The effect of different oxygen concentrations on oxidative stress in anesthetized horses

by

Mônica Midon

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 7, 2021

Keywords: Equine anesthesia, Isoprostanes, anesthetic recovery, oxidative stress

Copyright 2021 by Mônica Midon

Approved by

Stuart C. Clark-Price, Chair, Associate Professor, Clinical Sciences
Hui-Chu Lin, Professor, Clinical Sciences
Kara M. Lascola, Associate Professor, Clinical Sciences
Lindsey Boone, Associate Professor, Clinical Sciences

Abstract

Anesthesia-associated mortality in horses is high when compared with other species, and may be related to development of hypoxemia during recumbency. To overcome this, high fractions of inspired oxygen (FiO_2 >95%) are used. However, this may lead to an increase in oxidative stress, free radical formation and tissue injury. Isoprostanes are a marker of oxidative stress and their quantification is a relatively new and reliable approach to evaluate oxidative stress status. The aims of this research were to evaluate changes in urine isoprostanes (IsoP), serum cardiac troponin I (cTnI), arterial blood parameters, and serum chemistry in horses anesthetized with FiO_2 of 0.45, 0.75, or >0.95, and also evaluate the quality of recover utilizing accelerometry.

Twenty-four healthy adult horses were anesthetized with isoflurane for 120 minutes. Horses were randomly assigned to receive a FiO_2 of 0.45 (n=8), or 0.75 (n=8), or >0.95 (n=8) during anesthesia. Horses were anesthetized with a standard anesthesia induction and maintenance protocol and placed in dorsal recumbency. Horses were mechanically ventilated during throughout anesthesia. After 120 minutes of anesthesia, horses were allowed to recovery unassisted in a dedicated recovery stall wearing an accelerometer. Urine, venous, and arterial blood samples were collected at baseline prior to anesthesia, after 60 minutes of anesthesia, at the end of anesthesia, after recovery, and at 24 hours after induction. Urine IsoP and creatinine concentrations were measured. Myocardial injury was assessed utilizing cTnI. Oxygenation status was evaluated by arterial blood gas analysis. All parameters were evaluated by time and by group effect using 2-

way ANOVA, followed by Friedman test, and Kruskal-Wallis followed by Dunn's test. A $p < 0.05$ was used for significance.

During anesthesia, all physiologic parameters monitored remained within normal range without differences among groups. No difference was found over time or among groups for isoprostanes or cTnI. Measured serum chemistry variables, except for serum iron, returned to baseline values at T24h. Serum iron was lower at T24h compared with T0 in all groups (G95 T0 = 148.00 [103.00; 171.00] and T24h = 60.50 [39.00; 102.00] mcg/dL; G75 T0 = 153.00 [130.00; 198.00] and T24h = 53.50 [41.00; 96.00] mcg/dL; and G45 T0 = 149.50 [103.00; 213.00] and T24h = 63.50 [35.00; 102.00] mcg/dL). PaO_2 was different among groups during anesthesia, but the PaO_2/FiO_2 relationship was not different among groups. End tidal CO_2 was not different among groups, however, $PaCO_2$ was different during anesthesia at specific time points between G95 and G45 (G95 T60 = 59.30 [50.50; 67.50]; T120 = 55.65 [52.20; 63.30] mmHg; G45 T60 = 50.20 [43.10; 55.70]; T120 = 46.90 [41.90; 55.90] mmHg). There was no different in recovery score between groups.

The fractions of oxygen used during anesthesia in horses does not appear to have an impact on oxidative stress measured by urine isoprostane concentrations or myocardial injury measured by cTnI. Additionally, the fractions of oxygen tested did not have an effect on recovery scores of healthy horses anesthetized with isoflurane for two hours in dorsal recumbency.

Acknowledgments

To the horses Annie, Beau, Betty, Bill, Charlie, Chic, Cletus, Doc 3, Frenchie, Kansas, Kesha, Kobi, Madi, Midge, Milo, Nina, Ren, Rum, Spaz, Spook, Sully, Superstar, TC, Wesley, and Winston who made possible this project to happen,

To my advisor Dr. Stuart Clark-Price, for all the guidance, teaching, help, and support during this project and during the past three years,

To my committee Dr Hui-Chu Lin, Dr Kara Lascola, and Dr Lindsey Boone, without who this research would not be possible to be accomplished,

To Katie Ray, my “battle-buddy”, for all the help, talks and friendship,

To Jessica Brown, Dr Jennifer Taintor and all the equine technicians for all the care and help with the horses,

To my resident-mates Noelia, Hisashi, and Katharine, and to the anesthesia technicians Katie T, Hannah, Priscilla, Hayden, Courtney, Jamie, Johnie, Maddy, and Kirby, who had welcomed me, taught me how to live in America, and kept me alive,

To Dr Jacob Johnson and Dr Erik Hofmeister, for all the knowledge shared during my residency,
To the people from Clinical Pathology laboratory, Central supply, and Equine Research Laboratory, who always received me with a smile,

To my family and friends in Brazil and around the world, who prove that distance is relative, and to all the amazing people of Auburn who I had the blessing to know, work and laugh with during my residency,

To the College of Veterinary Medicine - Animal Health and Disease Research Program (CVM – AHDR) for the funding for this research,

Thank you!

Table of Contents

Abstract 2

Acknowledgments 4

List of Tables 8

List of Abbreviations 9

1. Introduction 14

2. Literature Review 16

2.1 Equine Anesthesia Mortality 16

2.2 Anesthesia Recovery 18

2.2.1 Methods of Evaluation 20

2.3 Oxidative Stress 23

2.3.1 Physiology and Pathophysiology 23

2.3.2 Methods of Detection 26

2.3.2.1 Isoprostanes 27

2.4 Anesthesia x Oxidative Stress 30

2.4.1 Fractions of Oxygen 31

2.4.2 Cardiac Troponin 34

3. Aims 37

4. Hypothesis........ 38

5. Material and Methods 39

5.1 Animals 39

5.2 Anesthetic Procedure 40

5.3 Experimental Design 42

5.4 Sample Collection and Analysis 42

5.5 Isoprostane Analysis 44

5.6 Anesthetic Recovery Evaluation 45

5.7 Statistical Analysis 45

6. Results........ 47

7. Discussion........ 54

8. Conclusion 60

9. References........ 61

List of Tables

Table 1. Median [min; max] of weigh, age, and temperament score for 24 horses (n = 8 in each group) 48

Table 2 Median [min; max] of physiologic parameters evaluated during 120 minutes of isoflurane anesthesia in 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%)........ 49

Table 3 Median [min; max] for total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK2), creatinine (Create), total calcium (Ca), magnesium (Mg^{++}), glucose (Glu), sodium (Na^+), potassium (K^+), chloride (Cl^-), anion gap (AG), and iron (S Iron) for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%)........ 49

Table 4 Median [min; max] for pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), base excess (BE), bicarbonate (HCO_3^-), lactate (Lact), cardiac troponin I (cTnI), and isoprostanes (Isop) for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%)........ 52

Table 5 Median [min; max] for time for first movement (1st mov), number of attempts to reach sternal recumbency (attempts to sternal), time to reach sternal recumbency, number of attempts to stand (attempts to stand), standing time, and recovery score for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%)........ 54

List of Abbreviations

%	per cent
˚C	Celsius degrees
∑UG	sum of the acceleration of any unsuccessful attempts to stand
AG	anion gap
ANOVA	analysis of variance
ASA	American society of anesthesiologists
AST	aspartate aminotransferase
ATP	adenosine-5-triphosphate
BE	base excess
bpm	beats per minute
Ca	total calcium
CEPEF	confidential enquiry into perioperative equine fatalities
CK2	creatine kinase
Cl^-	chloride
cm	centimeter
cmH_2O	centimeter of water
COX	cyclooxygenases
Create	creatinine
cTnI	cardiac troponin I

DAP	diastolic arterial pressures
DNA	Deoxyribonucleic acid
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
et al	*et alia*
$ETCO_2$	end-tidal carbon dioxide
ETISO	end-tidal isoflurane
FiO_2	fractions of inspired oxygen
G	gauge
g/dL	grams per deciliter
GC/MS	gas chromatography / mass spectrometry
Glu	glucose
h	hour
HCl	Hydrochloric acid
HCO_3^-	bicarbonate
HR	heart rate
HYPP	hyperkalemic period paralysis
Isop	isoprostanes
IV	intravenously
K^+	potassium
kg	kilogram
L	liter
L/min	liter per minute

L/min	liters per minute
Lact	lactate
LOX	lipoxygenases
m/z	mass-to-charge ratio
MAC	minimal alveolar concentration
MAP	mean arterial pressure
max	maximum
mcg/dL	microgram per deciliter
mcg/kg/min	microgram per kilogram per minute
MDA	malondialdehyde
mg/kg	milligram per kilogram
Mg^{++}	magnesium
min	minimum
min	minute
ml	milliliter
ml/kg	milliliter per kilogram
ml/kg/h	milliliter per kilogram per hour
mmHg	millimeters of mercury
mmol/L	millimole per liter
N	normality
n	sample size
Na^{+}	sodium
NADPH	nicotinamide adenine dinucleotide phosphate

ng	nanogram
ng/mg	nanogram per milligram
O_2	oxygen
O_2•-	superoxide anions
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
pH	potential of hydrogen
PIP	peak inspiratory pressure
PSSM	polysaccharide storage myopathy
RNS	reactive nitrogen species
ROS	Reactive oxygen species
RS	recovery score
S Iron	iron
SaO_2	arterial partial saturation of oxygen
SAP	systolic arterial pressure
SD	standard deviation
SG	acceleration of the successful attempt to stand
SpO_2	pulse oximetry
TV	tidal volume
U/L	units per liter
V/Q	ventilation/perfusion
V:V	volume to volume
VAS	visual analogic scale

Vd	volume of dead-space
Vmax	maximum velocities
Vt	tidal volume

1. Introduction

Horses have a high anesthetic morbidity and mortality rate compared to other domestic species. Although multifactorial, due to their unique anatomical and physiological make up, they have a greater potential to develop hypoxemia during general anesthesia, which may lead to adverse outcomes. Hypoxemia could compromise the oxygen delivery to various tissues and is thought to increase the risk for development of harmful anesthetic complications such as post anesthetic myopathy and poor recovery quality (Manohar et al. 1987). Recovery from general anesthesia is one of the most crucial phases of an anesthetic event in horses. Nearly 33% of anesthesia-associated death in horses occurs during the recovery period and can result from bone fractures while attempting to stand, myopathy, and cardiac arrest (Johnston et al. 2002). The impact of any anesthetic modification on recovery quality should be always considered. However, one substantial limitation is how to evaluate recovery quality, since most studies utilize subjective evaluation based on scoring systems.

Dogmatic practice and available apparatus for anesthetic delivery have resulted in the use of oxygen as the sole carrier gas for anesthesia in horses. While this approach often provides plenty of oxygen for tissue delivery, the use of high oxygen inspired fractions could increase cellular oxidative stress, leading to a peroxidation of membrane lipids, potentially inducing an impairment in cellular function and tissue damage. Studies

investigating the use of a lower FiO2 during equine anesthesia are contradictory about its benefit, necessitating further clarification (Marntell et al. 2005, Altemeier & Sinclair 2007, Crumley et al. 2013, Dang et al. 2017).

