvania (protocol number 806775-aaecgbc). A statistical a priori power analysis (type II error, 0.2; type I error, 0.05) revealed that 6 animals would be required to detect clinically significant changes in PaO2 and Cdyn, assuming an SD of 15%.

Six healthy (based on preanesthetic physical examination) university-owned horses with a mean (± SD) bodyweight of 582 ± 38 kg and a mean age of 7 ± 6 years were used in this experimental cross-over prospective study. Horses were housed in stalls 12 to 24 hours prior to each anesthetic event and were offered hay and water ad libitum. Food, but not water, was withheld 8 hours before experimentation. Horses were anesthetized 3 times, with a wash-out period of at least 1 week between anesthetic events. During each anesthetic period, every animal was ventilated using FLEX50, FLEX100, and VCV for 1 hour per ventilation mode. The order of the ventilation modes per anesthetic event was randomized using a computer-generated randomization list (Supplementary Figure S1).

Anesthesia

Prior to anesthesia, the skin over the left or right jugular vein was clipped and aseptically prepared for catheter placement. After local infiltration of the skin with a 2% lidocaine solution, a 14-gauge catheter was placed into the prepared jugular vein.

All horses were premedicated with 0.8 mg/kg xylazine, IV, and they were induced with 0.05 mg/kg midazolam, IV, and 2.2 mg/kg ketamine, IV. The animals were orotracheally intubated with a Murphy cuffed endotracheal tube (internal diameter of 24 mm) and were then positioned in dorsal recumbency on a thick foam mat. Isoflurane in oxygen, with a target end-tidal isoflurane concentration of 1.2% and inspired concentration of O2 (FiO2) of > 0.9, was used for maintenance of general anesthesia. Isotonic crystalloid solution was administered at a delivery rate of 5 mL/kg/h, IV. Dobutamine was administered at an infusion rate of 0.5 µg/kg/min and was titrated to maintain a mean arterial blood pressure (MAP) above 60 mm Hg during anesthesia. An arterial catheter (20-gauge over the needle) was placed in either the left or right facial artery for invasive blood pressure monitoring and arterial blood sampling. The arterial catheter was attached to a calibrated pressure transducer via rigid extension lines prefilled with heparinized saline. The shoulder joint was used as the reference point for system zeroing to atmospheric pressure. In addition to invasive blood pressure, all horses had standard ECG, pulse oximetry, and body temperature monitoring. At the conclusion of anesthesia, 0.2 mg/kg xylazine, IV, was administered for sedation. The animals recovered with head- and tail- rope assistance. Following the resumption of spontaneous ventilation, the orotracheal tube was removed, and the horses were nasotracheally intubated to facilitate nasal O2 insufflation for the duration of the recovery period (15 L/min). The nasotracheal tube was removed after achievement of the stable standing position.

Ventilation strategy and experimental design

The Tafonius Large Animal Anesthesia Workstation (Hallowell EMC) was used for mechanical ventilation.
This system has an integrated piston-driven ventilator. The Tafonius software had been modified, as has been previously described, for FLEX ventilation. Prior to the experiment, the software of the ventilator was additionally modified to allow linear release of the delivered tidal volume over operator-directed portions of the expiratory phase. Following intubation, the endotracheal tube was connected to the anesthesia workstation, and all horses spontaneously breathed during the first 30 minutes of instrumentation. After the initial 30 minutes, continuous mandatory ventilation (mechanical ventilation) was initiated using an inspiratory-to-expiratory ratio of 1:2. A tidal volume of 14 mL/kg was delivered consistently throughout the experimental phases. The respiratory rate was adjusted to maintain an end-tidal CO₂ tension of 35 to 40 mm Hg. In a randomized order (Supplementary Figure S1), each horse was ventilated using conventional VCV as well as 2 different modes of FLEX ventilation (FLEX50 and FLEX100). Horses were mechanically ventilated using each ventilatory mode for 60 minutes.

**Respiratory and pulmonary mechanics**

Airway pressures and dynamic lung compliance (Cdyn) were recorded every 15 minutes (4 times during each 60-minute ventilatory mode). Airway pressures (peak, plateau, and mean pressures) and the Cdyn were measured using a Pitot-based flow meter that was connected between the orotracheal tube and the Wye piece of the anesthesia breathing system. The flowmeter was calibrated with a 7-L calibration syringe (Hallowell EMC) prior to each experiment. Spirometry measurements were performed using a Datex Ohmeda S/5 multiparameter monitor (GE HealthCare).