Oxidative stress is an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. The use of a high inspired oxygen fraction results in oxidative stress in many tissues, particularly in lung tissues and myocardium (Inoue et al. 2002; Altemeier et al. 2007). Measurement of oxidative stress has been a challenge for researchers as it was initially performed via the investigation of lipid peroxidation, the oxidative degradation of the lipid in cell membranes. Assays used for analysis have poor specificity and it have proved to be challenging to establish normal reference ranges. However, an alternate pathway for the investigation of oxidative stress surged with the discovery of the isoprostanes. Isoprostanes are a prostaglandin-like compound produced independently of cyclooxygenases (COX), by free radical-induced peroxidation of arachidonic acid, representing the most reliable approach to evaluate oxidative stress (Morrow et al. 1990).

With the development of this newer technology, research evaluating the effects of different fractions of oxygen during anesthesia in horses in oxidative stress becomes feasible and is necessary. Additionally, evaluation of the potential impact of different fractions of oxygen during anesthesia on recovery is essential before any conclusions can be made.

2. Literature Review

2.1 Equine Anesthesia Mortality

The mortality rate associated with anesthesia is higher in horses when compared with other species. The anesthetic mortality rate of healthy humans, dogs, cats, and rabbits is reported to be approximately 0.0004%, 0.05%, 0.11%, and 0.73%, respectively (Brodbelt et al. 2008; Matthews et al. 2017, Gottschalk et al. 2011). In horses, however, the most commonly accepted number is 0.9% for elective procedures, meaning approximately 1 of every 100 horses presents with a fatal complication related to anesthesia, and that number increases to 1.9% (1 of every 50 horses) for emergency procedures, such as colic surgeries (Klinger 2011).

The reported equine mortality rate can vary from as low as 0.12% or as high as 3.6% (Mee et al. 1998; Bidwell et al. 2007) depending on the study. Several factors influence this rate, including the number of the days post-anesthesia where death is considered anesthetic related, specific location profile since the complexity of surgery / patients can be different between hospitals, and the inclusion or exclusion of euthanasia.

One of the earliest studies about equine mortality, a single center retrospective study including 7 years and 473 anesthetic episodes, found a general mortality risk of 1.47% with mainly elective procedures (Mitchell 1969). In contrast, another retrospective study from a single hospital and including just abdominal surgeries due to colic (300 anesthetic

procedures) during 6 years, found a mortality rate of 50% (Pascoe et al. 1983). In 1995, the first confidential enquiry into perioperative equine fatalities (CEPEF) was published, being the first multicenter prospective study. Data from 62 clinics over 2 years and 1 month, with a total of 6255 anesthetic episodes, reported an anesthetic-related mortality rate of 1.6% in general, and 0.9% when colic and foal delivery procedures were excluded (Johnston et al. 1995). Seven years after, CEPEF 1 and 2 were published together, including prospective data from 6 years and 41824 anesthetic episodes, finding an overall death rate of 1.9% (Johnston et al. 2002). In more recent studies, the reported mortality rate ranged from 0.24% (Bidwell et al. 2007) to 1.1% (Dugdale et al. 2016). A fourth CEPEF is currently being conducted.

When the causes of mortality related to anesthesia in horses were investigated, the occurrence of fractures by unsuccessful attempts to stand during recovery correspond to the second leading cause of death, representing 12.5 – 38% of anesthetic-related deaths (Young & Taylor 1993, Mee et al. 1998, Johnston et al. 2002, Johnston et al. 2004, Bidwell et al. 2007). Post-anesthetic myopathies had a higher incidence in the past, being responsible for numbers up to 44% of cause of death (Young & Taylor 1993), however more recent studies demonstrate a decrease in this rate, reported to be between 7 to 14% (Johnston et al. 2002, Bidwell et al. 2007). Together, fractures and myopathies correspond to one third of the anesthesia related deaths in anesthetized horses (Johnston et al. 2002), emphasizing how important the recovery period is on the context of equine anesthesia.

2.2 Anesthesia Recovery

Several factors can influence the recovery of anesthesia in equine patients. In general, horses have a flight instinct when in unfamiliar situations, and this likely contributes to the challenge of recovery (Hubbell, 2007). Factors such as age, breed, weight, temperament, type of drugs used for anesthesia, anesthesia duration, recovery room configuration, comfort level during recovery, manually assisted or unassisted recovery, hypotension or hemorrhage in the intraoperative period, and experience of staff are among some of the influences on quality of anesthetic recovery (Matthews et al. 1992, Hubbell & Muir 2009).

In an ideal recovery, a horse will regain consciousness in 10 to 20 minutes after the discontinuation of the inhalant anesthetic, have coordinated movements of head and limbs to reach sternal recumbency at approximately 30 minutes, remain in sternal for a few moments, and then, with coordinated and systematic movements, will stand in one attempt (Hubbell 2007, Whitehair et al 1993, Donaldson et al. 2000). Anesthetic recovery in horses can be divided in six phases: transition from anesthesia to recovery, first movement, movement to sternal recumbency, first attempt to stand, initial phase of standing, and complete recovery (Clark-Price 2013).

Horses that try to stand too early during recovery have a higher risk of injuries, since residual effects of the anesthetic drugs are still present, causing incoordination (Bidwell et al. 2007). Attempts to accelerate the standing process should be avoided, since this can lead to a long period of balance instability in a standing horse (Hubbell 2007). On the other hand, an overly prolonged recumbency period may promote recumbency related

injury (anesthetic myopathy) and failure to stand can be indicative of other conditions such as electrolyte disturbance or other disease conditions such as hyperkalemic period paralysis (HYPP) or polysaccharide storage myopathy (PSSM), or positional myopathy or neuropathy.

An inappropriate environment can also interfere with recovery quality. An adequately padded and not-slippery floor, in a quiet environment usually leads to smoother recoveries (Hubbel 2007). Ambient lighting does not appear to interfere with recovery as differences between horses that recovered in a dark or lighted recovery stall have not been observed (Clark-Price et al. 2008).

In a recent study, the use of re-sedation at the beginning of recovery was associated with better recoveries, while horses with a higher body mass, ASA classification greater than 2, longer anesthetic times, and surgeries performed at night tended to have worse recoveries (Dugdale et al. 2016). Factors related to the surgical procedure, such as postoperative pain, should be considered separately from the anesthesia intrinsic factors, but not dismissed when recovery strategies are being planned (Matthews et al. 1992). Horse temperament is an important factor that influences recovery, with anxious and/or aggressive horses tending to have worse recoveries. Temperament assessment before sedation should be performed in order to increase the success of anesthesia (Donaldson et al. 2000, Leece et al 2008, Valverde et al. 2013). Other relevant factors associated with anesthesia protocol that probably contribute to the quality of recovery are quality of preanesthetic sedation, induction period, and stability of anesthetic maintenance (Donaldson et al. 2000, Leece et al. 2008).

Considering the transition from unconsciousness to awake that happens on recovery, an increase in metabolic rate is expected to occur. Anesthetic drugs lead to respiratory depression in a dose-dependent way, acting on the respiratory centers and reducing minute ventilation. Together, with an increase in ventilation/perfusion (V/Q) mismatching commonly observed in horses under general anesthesia, an imbalance between oxygen demand and delivery can occur. In an attempt to counter this, supplementation of 10 L/min of oxygen via nasal insufflation during recovery was evaluated. However, there was no differences in PaO_2 were observed (Mason et al. 1987). Healthy horses have the ability of alveoli auto-recruitment during recovery, when with the change in respiratory rhythm – increase of the inspiratory pause – collapsed alveoli from the dorsal dependent lung lobes are re-expanded, suggesting a capability of auto pulmonary redistribution and homeostasis return (Mosen et al. 2016).

As described, the recovery period is a crucial phase of an anesthetic procedure in horses, and many factors contribute to the outcome. Clearly, anesthetic management of horses is not exclusively related to drug protocols, but also includes ventilatory strategies, that can potential improve or worsen recovery quality. As such, research related to anesthesia of horses should include recovery evaluation.

2.2.1 Methods of Evaluation

Considering anesthetic recovery is a multifactorial event, the nearly infinite interactions among these factors make the evaluation of quality of recovery particularly challenging. Several scales have been reported to fulfill this purpose. These include a simple visual analogic scale (VAS), simple descriptive scales, and more complex composite scales

(Whitehair et al. 1993, Matthews et al. 1998, Donaldson et al. 2000, Bettschart-Wolfensberger et al. 2001, Santos et al. 2003, Mama et al. 2005, Valverde et al. 2005, Clark-Price et al. 2008, Leece et al. 2008). Besides quality, time of first voluntary movement, time to sternal recumbency, and time to stand, together with number of attempts to reach sternal recumbency, and number of attempts to stand are frequently considered when evaluating anesthetic recovery (Wagner et al. 2008).

The VAS was first used in 1921 by two employees of the Scott Paper Company as a method for supervisors to rate their workers (Hayes & Patterson 1921). Two years later, guidelines for the construction and use of this scale were published, highlighting the use of a line no longer of 5 inches (127 millimeters), with no breaks or divisions, and the use of anchor words to represent the extremes (for example, 0 represents the worse recovery, and 10 the best recovery). It was also suggested that the scales should occasionally vary in the direction of the extremes to prevent a tendency of marking one margin of the page (Freyd 1923).

The simple qualitative scale usually ranges from 1 to 5, with basic descriptions, for example 1 corresponds to excellent, 2 corresponds to good, 3 corresponds to fair, 4 corresponds to poor, and finally 5 corresponds to unacceptable. This type of scale is very friendly and easy to use even for people without experience. However, the standpoints are completely subjective, allowing for tremendous variability in the results. Because this scale is simplistic to use, it is still widely used in clinical and research settings (Young & Taylor 1993, Wagner et al. 2002).

The first composite objective scale to evaluate equine anesthetic recovery was published by Donaldson and co-workers and consists of ten categories where different

characteristics are described and evaluated individually (Donaldson et al. 2000). The categories are recognizable components of the recovery process and are attitude, activity in recumbency, transition to sternal, number of attempts to sternal, sternal phase, transition to stand, number of attempts to stand, strength, balance and coordination, and knuckling of the fetlocks, each one with specific descriptors. Since then, some modifications of this scale, for example refining the number of attempts to stand and adding an additional category, occasionally appear in literature aiming to adapt the scale for the specific clinical condition or project (Clark-Price et al. 2008).

A comparison of four different systems to evaluate equine recovery was performed by Vettorato and co-workers, where the Donaldson scale, the Young and Taylor method, the Edinburgh system, and VAS all showed reliability of purpose, with the Donaldson scale having better categorization of the stages (Vettorato et al. 2010). However, even in Donaldson scale, words such as “good” or “excellent” are still present, providing an inherent level of subjectivity, which can potentially lead to different conclusions.

To avoid this bias, an objective evaluation system utilizing accelerometry was recently described. This system uses 3-axis accelerometry to provide a single recovery score based on the change in acceleration of horses while attempting to stand (Clark-Price et al. 2017). This system holds great promise to allow for objective recovery evaluation and the ability to compare results between studies as well as compare results from different institutions.

2.3 Oxidative Stress

Oxidative stress is an imbalance between the systemic production of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. In fact, oxidative stress can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA (Chandra et al. 2015, Zhang 2018). Oxidative stress associated with the delivery of anesthesia can result in cardiovascular, neurological, and renal tissue injury, cognitive dysfunction, immune suppression, and delayed wound healing (Cruzat et al. 2014, Hill et al. 2018, Meephansan et al. 2017, Zhang et al. 2018[a], Zhang et al. 2018[b]). In dogs and pigs, exposure to inhalant anesthetics can induce oxidative stress (Allaouchiche et al. 2001, Lee 2013). In horses, oxidative stress can result after anesthesia for routine procedures such as castration (Tsuzuki et al. 2016). Consequently, evaluation of oxidative stress in horses undergoing anesthesia is necessary, as it will allow for the development of anesthetic techniques that may reduce the risks of morbidity and mortality to the individual horse as well as reduce the cost of medical care that can affect the equine industry.