The spirometry-generated pressure-volume (PV) loops were video recorded during the experiment for subsequent analysis. At the above-described timepoints, 3 consecutive PV curves were analyzed using a previously described technique. For the 3 consecutive PV curves that were analyzed, the generated mean values were then used for further comparison and analysis. For hysteresis and maximal distance measurements, pictures of the video-recorded PV curves were uploaded and analyzed using ImageJ software. Arterial blood was sampled every 15 minutes (4 times during each 60-minute ventilatory mode) and was analyzed immediately using a blood gas analyzer (Opti CCA-TS2; Opti Medical Systems) to measure the PaO₂ and PCO₂. The FiO₂ at the time of sample collection was recorded to determine the PaO₂-FiO₂ ratio. Heart rate and MAP were also recorded at these same timepoints. The mean values of the measurements for each ventilatory mode were then used for subsequent analysis. All measurements and analyses were performed by individuals blinded to the treatment.

**Statistical analysis**

The data were analyzed using the statistical software packages SAS, version 9.3 (SAS Institute Inc) and GraphPad Prism, version 7 (GraphPad Software Inc). Visual evaluation of the q-q plots and the Shapiro-Wilk test were used to confirm normal distribution of the model residuals of the dependent variables. Variables were compared within the same group over time and between groups using the mean of the 4 measured timepoints using a two-factorial variance analysis for repeated measurements and Bonferroni correction for multiple comparisons. The level of significance was set to 5% (P < .05).
Results

All horses completed every phase of the study. There were no significant differences in heart rate or MAP between horses ventilated using FLEX50, FLEX100, or VCV (Table 1).

There were no differences in airway pressures, C<sub>dyn</sub>, hysteresis, or alveolar dead space within the same group over time when using the same ventilation mode. However, the FLEX50 and FLEX100 ventilation modes resulted in a lower peak airway pressure and plateau airway pressure and significantly higher mean airway pressure when compared to VCV (Table 1). Horses ventilated using FLEX50 and FLEX100 demonstrated significantly higher C<sub>dyn</sub> and significantly lower hysteresis values compared to horses ventilated using VCV (Table 1). The hysteresis ratio, the normalized maximal distance ratio, and the normalized maximal width ratio were significantly lower during FLEX50 and FLEX100 ventilation (Table 1). Horses ventilated using FLEX50 had significantly lower alveolar dead space compared to horses ventilated using FLEX100 or VCV. Horses ventilated using FLEX100 had significantly lower alveolar dead space compared to horses in the VCV group (Table 1). Horses ventilated with both FLEX50 and FLEX100 had higher PaO<sub>2</sub>/FiO<sub>2</sub> ratios compared with VCV.

Discussion

This study was designed to investigate alterations in FLEX ventilation exhalation speed on respiratory and pulmonary mechanics in healthy horses anesthetized in dorsal recumbency. The major findings of this study revealed increased C<sub>dyn</sub> and reduced hysteresis in horses ventilated with either FLEX50 or FLEX100 relative to traditional VCV, which supported our hypotheses. These enhancements in compliance and hysteresis with FLEX ventilation relative to conventional VCV reflect improvements in V/Q matching in healthy horses in dorsal recumbency, which were supported by significant reductions in alveolar dead space and increased PaO<sub>2</sub>/FiO<sub>2</sub> ratios. This data further builds upon previous preliminary investigations on FLEX ventilation in horses to specifically explore the effects of manipulation of expiratory airflow on mechanics.17,19,21

Recent strategies for combating the ventilatory challenges for equine patients under general anesthesia have used adjustments to tidal volume and positive pressures, with a focus on lung inflation and re-inflation.1,2 However, alveolar overdistension can cause lung injury, and the repeated recruitment and collapse of alveoli can also lead to lung damage (atelectotrauma).15 The use of PEEP is believed to reduce atelectotrauma,15,28 and it has been shown in multiple equine studies to improve V/Q mismatching and oxygenation.10–14,29 However, studies have shown deleterious effects of PEEP, including pulmonary damage and cardiovascular compromise, and the optimal PEEP setting is dependent on species, context, and concurrent pathophysiologic processes.16,30 It can be particularly challenging in situations with inhomogeneous lung pathology, such as the horse in dorsal recumbency or in a patient with acute respiratory distress syndrome (ARDS), to titrate ventilatory strategy and minimize further lung pathology.31 In these contexts, the use of spirometry to provide respiratory mechanical data can assist with targeted intervention strategies.