2.3.1 Physiology and Pathophysiology

Oxygen is a requirement for many biological systems, being essential for energy generation, but oxygen also has potentially damaging side-effects through direct reaction with proteins, lipids, and even DNA. Through oxidative phosphorylation, oxygen participates in the high-energy electron transfer reactions within the mitochondria, resulting in adenosine-5-triphosphate (ATP) production. However, this process is not

completely efficient, with some electrons escaping and binding to molecular oxygen, creating superoxide anions (O_2•-) (Burton & Jauniaux 2011). Superoxide anions are the most common oxygen free radical, and the mitochondria is the principal source. Superoxides can also be generated in other locations including the endoplasmic reticulum (responsible for 25% of superoxide anions production), cytochrome P450 and other oxido-reductases enzymes, reactions of disulfide bonds during protein folding , and reactions of nicotinamide adenine dinucleotide phosphate (NADPH) enzyme (Droge 2002, Tu & Weissman 2004, Raijmakers et al. 2006).

Reactive oxygen species (ROS) refer to both free radicals and non-radical intermediates. Free radicals are species containing one or more unpaired electrons, that confer high reactivity. ROS were first recognized as a potentially harmful by-product of aerobic metabolism, however, they are also is believed to have an important role as secondary messengers in many intracellular signaling pathways (Burton & Jauniaux 2011). Free radicals can potentially be generated from many different elements, but the two most important in animals are those involving oxygen and nitrogen (Burton & Jauniaux 2011). Superoxides are detoxified by superoxide dismutase enzymes resulting in hydrogen peroxide (also a ROS). Hydrogen peroxide is non-polar, can diffuse through membranes, and has an important function as second messenger in signal transduction pathways, being detoxified by the enzymes catalase and glutathione peroxidase to water. An imbalance between superoxide anions and hydrogen peroxide leads to the formation of a hydroxyl anion, which is a much more reactive and dangerous molecule (Droge 2002). Organisms are under constant oxidative attack, and as a result, a complex system of antioxidant defenses has evolved, including enzymatic and non-enzymatic processes. In

enzymatic processes that utilize superoxide dismutase and glutathione peroxidase as mentioned above, a transition metal (i.e. zinc or manganese) is always involved, while in non-enzymatic processes, ascorbate (vitamin C) and α-tocopherol (vitamin E) are involved. Polymorphisms in the antioxidant enzymes or dietary restrictions of micronutrients can predispose to an imbalance in this system leading to oxidative stress (Burton & Jauniaux 2011).

Under normal conditions, 2% of oxygen consumed is converted to O_2•- in the mitochondria, and since it is membrane impermeable, it remains in the mitochondrial matrix. The rate of O_2•- formation is influenced by the number of electrons present in the chain, being increased during hyperoxia and hyperglycemia, but is paradoxically also increased during hypoxia since the reduced availability of oxygen to act as final acceptor induces an accumulation of electrons. Under pathologic conditions the enzyme xanthine dehydrogenase plays an important role on ROS generation, especially during ischemia-reperfusion injuries. In hypoxic conditions, xanthine dehydrogenase is cleaved to the oxidase form, donating electrons to molecular oxygen (Burton & Jauniaux 2011).

Oxidative stress by definition is an imbalance between the oxygen reactions and the natural defenses, leading to an alteration in the pro-oxidant-antioxidant balance trending towards pro-oxidant production and, favoring tissue damage (Sies 1985).

Various ROS and reactive nitrogen species (RNS) have proteins, carbohydrates, nucleic acids, and lipids as targets, leading to oxidation. Lipid peroxidation induces disturbances of the structure and function of membranes, changing the integrity, fluidity, permeability, and functionality (Niki 2009). Direct and secondary products of lipid peroxidation have been demonstrated to have carcinogenic and mutagenic properties, modifying essential

molecules such as proteins and DNA (West & Marnett 2006). Therefore, it is not surprising that lipid oxidation has been associated with the pathophysiology of several diseases, such as cardiovascular, neurological, pulmonary disorders, cancer, and aging (Basu 2008, Morrow & Roberts 2002, Burton & Jauniaux 2011).

Enzymatic lipid peroxidation is an important type of oxidation, including the reaction of lipoxygenases (LOXs) and cyclooxygenases (COXs) with arachidonic acid, which creates hydroperoxyeicosatetraenoic acid, prostaglandins, prostacyclin, thromboxane, and leukotrienes. COX and LOX reactions are region, stereo, and enantiomer-specific (Schneider et al. 2007). Isoprostanes are a product of lipid peroxidation formed by the peroxidation of arachidonic acid, independently of cyclooxygenase (Morrow et al 1990).

2.3.2 Methods of Detection

Considering the key role oxidative stress plays in many pathologies, a method to measure it is essential. Direct measurement of ROS would be ideal, but they are labile compounds, and thus direct detection and quantification is difficult. In fact, the only method currently available is electron paramagnetic resonance. The production of oxidant species by stimulated phagocytes can be measured by chemiluminescence, however this is an ex-vivo technique, does not allow the identification of ROS produced, and can include incidental oxidation. Alternatively, measuring the antioxidant capacity is possible, where the consumption or changes in activity or expression of antioxidant enzymes are measured. The simplest way is quantifying some antioxidant molecule in plasma. Unfortunately, the relationship between circulating levels of antioxidants enzymes and the magnitude of oxidative stress has not been well established. In addition, measuring

the antioxidant capacity does not provide information about ROS production (Preiser 2012).

Indirect measurement using biomarkers of oxidative stress is an alternative to in vivo assessment. A biomarker can be defined as a specific substance that can be measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention (FDA-NIH *BEST resource*, 2016). Due to the large number of interactions of ROS with biological molecules, many different biomarkers have been identified, the most common being nitrated proteins, lipid peroxides, conjugated dienes, chlorinated lipids, oxidized glutathione, and malondialdehyde (MDA). These derivatives of ROS are considerably more stable and have a longer half-life than ROS per se, making them possible to be quantified and monitored repeatedly. However, they also have several limitations, primarily that they are nonspecific to peroxidation, have low sensitivity for detection in normal subjects leading to a lack of reference range, are subject to external factors that influence the concentrations, and require techniques that are too invasive to be used in clinical scenarios (Czerska et al. 2015).

2.3.2.1 Isoprostanes

A relatively new biomarker for oxidative stress was introduced in 1990, the isoprostanes (IsoP), with promising characteristics. The first report on the potential of isoprostanes existence was in 1975, when methyl linolenate was autoxidized, originating from a bicyclic endoperoxide precursor of prostaglandin-like compounds (Pryor & Stanley 1975). Thirteen years later, it was demonstrated that some side chains of prostaglandins could form isomers under certain conditions (Wendelborn et al. 1988). In 1990 these

isomer compounds were characterized by mass spectrometry and at the same time it was discovered that concentrations of these molecules in frozen plasma samples increase after storage. The addition of antioxidants to these samples suggested an enzyme independent route of formation. Since these compounds originated from prostaglandin F2, they were named F2-isoprostanes (Morrow et al 1990). Shortly after, the same group was able to determine the formation of isoprostanes in vivo (Morrow et al. 1994).

F2-isoprostanes are derived from arachidonic acid, but are not the only type. Omega-3 fatty acids eicosapentaenoic and docosahexaenoic acids can generate F3- and F4-isoprostanes, and even plants can produce isoprostanes from plant-based omega-3 fatty acid α-linolenic acid, generating phyto-isoprostanes (Nourooz-Zadeh et al. 1997, Nourooz-Zadeh et al. 1998, Parchmann & Mueller 1998). Since the different types of isoprostanes are beyond the scope of this work, the word “isoprostanes” and the abbreviation “IsoP” will refer to F2-isoprostanes exclusively.

Isoprostanes are prostaglandin isomers generated from peroxidation of arachidonic acid by free radicals and ROS, in a non-enzymatic pathway and have unique characteristics that make them an excellent biomarker for oxidative stress (Czerska et al. 2015). They are stable compounds, specific products of ROS peroxidation, are found in detectable quantities in biological fluids, and are unaffected by diet (Roberts & Morrow 2000). There are four subfamilies of isoprostanes (series 5, 8, 12, and 15), each one with 16 diastereoisomers, resulting in a total of 64 F2-isoprostane isomers potentially being formed during peroxidation (Nikki & Yoshida 2005, Milne et al 2008).

Isoprostanes are present in urine, plasma, cerebrospinal fluid, and exhaled breath condensate. Plasma samples should preferentially be analyzed immediately after

collection, since autoperoxidation can continue falsely elevating isoprostane concentrations (Barden et al 2014). Urine samples are usually preferred because collection is non-invasive and isoprostanes concentration is very stable (Putman et al. 2021, Cracowski et al. 2002).

Detection of a single specific isoprostane isomer is challenging because of a high structural similarity between stereoisomers and their metabolites present in samples. Currently, techniques used to measure isoprostanes in urine include gas-chromatography with mass spectrometry detection and enzyme-linked immunosorbent assay (ELISA) (Il'yasova et al. 2012). The initial discovery of isoprostanes was performed using mass spectrometry analysis, and while expensive and time consuming, this method is extremely specific and sensitive. An ELISA has been developed and is a faster and less expensive method. However, because of interference of other biological substances, false results are commonplace and therefore this method is not considerate appropriated. In fact, when the ELISA is compared with mass spectrometry for isoprostanes analysis, obtained results were highly inconsistent in humans, dogs, cattle and horses (Proudfoot et al. 1999, Soffler et al. 2010, Smith et al. 2011).

In horses, very few studies have been performed investigating the use of isoprostanes as an indicator of oxidative stress. In humans, acute lung injury, pulmonary arterial hypertension, chronic obstructive pulmonary disease, interstitial lung disease, and cystic fibrosis are correlated with an increase in isoprostanes concentrations (Janssen 2008). Similarly, in horses with chronic airway inflammation, elevated concentrations of isoprostanes were observed after exercise and were dependent on severity of airway disease (Kirschvink et al. 2002[a]). Additionally, oxidant status was related to pulmonary function parameters

including arterial partial pressure of oxygen (PaO_2) and mechanics of breathing (Kirschvink et al. 2002[b]). In horses, a positive correlation exists between plasma and pulmonary isoprostane concentration and oxidative stress induced by strenuous exercise, chronic obstructive airway disease or both (Kirschvink et al. 1999). A single study investigating isoprostanes as a potential predictor for surgical intervention in horses with colic revealed a potential therapeutic tool for early recognition of the need for surgical correction, and as a measurable diagnostic tool for the prevention of further systemic and gastrointestinal tissue injury in those patients (Noschka et al. 2011).

2.4 Anesthesia x Oxidative Stress

The anesthetic procedure by itself represents a physiologic challenge for all the body systems since it induces an artificial sleeping state. The four traditional pillars of anesthesia include unconsciousness, immobility, muscle relaxation, and analgesia, are ideally provided while attempt to maintain homeostasis of the patient, often in the face of other systemic compromises. Therefore, it is not surprising that oxidative stress can occur with anesthesia. In humans, oxidative stress associated with the delivery of anesthesia can result in cardiovascular, neurological, and renal tissue injury, cognitive dysfunction, immune suppression, and delayed wound healing (Cruzat et al. 2014, Hill et al. 2018, Meephansan et al. 2017, Zhang et al. 2018[a], Zhang et al. 2018[b]).