Spirometry provides information regarding pulmonary compliance. In our study, dynamic compliance was calculated using a Pitot-type spirometer that measured the change in volume per unit change in pressure.32 Compliance reflects the ability of the lungs to distend, and graphing pressure versus volume during the respiratory cycle will generate PV loops, the slope of which is the compliance.33 Under
normal physiological conditions, the compliance of the lung is mainly affected by its elastic properties, surfactant, and the lung-volume-to-tidal-volume ratio. Pathologic conditions such as pulmonary fibrosis, atelectasis, or severe ARDS can all negatively impact the compliance. In our study, horses ventilated using FLEX50 and FLEX100 demonstrated significantly higher $C_{dyn}$ relative to horses ventilated using VCV. Since all horses were considered healthy and free of pulmonary disease, it is unlikely that the observed difference in compliance between FLEX and VCV was due to alterations in elastic properties or surfactant. Instead, we propose the observed difference in $C_{dyn}$ to be due to reduced atelectasis with FLEX ventilation. A reduction in atelectasis has also been observed in other species. In pigs and in people, FLEX ventilation caused a more homogeneous distribution of ventilation and gas flow and improved alveolar recruitment. This proposed alveolar recruitment and reduction of atelectasis would explain the increase in lung compliance observed in our study because lower pressures were required to ventilate recruited lungs at a constant tidal volume. Therefore, we conclude that the improvement in $C_{dyn}$ indicated a reduction of atelectasis and more complete lung ventilation in these healthy horses in dorsal recumbency.

Spirometry and PV loops also provide information regarding hysteresis, which is the energy applied during the inspiratory phase that is not recovered during the expiratory phase in the imperfect elastic lung. Alternatively, it has been described as the additional energy needed during the inspiratory phase to inflate and recruit alveoli. Hysteresis occurs due to the collapse of smaller airways and from surface tension in the alveoli, with loss of energy into the respiratory system, and it is graphically the area between the inspiratory and expiratory curves of the PV loop. While compliance is a reflection of lung distensibility, hysteresis is an indicator of lung recruitability. While compliance and hysteresis are compared, hysteresis measures are used to standardize the hysteresis measurements and to account for possible differences in tidal volumes. These calculations are particularly useful when different populations and lung conditions are compared. However, as our study population included horses of similar phenotype, size, and age with no known lung pathology, the comparison of hysteresis between groups was more reliable. The results demonstrated significantly reduced hysteresis, hysteresis ratio, normalized maximal distance ratio, and normalized maximal width ratio for FLEX ventilation modes relative to VCV. Hysteresis and hysteresis ratio were reduced by approximately 50% in FLEX modes compared with VCV. The lower hysteresis in FLEX ventilation indicated reduced recruitment and, thus, increased mechanical efficiency compared with VCV. By dissipating less energy into the lung, the control of expiratory flow may decrease microatelectasis and tissue injury and affect the deflationary stresses that occur as the lower airways deform during exhalation.

Clinical studies of ARDS have shown that hysteresis, as well as the normalized maximal distance ratio, are acceptable predictors of lung aeration and improved pulmonary mechanics. In contexts of pulmonary fibrosis or emphysema, hysteresis can further give information about the viscoplastoelastic properties of the lungs, and it has been shown to be higher in affected lungs than in healthy lungs. Furthermore, previous studies have shown a reduction in hysteresis ratio and normalized maximal distance ratio in patients ventilated with PEEP as a result of reduced airway closure and reduced atelectasis formation. To the authors’ knowledge, this is the first study to investigate hysteresis behavior between ventilatory modes in horses under general anesthesia. Since techniques used in other species for the assessment of lung aeration, such as computed tomography, are limited in horses, our results demonstrate that hysteresis can be used in horses to demonstrate lung recruitment in ventilatory studies. The main principle of FLEX is the continuous and linear release of pressure during expiration. This leads to a more homogenous gas flow and, thus, more organized emptying of the lungs, with subsequent preservation of energy. The more homogenous expiration during FLEX ventilation may explain how FLEX stabilizes dependent lung areas and prevents these lung areas from collapse during expiration. In agreement with previous studies, the results of our study also demonstrated that the FLEX ventilation modes resulted in a lower peak airway pressure and plateau airway pressure and significantly higher mean airway pressure relative to VCV. It has been proposed that the maintenance of higher mean pressures during FLEX ventilation provides a mechanism for sustained aeration of the lung, with a subsequent increase in alveolar surface area. By reducing atelectasis and airway collapse, FLEX ventilation can lead to improvements in V/Q matching. This effect was also demonstrated by the observed increase in higher $P_{aO_2}/FiO_2$, which has been previously documented in pigs and in horses ventilated with FLEX.