Similar evidence has been found in animals. In pigs anesthetized with propofol, sevoflurane or desflurane, an increase in systemic and pulmonary oxidative stress biomarkers were found with desflurane anesthesia (Allaouchiche et al. 2001). Propofol in other hand has demonstrated antioxidant properties, as evidenced in dogs that showed

higher levels of oxidative stress when anesthetized with thiopental when compared with propofol (Lee 2012). In contrast, propofol had no differences on the reduction of antioxidant biomarkers in dogs with early stages of cardiac diseases when compared with sevoflurane, but beagles that received propofol had increased activity of antioxidative stress enzymes when compared with isoflurane (Lee & Kim 2012, Tomsic et al. 2018). Isoflurane itself appears to have a pro-oxidative stress effect, as demonstrated in dogs anesthetized with isoflurane, where biomarkers for oxidative stress increased in a time and dose dependent manner (Lee 2013).

The invasiveness of a surgical procedure also affects oxidative stress production, as evidenced in dogs submitted to ovariectomy, where laparoscopic surgeries induced less oxidative stress when compared to traditional laparotomies (Lee & Kim 2014). In horses, antioxidant activity was reduced after castration with isoflurane anesthesia (Tsuzuki et al. 2016). Similarly, antioxidant biomarkers were reduced after arthroscopy in horses, however oxidative stress biomarkers were also reduced when sevoflurane or propofol were used to maintain anesthesia (Kambayashi et al. 2018).

2.4.1 Fractions of Oxygen

Anesthesia poses unique challenges to the veterinary patient due to species factors not found in human patients. Horses can be exceptionally challenging to anesthetize due to anatomical factors. Horses placed in dorsal recumbency for long durations is not a natural body positions for horses, and potentially leads to deleterious cardiopulmonary changes during anesthesia (Hubbell 1999). For example, anesthetized horses in dorsal recumbency tend to develop hypoxemia, ($PaO_2 < 60$ mmHg), necessitating the use of supplemental

oxygen (Gleed 1988). Due to body weight and anatomical conformation, recumbency in horses results in an increase in mismatching between alveolar ventilation (V) and perfusion (Q) in the lung. Some individual horses have high V/Q mismatching even when standing (Hedenstierna et al. 1987).

During general anesthesia, horses develop large areas of shunt, and shunt formation is usually greater in dorsal recumbency compared to lateral (Nyman & Hendenstierna 1989). The resulting hypoxemia compromises oxygen delivery to various tissues and is thought to increase the risk for development of harmful anesthetic complications. As such, it is recommended that horses should not remain under general anesthesia for more than 1 hour without oxygen supplementation (Hubbell 1999). Additionally, the use of controlled positive-pressure ventilation during equine anesthesia improves PaO_2 and allows for maintenance of more normal ranges of arterial carbon dioxide ($PaCO_2$) (Steffey et al. 1977).

In horses, a maximal fraction of inspired oxygen (FiO_2) of > 95% is commonly used during anesthesia to maintain an acceptable PaO_2. The use of a maximal FiO_2 is commonplace in equine anesthesia for two main reasons: minimizing hypoxemia and that most anesthesia delivery machines designed for large animal are equipped with an ability to deliver only oxygen as a carrier gas for anesthetic. This results in supraphysiologic concentration of oxygen and a hyperoxic PaO_2 in anesthetized horses.

The use of high inspired oxygen fraction however results in oxidative stress in many tissues. For example, exposure to high fractions of oxygen during delivery of anesthesia can induce lung tissue damage, thought to be caused by excessive production of intermediate reactive oxygen species (Altemeier & Sinclair 2007).

In pulmonary tissue, hyperoxia (FiO_2 > 95%) markedly inhibits mRNA and protein expression in alveolar epithelia cells and increases the rate of apoptosis (Dang et al. 2017). The myocardium also appears to be sensitive to hyperoxic injury. In humans recovering from cardiac bypass, those receiving supraphysiologic oxygen during reperfusion after bypass surgery had higher levels of circulating troponin indicating myocardial injury and had worse hemodynamic parameters and myocardial performance than patients receiving a more physiologic concentration of oxygen (Inoue et al. 2002).

Providing physiologic concentrations of FiO_2 during anesthesia appears to reduce oxidative stress. Recently a new apparatus (Tafonius large animal anesthesia machine, Hallowell EMC) has become available for equine anesthesia, which allows for the ability to mix medical air and oxygen to achieve a variable FiO_2. Mechanical ventilation is associated with formation of atelectatic areas of lung due to loss of airway nitrogen that acts to keep alveoli open, in addition to a reduction of venous return and a subsequent reduction of cardiac output (Lumb 2005).Diverse techniques have been described for use in anesthetized horses, including the use of various FiO_2, to minimize atelectasis formation.

Currently, reported results are contradictory and no consensus on the ideal FiO_2 to use in anesthetized horses exists. For example, during mechanical ventilation in dorsally recumbent horses, the use of a FiO_2 of 50% compared to >95% did not result in changes in alveolar dead space or pulmonary shunt fraction (Hubbell et al. 2011). In spontaneous breathing horses receiving either 50% or >95% FiO_2, horses in the 50% group had a lower blood oxygen content, lower SaO_2 and PaO_2 values, and demonstrated no improvement in respiratory rhythm (Crumley et al. 2013). On the other hand, the use of FiO_2 >95% in horses lead to an increase in clinically relevant pulmonary shunting, which remained high even

after FiO_2 was reduced to 21%, when compared with horses that were administered only a FiO_2 of 21% (Marntell et al. 2005). Similarly, a FiO_2 >95% has been associate with a greater degree of atelectasis in horses, dogs and cats when compared with a FiO_2 of 50% (Staffieri et al. 2007, Staffieri et al. 2009, Staffieri et al. 2010).

Few studies have been performed examining the effect of FiO_2 on oxidative stress in horses. In one study, horses anesthetized with a FiO_2 of 21% had a greater resistance to free radical induced red cell lysis. However, compared with horses receiving a maximal FiO_2 of >95%, horses receiving 21% O_2 had poor muscle oxygenation (Portier et al. 2009). Clearly more research is necessary to determine an optimal FiO_2 in horses that maintains appropriate tissue oxygen delivery while minimizing oxidative stress and tissue injury.

2.4.2 Cardiac Troponin I

Considering that horses appear to be more prone to hypoxemia during anesthesia than other domestic veterinary species, the use of lower fractions of oxygen could lead to potential tissue damage. The heart is of great concern as it is one of the first organs to suffer when aerobic metabolism is not possible due to lack of oxygen. Troponins are globular proteins found in striated muscle that regulate the calcium-dependent myosin-actin interaction that allows for muscle contraction (Rossi et al. 2014). There are three types of troponin, I, T, and C. The potential use for troponin-I as a cardiac biomarker was first recognized in 1987. There is a great amount of structural similarity among mammals (DNA homology for cardiac troponin-I between humans and horses is greater than 90%), thus there is potential for human assays to be useful in other species

(Cummins et al. 1987, Cummins & Cummins 1987, Rishniw & Simpson 2005, Rossi et al. 2014).

Cardiac troponin I (cTnI) is not expressed in either healthy or disease skeletal muscle, unlike cardiac troponin T and C (Schreier et al. 1990, Bodor et al. 1995, Aggarwal et al. 2009). In horses, plasma cTnI concentration measurement is used as a diagnostic and monitoring tool in horses with suspected or known cardiac injury. In fact, a portable point-of-care diagnostic tool has been evaluated and validated for use in horses and this has greatly increased the utility of this test (Kraus et al. 2010). Horses with various cardiac diseases such as piroplasmosis, ruptured aortic jet lesions, structural cardiac disease and neoplasia, as well as after cardioversion show increased blood concentrations of cTnI (Diana et al. 2007, Cornelisse et al. 2000, McGurrin et al. 2008, Fennell et al. 2009, Nath et al. 2012[a]).

Cardiac troponin I is also elevated in horses with other diseases and not just primary cardiomyopathies. Significant increases in cTnI, acute-phase proteins, and oxidative stress biomarkers were founded in mares with pyometra (El-Bahr & El-Deeb 2016). In horses with colic, a positive correlation was found between increased cTnI concentrations and the incidence of ventricular arrhythmias. Additionally, there was a correlation between high cTnI concentrations and severity of illness (strangulating or inflammatory lesions), leading to a less favorable prognosis (Nath et al. 2012[b], Seco-Diaz et al. 2014). In fact, cTnI concentrations are higher in non-survivors in horses admitted for emergency abdominal surgery (Radcliffe et al. 2012).

In horses undergoing elective procedures, general anesthesia did not result in cTnI elevations in the first 24 hour after surgery (Slack et al. 2011). In dogs anesthetized with

the combination of medetomidine and propofol, with or without sevoflurane or oxygen supplementation, an increase in cTnI was observed on the group that did not receive oxygen supplementation, suggesting myocardial hypoxic injury. (Vasiljevic et al. 2018). Myocardial injury is not limited to hypoxic states. Both hypoxia and hyperoxia have been associated with changes in proteins and other biochemical markers that are consistent with cardiac injury (Gyongyosi et al. 2018).

3. Aims

- To evaluate change in urine isoprostanes as a marker of oxidative stress in healthy, adult horses anesthetized with an FiO_2 of 0.45, 0.75, and >0.95.
- To evaluate changes in cTnI in healthy, adult horses anesthetized with an FiO_2 of 0.45, 0.75, and >0.95.
- To evaluate the effect of anesthesia delivered in a FiO_2 of 0.45, 0.75, and >0.95 on accelerometry scores during recovery in healthy, adult horses.
- To evaluate change in urine isoprostanes as a marker of oxidative stress during recovery from anesthesia in healthy, adult horses.

4. Hypothesis

- Horses anesthetized with isoflurane delivered in a FiO_2 of 0.45 or >0.95 will have increased concentrations of urine isoprostanes and serum cTnI compared to horses anesthetized with isoflurane delivered in a FiO_2 of 0.75.
- Horses anesthetized with isoflurane delivered in a FiO_2 of 0.45 or >0.95 will have a higher accelerometry recovery score indicating a lower quality recover compared to horses anesthetized with isoflurane delivered in a FiO_2 of 0.75.

5. Material and Methods

This research was approved by the Institutional Animal Care and Use Committee under protocol number 2019-3503. A sample size calculation was performed to detect a difference in urine isoprostanes concentration of 1.5 ng/mg creatinine, utilizing a sigma of 1.39 (Noschka et al. 2011), an alpha value of 0.05, and a power of 0.8. This resulted in a sample size (n) of seven horses per group. Each group was increased to eight horses to account for the potential of missed sample times or technical error.

5.1 Animals

Twenty-four adult horses, 8 females and 16 geldings, with a mean ± SD [range] age of 13.3 ± 5.0 [6 - 21] years old and weighting 545.2 ± 33.3 [325 - 640] kg were used for this research. Horses were determined to be healthy by normal findings on physical examination, complete blood count, and serum chemistry panel.

Animals were confined to a stall 24 hours prior to the anesthetic episode. Feed but not water was withheld for 8 hours prior to anesthesia. Horses had their temperament assessed via the follow previously described scale: 1- Calm, well handled; 2- Restless, anxious; 3- Aggressive; 4- Unhandled, requiring sedation (Leece et al. 2008). After anesthesia, animals were kept in the stall overnight to monitor for possible complications

and collection of final blood and urine samples. Horses were then returned to their regular pastures.

5.2 Anesthetic Procedure

An area over the jugular vein was clipped, aseptically prepared and a 14G intravenously catheter (Milacath 14G 13cm, Mila International, KY, USA) was placed and secured to the skin with suture. Eighteen animals required sedation to allow urinary bladder catheterization and baseline urine sample collection (described on section 5.4 Sample Collection). Xylazine 0.8 mg/kg (Xylamax, Bimeda Canada, Ont, Canada) was administered intravenously (IV), and also served as premedication. After urine sample collection, horses were led to the induction stall and sedated with xylazine (as previous described), if not already administered. Anesthesia was induced with ketamine (2.2 mg/kg IV) (Ketaset, Zoetis, NJ, USA) and midazolam (0.1 mg/kg IV) (Midazolam Hydrochloride Injection, Westward, NJ, USA). After induction horses were positioned in lateral recumbency and orotracheally intubated with an appropriate size cuffed endotracheal tube.

Horses were then hoisted and positioned in dorsal recumbency on a padded equine specific operating table. Anesthesia was maintained with isoflurane (Isoflurane, Akorn, IL, USA) delivered in a FiO_2 designated by group assignment, targeted to 1.2 times the minimal alveolar concentration (MAC) of horses (Steffey et al. 1977). Isoflurane deliver concentration was adjusted during anesthesia period based on anesthetic depth assessment (eye position, palpebral reflex, neck muscles relaxation, nystagmus, spontaneous movement, and cardiovascular variables). Cumulative MAC hours were

calculated as end-tidal isoflurane (ETISO) divided by the MAC of isoflurane for horses (1.31) (Steffey et al. 1977) at each five-minute interval, giving the fraction of MAC of isoflurane for each horse. The value for each interval was divided by 12 (1/12 of an hour). All values for each interval were then summed to give the cumulative MAC hours for each horse.

Anesthesia was maintained for 120 minutes. Horses were mechanically ventilated with a large animal anesthesia machine (Tafonius Junior Large Animal Anesthesia Machine, Hallowell EMC, MA, USA), with a respiratory rate of 6 – 8 breaths per minute, tidal volume (TV) of 12 – 15 ml/kg, peak inspiratory pressure (PIP) of 15 – 40 cmH_2O, adjusted to maintain an end-tidal carbon dioxide ($ETCO_2$) between 35 – 45 mmHg. Acetate Ringer's solution (Vetivex, Dechra, KS, USA) was administered at 5 ml/kg/h. A urinary catheter (Stallion urinary catheter 6.6mm x 137cm, Jorgensen Laboratories, CO, USA) was placed to allow samples collection, and the bladder was emptied immediately before moving the horse to the recovery stall.

During anesthesia, heart rate (HR), electrocardiogram (ECG), pulse oximetry (SpO_2), capnography ($ETCO_2$), ETISO, FiO_2, and systolic, mean and diastolic arterial pressures (SAP, MAP, and DAP, respectively) were monitored with a multiparameter monitor (Mindray Passport 12, Mindray North America, NJ, USA) and recorded at 5 minutes intervals. An arterial catheter (Surflash 20G x 1", Terumo, NJ, USA) was aseptically placed in the facial artery to allow direct blood pressure measurement and collection of arterial blood samples for blood gas analysis. The catheter was connected to a transducer previously zeroed at heart level. Dobutamine (Dobutamine, Hospira – Pfizer, USA) was administered IV at 0.5 – 5 mcg/kg/min to maintain the MAP between 70 and 90 mmHg.

At the conclusion of anesthesia, the arterial and urinary catheter were removed, horses were weaned of the ventilator using the “assisted mode” (respiratory rate reduced by 1/3, and a flow-triggered assisted breath delivered) until breathing spontaneously. They were moved to a padded recovery stall and fitted with an accelerometer attached to a surcingle (described on section 5.5 Recovery Evaluation).

5.3 Experimental Design

Horses were anesthetized once and randomly allocated in one of the three experimental groups via an online random number generation (random.org): group FiO2 = 0.45 (G45, n = 8), group FiO2 = 0.75 (G75, n = 8), or group FiO2 > 0.95 (G95, n = 8).

Fresh gas flow was adjusted to 6 L/min for all horses. For G95, medical oxygen (O_2) was used as deliver gas. For G75, a mix of oxygen with medical air (ratio of 70% of O_2: 30% of medical air) was used. For G45, a mix of oxygen with medical air (ratio was 30% of O_2: 70% of medical air) was used.

The fraction of inspired oxygen was monitored with the same multiparameter monitor used for physiologic data. The accuracy of the gas analyzer was verified monthly with a standard gas mixture, following the manufacturer’s recommendations.

5.4 Sample Collection and Analysis

For each experimental group, urine, venous and arterial blood were collected at baseline prior to induction of anesthesia (T0), 60 minutes after induction of anesthesia (T60), at the conclusion of anesthesia (T120), immediately following recovery (TREC), and 24

hours after T0 (T24). End of recovery was defined as when the horse stood up and was stable enough to allow sample collection.

Venous blood samples were collected via jugular venipuncture (5 ml per sample), transferred to a lithium-heparin tube, and immediately submitted to the Auburn University Clinical Pathology Laboratory for serum chemistry analysis. The serum chemistry analysis included total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK2), creatinine (Create), total calcium (Ca), magnesium (Mg^{++}), glucose (Glu), sodium (Na^+), potassium (K^+), chloride (Cl^-), anion gap (AG), and iron (S Iron). Additionally, venous blood was analyzed immediately after collection for cardiac troponin I (cTnI) concentration with a point-of-care analyzer (I-Stat, Abaxis).

Arterial blood samples during anesthesia were collected from the facial artery catheter. For T0, TREC and T24, the sample was obtained via direct puncture of the transverse facial artery. Samples were immediately analyzed with the same point-of-care analyzer (I-Stat, Abaxis). Recorded variables included pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), base excess (BE), bicarbonate (HCO_3^-), and lactate (Lact). The Enghoff equation was applied to estimate dead-space ($Vd/Vt = (PaCO_2 – ETCO_2) / PaCO_2$, where Vd is the volume of dead-space and Vt the tidal volume).

Urine samples were collected by urethral catheterization after aseptic preparation. Samples were stored at -80 ˚C until completion of all sample collection and then shipped on dry for isoprostane and creatinine quantification.

5.5 Isoprostane Analysis

Isoprostanes (Isop) were analyzed in the Eicosanoid Core Laboratory at Vanderbilt University Medical Center using a previously described gas chromatography / mass spectrometry (GC/MS) method (Milne et al. 2007; Milne et al. 2013). In short, samples were prepared by diluting 0.25 ml of urine in 10 ml of water and acidifying to a pH of 3.00 with 1N of HCl. Sample purification was performed by adding 1 ng of the internal standard ($[^2H_4]$-15-F_{2t}-IsoP) and vortexed. A C-18 Sep-Pak cartridge was preconditioned with 5 ml of methanol and 7 ml water with a corrected pH of 3. The sample was applied to the cartridge and washed with 10 ml of pH 3 water and 10 ml of heptane. Isoprostanes were eluted with 10 ml of ethyl acetate/heptane (50:50, V:V) into a glass scintillation vial. Residual water from the sample was removed with sodium sulfate. Samples were transferred to a preconditioned silica Sep-Pak, washed with 5 ml of ethyl acetate, and eluted with 5 ml of ethyl acetate/methanol (1:1, V:V) into a react-a-vial. Eluant was evaporated by nitrogen and isoprostanes were converted to the corresponding pentafluorobenzyl esters to facilitate analysis. Samples were transferred to an autosampler vial for GC-MS. For quantification, the height of the peak of m/z 569 (Isoprostanes) was compared with the height of m/z 573 (internal standard).

Urinary creatinine concentration was measured using a test kit from Enzo Life Sciences. The isoprostanes concentrations in each sample were normalized using the urinary creatinine concentration of the sample and expressed in ng/mg creatinine. This method has an accuracy of 96%, precision of 6%, and the lower limit of quantification if 0.002 ng/ml.

5.6 Recovery Evaluation

At the conclusion of anesthesia, horses were transported to a padded recovery stall and positioned in right lateral recumbency. An accelerometer (GP1 programmable accelerometer, Sensor, Monitoring Technologies, TX, USA) was attached to a surcingle at the level of the withers and activated. Once horses were verified to have spontaneous breathing, the endotracheal tube removed. The accelerometer was programed to record the maximum velocities (Vmax) in three axes during the recovery period at one-second intervals. Vmax data were used to determine a recovery score as previously described (Clark-Price et al. 2017). The recovery score (RS) is calculated as: $RS = 9.998 \times SG^{0.633} \times \sum UG^{0.174}$, where SG is the acceleration of the successful attempt to stand, and $\sum UG$ is the sum of the acceleration of any unsuccessful attempts to stand. Recoveries were video recorded to capture recovery characteristics. These characteristics included the number of attempts to reach sternal recumbency, the number of attempts to stand, time to reach sternal recumbency (defined as the time from extubation to when the horse reached sternal recumbency), and the time to stand (defined as the time from extubation to the successful attempt to stand). After recovering, the surcingle and the jugular catheter were removed, and horses were returned to their stall.

5.7 Statistical Analysis

Data were analyzed for normality with Kolmogorov-Smirnov test, and are reported as median [min; max]. All parameters were evaluated by time and by group effect using 2-way ANOVA, followed by Friedman test, and Kruskal-Wallis followed by Dunn's test. Recovery parameters, weight, age, and temperament scores were analyzed among groups

with Kruskal-Wallis followed by Dunn's test. Analysis was performed with GraphPad Prism 6 and Instat by GraphPad 3.10 software. A $p < 0.05$ was used for significance.

6. Results

All horses completed the study without major complications. One horse had a minor laceration on the right hind limb, and two horses had corneal abrasions acquired during unsuccessful attempts to stand during recovery. All were successfully managed medically during the following days. There was no difference among groups for age, weight, or temperament score (Table 1). Over the 120 minutes of general anesthesia, all physiologic parameters monitored remained within the normal range for the species (Table 2). FiO2 was different between G95 and G45.

Table 1. Median [min; max] of weigh, age, and temperament score for 24 horses (n = 8 in each group)

	G95	G75	G45
Weight (kg)	575.5 [325.0; 640.0]	582.5 [457.0; 617.0]	536.0 [498.0; 590.0]
Age (years)	12.0 [6.0; 20.0]	16.5 [11.0; 23.0]	12.0 [10.0; 20.0]
Temp Score	1 [1;3]	1 [1;2]	1 [1;2]

The values for serum chemistry analysis are presented in Table 3. Total protein was different between groups at Trec between G75 (6.45 [6.02; 6.62] g/dL) and G45 (5.87 [5.59; 6.49] g/dL) but was not considered clinically important. Within groups, several parameters differed from baseline over time but returned to baseline by T24. The only exception was observed in serum iron, where significantly lower values were observed at T24h when compared with T0 in all groups.

Table 2. Median [min; max] of physiologic parameters evaluated during 120 minutes of isoflurane anesthesia in 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%).