Enhanced ventilatory efficiency with FLEX ventilation was also observed in the significant reduction in alveolar dead space with FLEX ventilation relative to VCV. The linearization of airflow during the expiratory phase has been shown to not only improve aeration of lung tissue to facilitate gas exchange but also redirect airflow to dependent lung areas. However, our documented improvement in alveolar dead space also suggests effects on the pulmonary vasculature. By linearizing airflow during expiration, FLEX may reduce alveolar overdistention and air trapping that can be observed with conventional VCV strategies using PEEP that leads to capillary compression. Additionally, by reducing alveolar collapse, local hypoxic pulmonary vasoconstrictive mechanisms may also be attenuated. Interestingly, we observed that horses ventilated using FLEX50 had significantly lower alveolar dead

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space compared to horses ventilated using FLEX100 or VCV. Horses ventilated using FLEX100 had significantly lower alveolar dead space compared to horses in the VCV group. These results suggest that by linearizing expiratory flow for only half of the expiration phase (FLEX50), which increases airflow speed, dead space is improved over the situation where airflow is extended over the entire expiration phase (FLEX100). Airflow during exhalation typically occurs in an exponential fashion, with an initial rapid period of airflow. The FLEX50 mode more closely resembles the normal expiratory pattern and may reduce air trapping, alveolar overdistention, and vascular compression relative to FLEX100. Additionally, it has been proposed that the recruiting effects of FLEX are time dependent on when lung volume falls below the closing airway capacity. By extending the expiratory airflow through linearization, this timepoint is delayed. It is unknown, however, when this critical timepoint occurs, and it may be variable depending on context and individual. Our data further support the proposed importance of the relaxation phase of the respiratory cycle for both gas exchange and perfusion and offers additional support for FLEX ventilation as an effective ventilatory strategy for horses in dorsal recumbency. However, future studies are required to further define the optimum expiration speed for FLEX ventilation. Our experimental study was limited by the standardized healthy animal group. Following the replacement, reduction, and refinement principles of animal research, the horses were ventilated using all modes in a random order on 3 occasions. Since the animals transitioned between ventilatory modes without a washout period under anesthesia, it is possible that there were residual effects of the previous ventilatory mode. However, alternating between ventilatory modes has been performed in prior FLEX studies, and the random order of modes was used to control for some of these influences. Three sequences (VCV-FLEX50-FLEX100, FLEX50-FLEX100-VCV, and FLEX100-VCV-FLEX50) were assigned randomly for each anesthetic event. The 3 assessed ventilation modes (VCV, FLEX50, and FLEX100) would have allowed 6 different sequences and 6 different groups. However, since each horse was only anesthetized 3 times, the authors decided to use the same 3 sequence orders for comparison. The results indicate only mild differences between FLEX50 and FLEX100 and significant differences relative to VCV. Thus, we postulate that the other 3 ventilation sequence orders would not have brought more insight. In addition, we did not measure expiratory flows, and future investigations into FLEX expiration speeds will include airflow measurements. FLEX permits the individual calculation of respiratory mechanics for inspiration and expiration separately. However, we also did not investigate these mechanical properties individually, and these measurements will be considered in future studies. Additionally, we did not measure cardiac output directly in these animals as pulmonary and respiratory mechanics were the focus of this study. Cardiac output has been previously measured in FLEX-ventilated horses, and it will be important to assess cardiac output again during future FLEX ventilation studies investigating the modality in critically ill and cardiovascually compromised horses. It is also unknown how respiratory and pulmonary mechanics are affected by using FLEX ventilation in horses with lung pathology. Future studies investigating this modality in pathologic conditions are required prior to the implementation of FLEX ventilation in clinical settings. Our results add to the body of evidence in support of FLEX ventilation as a strategy to improve ventilation in anesthetized horses in dorsal recumbency. The results demonstrate an improvement in mechanical efficiency that facilitates gas exchange using FLEX ventilation. V/Q matching is improved, and horses ventilated with FLEX had less alveolar dead space. The manipulation of the speed of the expiration phase using FLEX50 offered additional improvements in dead space. By linearizing airflow during the expiratory phase, FLEX improves oxygen delivery for horses under anesthesia, which is essential as hypoxemia is a common complication of equine anesthesia. Investigations into FLEX ventilation continue to demonstrate its efficacy as a promising, emerging technique.

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Disclosures

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Supplementary Materials
Supplementary materials are posted online at the journal website: avmajournals.avma.org.