	G95	G75	G45
HR (bpm)	39 [34; 63]	39 [35; 47]	45 [39; 51]
SpO2 (%)	97 [86; 99]	96 [91; 98]	95 [91; 96]
ETCO2 (mmHg)	41 [35; 46]	37 [35; 41]	37 [30; 43]
MAC/hour	2.14 [2.03; 2.24]	2.07 [1.91; 2.26]	2.12 [1.93; 2.19]
SAP (mmHg)	97 [89; 110]	101 [83; 106]	97 [88; 102]
MAP (mmHg)	72 [68; 77]	72 [70; 74]	72 [65; 77]
DAP (mmHg)	57 [55; 66]	59 [52; 61]	58 [50; 68]
FiO2 (%)	94 [93; 95]^{A}	75 [73; 77]^{AB}	43 [41; 47]^{B}
RR (breaths / min)	6 [6; 8]	6 [6; 6]	6 [6; 7]
Peak Pressure (cmH_2O)	26.5 [16.0; 37.4]	25.0 [19.7; 29.2]	22.7 [15.8; 31.4]
TV (L)	7.45 [5.03; 9.50]	7.72 [6.50; 9.20]	7.18 [6.80; 8.00]
TV (ml/kg)	13.3 [12.3; 15.5]	13.6 [12.4; 15.0]	13.4 [12.7; 14.5]

Difference among groups ($p < 0.05$) are indicated with uppercase letters.

Table 3. Median [min; max] for total protein, albumin, aspartate aminotransferase (AST), creatine kinase (CK2), creatinine (Create), total calcium (Ca), magnesium (Mg^{++}), glucose (Glu), sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}), anion gap (AG), and iron (S Iron) for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%).

Total protein (g/dL)		G95	G75	G45
	T0	6.81 [6.27; 7.68]^{a}	6.66 [6.48; 7.79]^{ac}	6.77 [5.98; 7.49]^{a}
	T60	6.34 [5.87; 7.21]^{ab}	6.44 [5.75; 6.85]^{ab}	6.45 [5.81; 6.94]^{ab}
	T120	6.29 [5.58; 7.03]^{b}	6.31[5.61; 6.89]^{b}	6.05 [5.66; 6.51]^{b}
	Trec	6.18 [5.72; 7.39]^{abAB}	6.45 [6.02; 6.62]^{abcA}	5.87 [5.59; 6.49]^{bB}
	T24h	7.01 [6.15; 7.93]^{a}	7.18 [6.32; 7.93]^{c}	6.82 [6.54; 7.78]^{a}
Albumin (g/dL)		G95	G75	G45
	T0	3.13 [2.60; 3.40]^{ac}	3.11 [2.74; 3.49]^{ac}	3.14 [2.88; 3.40]^{a}
	T60	2.84 [2.46; 3.08]^{ab}	2.86 [2.56; 3.10]^{b}	2.89 [2.76; 3.23]^{ab}
	T120	2.76 [2.38; 3.04]^{b}	2.86 [2.42; 3.10]^{b}	2.73 [2.56; 3.02]^{b}
	Trec	2.87 [2.51; 3.00]^{abc}	2.96 [2.59; 3.11]^{ab}	2.73 [2.55; 3.24]^{b}
	T24h	3.15 [2.69; 3.34]^{c}	3.24 [2.97; 3.54]^{c}	3.24 [3.08; 3.36]^{a}
AST (U/L)		G95	G75	G45
	T0	275.5 [242.0; 401.0]^{ac}	284.5 [246.0; 343.0]^{ac}	278.0 [251.0; 335.0]^{ac}
	T60	247.0 [212.0; 364.0]^{ab}	263.5 [223.0; 307.0]^{ab}	274.5 [244.0; 294.0]^{abc}

	T120	242.5 [209.0; 354.0]^{b}	263.0 [216.0; 306.0]^{b}	258.0 [223.0; 283.0]^{b}
	Trec	254.5 [234.0; 371.0]^{ab}	266.0 [231.0; 320.0]^{ab}	266.5 [223.0; 298.0]^{ab}
	T24h	374.0 [294.0; 442.0]^{c}	396.5 [299.0; 545.0]^{c}	409.5 [298.0; 571.0]^{c}
CK2		G95	G75	G45
(U/L)	T0	368.5 [262.0; 885.0]^{abc}	353.0 [213.0; 894.0]^{ab}	344.5 [221.0; 574.0]^{abc}
	T60	374.5 [179.0; 1076.0]^{c}	285.0 [183.0; 423.0]^{a}	306.5 [192.0; 925.0]^{ac}
	T120	378.0 [173.0; 641.0]^{bc}	282.5 [171.0; 1066.0]^{a}	284.0 [177.0; 498.0]^{a}
	Trec	565.0 [345.0; 699.0]^{ac}	430.0 [224.0; 771.0]^{ab}	451.0 [354.0; 1066.0]^{bc}
	T24h	830.0 [359.0; 1303.0]^{a}	918.0 [288.0; 2346.0]^{b}	914.5 [417.0; 1923.0]^{b}
Create		G95	G75	G45
(mg/dL)	T0	1.50 [0.80; 1.70]^{a}	1.45 [1.10; 2.00]^{ab}	1.40 [1.20; 2.10]^{a}
	T60	1.45 [0.90; 1.60]^{a}	1.35 [1.00; 2.00]^{a}	1.50 [1.00; 2.00]^{a}
	T120	1.50 [0.90; 1.60]^{ab}	1.45 [1.00; 2.00]^{a}	1.50 [1.10; 2.00]^{ab}
	Trec	1.85 [1.10; 2.00]^{b}	1.75 [1.30; 2.60]^{b}	1.90 [1.50; 2.50]^{b}
	T24h	1.35 [0.80; 1.50]^{a}	1.25 [1.00; 2.00]^{a}	1.40 [1.00; 1.80]^{a}
Ca		G95	G75	G45
(mg/dL)	T0	12.20 [11.40; 13.00]^{a}	12.25 [11.00; 13.00]^{a}	12.25 [11.30; 12.40]^{ac}
	T60	10.15 [9.40; 11.40]^{bc}	10.35 [9.70; 10.90]^{bc}	10.40 [9.50; 11.60]^{abc}
	T120	9.95 [9.00; 11.10]^{c}	10.05 [9.30; 11.00]^{c}	10.05 [9.30; 10.90]^{b}
	Trec	10.35 [9.20; 11.60]^{bc}	10.50 [9.20; 10.90]^{bc}	10.00 [9.30; 11.30]^{b}
	T24h	11.65 [11.10; 12.30]^{ab}	11.95 [10.90; 12.30]^{ab}	11.85 [11.40; 12.30]^{c}
Mg^{++}		G95	G75	G45
(mg/dL)	T0	1.85 [1.50; 2.40]	1.90 [1.50; 2.40]^{a}	1.85 [1.60; 2.00]
	T60	1.65 [1.50; 1.90]	1.75 [1.50; 1.90]^{b}	1.65 [1.40; 2.00]
	T120	1.70 [1.50; 2.10]	1.80 [1.60; 2.20]^{ab}	1.75 [1.50; 2.00]
	Trec	1.65 [1.40; 2.00]	1.65 [1.50; 1.90]^{ab}	1.75 [1.30; 2.00]
	T24h	1.70 [1.50; 1.90]	1.75 [1.50; 2.00]^{ab}	1.65 [1.40; 2.00]
Glu		G95	G75	G45
(mg/dL)	T0	96.0 [77.0; 102.0]^{a}	99.5 [84.0; 109.0]^{a}	99.5 [82.0; 130.0]^{a}
	T60	126.5 [89.0; 179.0]^{b}	146.5 [132.0; 173.0]^{b}	140.0 [126.0; 153.0]^{b}
	T120	110.5 [91.0; 145.0]^{ab}	125.5 [112.0; 150.0]^{ab}	126.0 [114.0; 170.0]^{b}
	Trec	121.0 [97.0; 154.0]^{ab}	143.0 [113.0; 171.0]^{b}	140.0 [121.0; 186.0]^{b}
	T24h	123.5 [98.0; 181.0]^{b}	118.0 [100.0; 152.0]^{ab}	125.0 [115.0; 150.0]^{ab}
Na^{+}		G1	G75	G45
(mmol/L)	T0	137.0 [135.0; 140.0]	138.0 [135.0; 139.0]	138.0 [134.0; 143.0]
	T60	139.0 [135.0; 142.0]	139.0 [136.0; 141.0]	139.5 [137.0; 144.0]
	T120	138.5 [135.0; 141.0]	139.5 [137.0; 141.0]	139.5 [136.0; 144.0]
	Trec	139.0 [135.0; 142.0]	140.0 [137.0; 141.0]	140.0 [137.0; 145.0]
	T24h	136.0 [134.0; 139.0]	138.5 [136.0; 141.0]	138.0 [135.0; 141.0]

K⁺ (mmol/L)		G95	G75	G45
	T0	3.95 [3.20; 4.60][a]	4.15 [3.20; 4.20][ac]	3.85 [3.20; 4.20][ab]
	T60	3.70 [3.00; 4.30][ab]	3.20 [2.90; 3.60][ab]	3.30 [2.80; 3.80][a]
	T120	3.55 [2.90; 4.00][b]	3.10 [2.90; 3.40][b]	3.35 [2.90; 4.30][ab]
	Trec	4.15 [3.60; 4.70][a]	4.00 [3.40; 4.30][c]	3.95 [3.50; 4.90][b]
	T24h	3.80 [3.30; 4.20][ab]	3.60 [3.00; 4.40][abc]	3.55 [3.30 ;3.80][ab]
Cl⁻ (mmol/L)		G95	G75	G45
	T0	99.0 [97.0; 101.0][ab]	98.0 [95.0; 101.0][a]	98.0 [95.0; 102.0][a]
	T60	97.5 [95.0; 100.0][ab]	98.5 [97.0; 101.0][ab]	99.0 [95.0; 100.0][ab]
	T120	97.0 [94.0; 99.0][a]	98.0 [97.0; 101.0][ab]	97.5 [94.0; 100.0][ab]
	Trec	97.0 [92.0; 100.0[a]]	97.0 [95.0; 100.0][a]	96.0 [93.0; 100.0][b]
	T24h	99.0 [97.0; 104.0][b]	102.0 [100.0; 106.0][b]	100.0 [97.0; 103.0][a]
AG (mmol/L)		G95	G75	G45
	T0	14.80 [12.30; 17.60]	15.00 [13.10; 18.90]	14.70 [12.90; 16.10][a]
	T60	15.30 [13.40; 18.00]	14.45 [11.80; 17.20]	15.15 [13.00; 17.90][ab]
	T120	15.65 [13.80; 18.80]	14.00 [12.50; 16.90]	15.55 [14.00; 19.60][ab]
	Trec	16.35 [13.00; 18.50]	16.05 [12.00; 27.10]	17.55 [16.20; 22.70][b]
	T24h	15.90 [11.20; 17.90]	14.95 [11.10; 16.60]	15.30 [11.40; 18.20][a]
S Iron (mcg/dL)		G95	G75	G45
	T0	148.0 [103.0; 171.0][a]	153.0 [130.0; 198.0][a]	149.5 [103.0; 213.0][a]
	T60	135.5 [104.0; 184.0][a]	131.0 [117.0; 179.0][ab]	142.0 [91.0; 166.0][a]
	T120	132.5 [100.0; 188.0][a]	134.5 [113.0; 166.0][ab]	133.5 [87.0; 161.0][ab]
	Trec	133.0 [106.0; 198.0][ab]	133.5 [112.0; 172.0][a]	131.5 [95.0; 166.0][ab]
	T24h	60.5 [39.0; 102.0][b]	53.5 [41.0; 96.0][b]	63.5 [35.0; 102.0][b]

Differences among groups are indicated by uppercase letters, and among times by lowercase letters ($p < 0.05$).

Blood gas analysis values as well as cardiac troponin and isoprostanes values are presented in Table 4. There was no difference over time or among groups for either cardiac troponin I or isoprostanes. Lactate values were increased at Trec within groups, but without difference among groups at any time point. Values returned to baseline at T24h Bicarbonate, base excess and pH had differences over time, but were never outside of normal reference ranges and all values returned to baseline at T24h. PaO_2 was different among groups during anesthesia based on oxygen fraction provided, but no

difference was observed among groups at Trec or T24h. Some horses in group G95 developed hypoxemia (PaO2 < 60 mmHg) during anesthesia. One horse had a PaO_2 of 44 mmHg at T60. However, the PaO_2/FiO_2 relationship was not different among groups. When categorizing the PaO_2/FiO_2 relationship, in G95, one horse had a value lower than 200 and one lower horse had a value lower than 60 at T60. Two horses had values lower than 100 at T120; in G75 2 horses had values lower than 200 and one horse lower than 100 at T60, while 3 horses had lower than 200 and 2 horses lower than 100 at T120; and in G45 4 horses had values lower than 200 at T60, 6 horses at T120, and no horse had values lower than 100. Although end tidal CO_2 did not differ among groups, $PaCO_2$ was different between G95 and G45 at some time points. Calculated Vd/Vt values were different at T60, G95 was significant higher from G45, but not from G75 (G95 = 0.27 [0.26;0.33], G75 = 0.26 [0.19; 0.35], and G45 = 0.24 [0.19; 0.25], and on T120 G45 was significantly lower than G95 and G75 (G95 = 0.33 [0.28; 0.39], G75 = 0.34 [0.24; 0.39] and G45 = 0.23 [0.11; 0.31])

Table 4. Median [min; max] for pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), base excess (BE), bicarbonate (HCO_3^-), lactate (Lact), cardiac troponin I (cTnI), and isoprostanes (Isop) for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%).

pH		G95	G75	G45
	T0	7.41 [7.37; 7.44]^{a}	7.42 [7.40; 7.46]^{ab}	7.44 [7.37; 7.45]^{ab}
	T60	7.35 [7.30; 7.39]^{bA}	7.38 [7.33; 7.44]^{aAB}	7.42 [7.38; 7.49]^{aB}
	T120	7.35 [7.26; 7.40]^{abA}	7.40 [7.33; 7.58]^{abAB}	7.43 [7.37; 7.47]^{abB}
	Trec	7.45 [7.39; 7.55]^{a}	7.46 [7.41; 7.51]^{b}	7.50 [7.44; 7.63]^{b}
	T24h	7.40 [7.34; 7.42]^{ab}	7.40 [7.37; 7.41]^{ab}	7.41 [7.36; 7.43]^{b}
$PaCO_2$ (mmHg)		G95	G75	G45
	T0	44.2 [38.5; 48.6]^{ab}	44.5 [41.0; 47.1]^{ac}	44.6 [41.4; 49.2]
	T60	59.3 [50.5; 67.5]^{aA}	53.5 [47.2; 58.5]^{bAB}	50.2 [43.1; 55.7]^{B}
	T120	55.7 [52.2; 63.3]^{aA}	52.3 [47.5; 56.0]^{abAB}	46.9 [41.9; 55.9]^{B}

	Trec	43.3 [36.5; 50.8]^{b}	43.6 [40.0; 52.3]^{abc}	44.0 [31.7; 46.3]
	T24h	40.6 [36.2; 45.2]^{b}	41.7 [35.8; 46.9]^{c}	44.0 [40.7; 46.7]
PaO_2 (mmHg)		G95	G75	G45
	T0	91.0 [76.0; 98.0]^{a}	90.0 [83.0; 97.0]	84.0 [71.0; 102.0]^{ab}
	T60	308.0 [44.0; 427.0]^{bA}	179.5 [73.0; 306.0]^{AB}	85.0 [73.0; 92.0]^{abB}
	T120	260.5 [59.0; 398.0]^{abA}	101.0 [58.0; 291.0]^{AB}	77.5 [68.0; 90.0]^{aB}
	Trec	90.0 [72.0; 103.0]^{a}	81.5 [74.0; 107.0]	89.0 [69.0; 108.0]^{ab}
	T24h	98.5 [88.0; 112.0]^{ab}	98.0 [73.0; 112.0]	97.0 [89.0; 107.0]^{b}
PaO2/FiO2		G95	G75	G45
	T0	433.3 [361.9; 466.7]^{ab}	428.6 [395.2; 461.9]^{ac}	400.0 [338.1; 485.7]^{ac}
	T60	322.8 [45.8; 454.3]^{a}	237.5 [97.3; 419.2]^{ab}	197.8 [172.3; 225.0]^{ab}
	T120	274.4 [62.1; 419.0]^{a}	133.9 [75.3; 393.2]^{b}	179.3 [151.1; 219.4]^{b}
	Trec	428.6 [342.9; 490.5]^{ab}	388.1 [352.4; 509.5]^{abc}	423.8 [328.6; 514.3]^{ac}
	T24h	469.1 [419.1; 533.3]^{b}	466.7 [347.6; 533.3]^{c}	461.9 [423.8; 509.5]^{c}
BE (mmol/L)		G95	G75	G45
	T0	3.50 [0.00; 6.00]^{ab}	4.50 [1.00; 7.00]^{ab}	5.50 [1.00; 6.00]^{a}
	T60	7.00 [3.00; 9.00]^{a}	6.50 [4.00; 10.00]^{a}	8.00 [4.00; 10.00]^{ab}
	T120	8.00 [0.00; 8.00]^{a}	6.50 [3.00; 10.00]^{a}	7.50 [5.00; 10.00]^{ab}
	Trec	8.50 [0.00; 11.00]^{a}	6.50 [3.00; 13.00]^{a}	9.00 [7.00; 13.00]^{b}
	T24h	0.00 [-2.00; 2.00]^{b}	0.50 [-3.00; 3.00]^{b}	3.00 [-1.00; 5.00]^{a}
HCO_3^- (mmol/L)		G95	G75	G45
	T0	28.25 [25.30; 30.60]^{ab}	28.75 [25.90; 31.10]^{ab}	29.55 [26.70; 31.10]^{a}
	T60	32.30 [25.60; 34.60]^{a}	31.75 [29.50; 34.20]^{a}	32.20 [28.50; 34.50]^{b}
	T120	33.15 [26.70; 33.80]^{a}	31.55 [29.20; 34.50]^{a}	31.75 [29.20; 34.40]^{ab}
	Trec	31.65 [24.90; 34.30]^{ab}	30.55 [27.30; 36.50]^{a}	32.05 [30.90; 35.70]^{b}
	T24h	24.80 [23.50; 26.50]^{b}	25.15 [21.70; 28.20]^{b}	27.65 [23.90; 29.30]^{a}
Lact (mmol/L)		G95	G75	G45
	T0	0.43 [0.00; 1.07]^{a}	0.18 [0.00; 0.56]^{c}	0.19 [0.00; 1.64]^{a}
	T60	1.02 [0.88; 2.25]^{b}	0.94 [0.56; 1.31]^{abc}	0.92 [0.58; 1.24]^{ac}
	T120	1.04 [0.79; 2.21]^{b}	1.03 [0.78; 1.64]^{ab}	1.22 [0.70; 1.80]^{bc}
	Trec	1.17 [0.00; 4.09]^{b}	2.60 [0.00; 6.98]^{b}	2.44 [0.85; 6.28]^{b}
	T24h	0.75 [0.35; 1.06]^{ab}	0.65 [0.41; 1.04]^{ac}	0.70 [0.31; 1.03]^{ac}
cTnI (ng/ml)		G95	G75	G45
	T0	0.00 [0.00; 0.02]	0.00 [0.00; 0.01]	0.00 [0.00; 0.01]
	T60	0.00 [0.00; 0.01]	0.00 [0.00; 0.03]	0.00 [0.00; 0.00]
	T120	0.00 [0.00; 0.02]	0.00 [0.00; 0.01]	0.00 [0.00; 0.00]
	Trec	0.00 [0.00; 0.01]	0.005 [0.00; 0.01]	0.00 [0.00; 0.01]
	T24h	0.00 [0.00; 0.03]	0.00 [0.00; 0.04]	0.00 [0.00; 0.05]
		G95	G75	G45

Isop (ng/mg creatinine)	T0	2.28 [1.25; 2.94]	2.30 [1.59; 3.81]	2.48 [1.60; 3.47]
	T60	2.92 [1.83; 3.37]	3.28 [2.03; 5.29]	2.37 [1.67; 4.10]
	T120	2.82 [1.52; 6.44]	2.95 [2.21; 5.47]	2.64 [1.75; 4.16]
	Trec	2.94 [1.57; 3.48]	3.70 [1.93; 4.82]	3.16 [1.93; 3.68]
	T24h	2.05 [0.84; 2.98]	2.00 [0.72; 4.54]	1.67 [0.49; 3.96]

Differences among groups are indicated by uppercase letters, and among times by lowercase letters ($p < 0.05$).

Results for recovery times and scores are presented in Table 5. No differences were found in any variable among groups.

Table 5. Median [min; max] for time for first movement (1st mov), number of attempts to reach sternal recumbency (attempts to sternal), time to reach sternal recumbency, number of attempts to stand (attempts to stand), standing time, and recovery score for 24 horses (n = 8 in each group), submitted to different fractions of oxygen (G95 = 95%, G75 = 75%, G45 = 45%).

	G95	G75	G45
1st mov (min)	9.91 [1.73; 13.17]	7.49 [2.65; 17.08]	3.69 [0.27; 15.70]
Attempts to sternal	2 [1; 25]	2 [1; 3]	1.5 [1; 9]
Time to sternal (min)	16.68 [10.20; 62.37]	9.19 [5.78; 24.48]	13.05 [7.48; 20.40]
Attempts to stand	6 [1; 13]	7 [4; 15]	8 [3; 12]
Standing time (min)	30.36 [18.5; 151.5]	34.63 [13.58; 107.4]	26.26 [14.52; 47.05]
Recovery Score	37.46 [21.27; 55.31]	27.40 [19.60; 66.50]	34.24 [25.20; 44.95]

7. Discussion

Administration of low (0.45) or high (> 0.95) fractions of oxygen during anesthesia with isoflurane does not result in higher production of isoprostanes or cardiac troponin I in healthy horses. Additionally, no differences were found between groups in recovery quality, number of attempts to stand, or recovery times. Therefore, both hypotheses tested were rejected.

Cardiac troponin was not different among groups, demonstrating that in healthy horses, under these experimental conditions, the lower PaO_2 associated with an FiO_2 of 0.45 was not sufficient to cause hypoxemic damage to the myocardium. A previous study analyzed post-anesthesia cTnI in horses undergoing various elective procedures and found no increase in this parameter (Slack et al. 2011). However, all horses in that study were submitted to high fractions of oxygen ($FiO_2 > 94\%$), so the effect of lower fractions on the cardiac muscle oxygenation was not evaluated. On the other hand, a progressive muscle deoxygenation was observed in anesthetized horses with the use of an FiO_2 of 0.21 (Portier et al. 2009). Unfortunately, this study used a lower fraction of oxygen than our study and did not evaluate cTnI. Based on the results of our study, hypoxic injury to the myocardium in healthy adult horses is not detected using cTnI with fractions as low as 0.45, but no extrapolation can be made for oxygen fractions lower than that or in sick animals.

Despite no change in cardiac troponin, serum lactate was increased in all groups immediately after recovery, being significant lower in G95 than G75 and G45. Lactate is traditionally considered an indicator of anaerobic metabolism, but it is also generally increased during exercise, especially short-term high-intensity exercises, and is now considered an important intermediate in several metabolic process, acting as a mediator of redox-state among cells (Gladden 2004). The smaller magnitude of increase found in G95 suggest that providing high fraction of oxygen during anesthesia can offer a potential oxygen reserve to be used during the recovery.

Isoprostanes concentrations were not different among groups, suggesting that even if an increase in anaerobic metabolism occurs during recovery, as suggested by the increase in lactate, healthy horses possess an ability to compensate to such a degree that significant hypoxemic oxidative stress does not occur. Values for urine isoprostanes are reported to be 1.89 ± 1.39 ng/mg creatinine in healthy horses, and 2.94 ± 1.69 ng/mg creatinine in horses with colic (Noschka et al. 2011). In our study, the median concentration for isoprostanes at baseline was 2.36 [1.25; 3.81] ng/mg creatinine. This value is considerably higher for healthy horses than in the previous report. However, the report by Noschka and co-workers is the only study reporting urine isoprostanes values in healthy horses with the same methodology from out study. This suggests that studies with a larger population of healthy horses may be necessary to establish the true normal range for the species. One potential bias could be that many of our baseline samples were collected from horses that had been sedated with xylazine, while the samples from the previous studies in humans and horses were obtained by free-catch without sedation

(Gniwotta et al. 1997, Morrow & Roberts 2002, Noschka et al. 2011). It is unknown if xylazine administration can affect urine isoprostane concentrations.

Unsuccessful attempts to stand resulted in minor injuries in two horses in this study, representing 8.33% of the enrolled animals. Minor injuries during recovery are reported to occur in 0.31% of horses in a retrospective clinical study (Barrio et al. 2018). Several factor can influence the anesthetic recovery in horses, as age, breed, weight, temperament, use of sedative during recovery, and assistance or not during recovery (Matthews et al. 1992, Dugdale et al. 2016). The horses in our study all had a similar temperament and were calm and easy to handle. However, two main factors could explain the high incidence of minor injuries when compared with the data from Barrio and co-workers: recoveries were unassisted and there was no administration of sedatives during recovery. The use of sedation at recovery is associated with better recovery scores (Dugdale et al. 2016). Sedation at recovery was not used in this study because of the potential to mask recovery differences that may have been detected between the different groups. Similarly, recoveries were unassisted to minimize external influences on the quality of recovery.

The recovery score was not different among groups. These findings are in agreement with previous research where horses anesthetized with FiO2 of 0.5 or >0.95 resulted in no differences in recovery variables (Hubbell et al. 2011). However, our horses had a median value of 6 attempts to stand while horses in Hubbell and co-workers had a median of 1. Horses in that study were sedated early in the recovery period which corroborates the positive effect of sedation in the early period of recovery (Dugdale et al. 2016).

Other interesting findings in our study include changes in serum iron concentrations and dead-space fraction among groups. With the exception of serum iron, all other measured biochemical variables were not different or returned to basal values by the day following anesthesia. Serum iron concentrations decreased and remained low at 24 hours. Iron is a negative acute phase reactant in several species, being a very sensitive indicator of systemic inflammation in horses. Serum iron also can reflect the intensity of a surgical trauma but returns to baseline after two days of the surgical procedure (Borges et al 2007, Jacobsen et al. 2009, Lilliehook et al. 2019). To our knowledge, this is the first study to report a decrease in serum iron associated with only an anesthetic procedure. Previous studies with horses indicated that general anesthesia does not increase fibrinogen or serum amyloid A, as markers of an inflammatory response (Allen & Kold 1988, Pepys et al. 1989). This may indicate that serum iron is a more sensitive marker of a stress response in horse, however, further studies are necessary.

Hypoxemia is defined as a PaO_2 less than 60 mmHg, however values less than 80 mmHg are considered to be not ideal (Hubbell & Muir 2015). Since different fractions of oxygen where utilized in this study, analysis of the relationship between PaO_2 and FiO_2 provided a more homogenous value to make comparisons among groups than the absolute PaO_2. Traditionally the PaO_2/FiO_2 is expected to be greater than 300, with values lower than 200 being indicative of pulmonary dysfunction (Karbing et al. 2007). Horses in G45 had PaO_2/FiO_2 below 200 at T60 and T120. However, there was no statistical difference when compared to the values of the other groups. There are four main factors identified as contributors to large differences between alveolar and arterial oxygen tensions: hypoventilation, diffusion impairment, shunt and V/Q mismatching (West 2008).

Diffusion impairment is not a significant contributor to hypoxemia in anesthetized horses free of pulmonary diseases (Hubbell & Muir 2015) and thus likely not an issue in the horses in this study. Hypoventilation by reduced minute ventilation due to positional and anesthetic drug effects was not considered to be a factor in our horses since all were mechanically ventilated. Shunts represents an extreme form of V/Q mismatch where blood flows through collapsed parts of the lung where no ventilation is present. In anesthetized horses compression atelectasis has been determined to be the main cause of atelectasis (Sorenson & Robinson 1980, Nyman & Hedenstierna 1989). Pulmonary blood in horses flows preferentially to the caudal lobe regardless of body position with the difference between alveolar and arterial oxygen tension being greater in dorsally recumbency animals compared to standing animals. Additionally, the proportion of shunts is greater in anesthetized than awake horses (Dobson et al. 1985). Dead-space, in the other extreme, represents portion of the lung that are ventilated but have no blood flow. Our findings that horses receiving a lower fractions of oxygen had less dead-space suggest that a reduction of absorbance atelectasis improves the balance between ventilated and perfused, but not enough to contra balance shunt formation. Use of lower oxygen fraction however, lead to lower values of PaO_2 even with mechanical ventilation. This is further supported by the findings of Crumley and co-workers (2013) that anesthetized horses submitted to a FiO_2 of 50% have increased risk of hypoxemia when compared to a FiO_2 >95% during spontaneous ventilation.

There are a few limitations to this study. First, the evaluators were not blinded to treatment. However, all variables were objectives parameters minimizing the subjective interference that awareness of the treatment could promote. Second, the recovery blood

and urine samples were not collected at the same period of time after discontinuation of anesthesia due to variability in time to stand. However, the main objective of the recovery samples was to evaluate if a correlation exists between rough recoveries with the variables. Regardless of differences in timing of sample collection immediately post recovery, differences in measured parameters associated with recovery score were not identified. The samples at 24 hours after the anesthetic episode were used to evaluate if variable returned to baseline values within one day. Also, despite being an objective tool to evaluate horse anesthetic recoveries, the accelerometry method is relatively new, thus it is difficult to make comparisons between its findings with previous publications when different evaluation methods were utilized. Horses did not serve as their own controls in our study. This would have strengthened the power of the study and minimized horse to horse differences. However, repeated anesthetic procedures are associated with improvement in recovery scores (Platt et al. 2018), and the main objective of this study was to evaluate the influence of different fractions of oxygen in recovery quality.
This project utilized healthy animals and care should be taken when translating our findings to clinical animals. Compensatory mechanisms for decreased FiO_2 may not be present in horses with systemic disease. Therefore, future studies should be performed to determine the impact of different fraction of oxygen during anesthesia in sick animals.

8. Conclusion

In conclusion, the use of relatively high (>95%) and low (45%) fractions of oxygen in anesthetized horses does not lead to an increase in oxidative stress in anesthetized horses. A FiO_2 of 0.45 does lead to myocardial injury from hypoxemia but it also does not improve pulmonary function. None of the fractions of oxygen tested demonstrated superiority with regards to recovery quality in healthy horses anesthetized with isoflurane for two hours in dorsal recumbency.

9. References

Aggarwal R, Lebiedz-Odrobina D, Sinha A et al. (2009) Serum cardiac troponin T, but not troponin I, is elevated in idiopathic inflammatory myopathies. J Rheumatol 36, 2711-2714.

Allaouchiche B, Debon R, Goudable J, et al. (2001) Oxidative stress status during exposure to propofol, sevoflurane and desflurane. Anesth Analg 93, 981-985.

Allen BV, Kold SE (1988) Fibrinogen response to surgical tissue trauma in the horse. Equine Vet J 20, 441-443.

Altemeier WA, Sinclair SE (2007) Hyperoxia in the intensive care unit: why more is not always better. Curr Opin Crit Care 13, 73-78.

Barden A, Mas E, Croft KD et al. (2014) Minimizing artifactual evaluation of lipid peroxidation products (F2-isoprostanes) in plasma during collection and storage. Anal Biochem 449, 129-131.

Barrio MCN, David F, Hughes JML et al. (2018) A retrospective report (2003-2013) of the complications associated with the use of a one-man (head and tail) rope recovery system in horses following general anaesthesia. Irish Veterinary Journal 71, 6.

Basu S. (2008) F2-isoprostanes in human health and diseases: from molecular mechanisms to clinical implications. Antioxid Redox Signal 10, 1405-1434

Bettschart-Wolfensberger R, Freeman SL, Nicola JS, et al. (2001) Infusion of a combination of propofol and medetomidine for long-term anesthesia in ponies. Am J Vet Res, 62, 500-507.

Bidwell LA, Bramlage LR, Rood WA (2007). Equine perioperative fatalities associated with general anaesthesia at a private practice – a retrospective case series. Vet Anaesth Analg, 34, 23-30.

Bodor GS, Porterfield D, Voss EM et al. (1995) Cardiac troponin-I is not expressed in fetal and healthy or diseased adult human skeletal muscle tissue. Clin Chem 41, 1710-1715.

Borges AS, Divers TJ, Stokol T et al. (2007) Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. J Vet Intern Med 21, 489-494.

Brodbelt DC, Blissit KJ, Hammond RA, et al. (2008) The risk of death: the confidential enquiry into perioperative small animal fatalities. Vet Anaesth Analg, 35, 365-373.

Burton GJ, Jauniaux E (2011) Oxidative Stress. Best Practice & Research Clinical Obstetrics and Gynecology 25, 287-299.

Chandra K, Syed SA, Abid M, et al. (2015) Protection against FCA induced oxidative stress induced DNA damage as a model of arthritis and in vitro anti-arthritic potential of costus speciosus rhizome extract. Int J Pharm Phyto Res 7, 383-389.

Clark-Price SC (2013) Recovery of horses from anesthesia. Veterinary Clinics: Equine Practice 29 (1), 223-242.

Clark-Price SC, Lascola KM, Carter JE, et al. (2017) Assessment of agreement among diplomats of the American College of Veterinary Anesthesia and Analgesia for scoring the recovery of horses from anesthesia by use of subjective grading scales and development of a system for evaluation of the recovery of horses from anesthesia by use of accelerometry. Am J Vet Res 78 (6), 668-676.

Clark-Price SC, Posner LP, Gleed RD (2008) Recovery of horses from general anesthesia in a darkened or illuminated recovery stall. Vet Anaesth Analg, 35, 473-479.

Cornelisse CJ, Schott HC, Oliver NB et al. (2000) Concentration of cardiac troponin I in a horse with a ruptured aortic regurgitation jet lesion and ventricular tachycardia. J Am Vet Med Ass 217, 231-235.

Cracowski JD, Durand T, Bessard G (2002) Isoprostanes as a biomarker of lipid peroxidation in humans: physiology, pharmacology and clinical implications. Trends Pharmacolol Sci 23 (8), 360-366.

Crumley MN, McMurphy RM, Hodgson DS, et al. (2013) Effects of inspired oxygen concentration on ventilation, ventilator rhythm, and gas exchange in isoflurane-anesthetized horses. Am J Vet Res 74 (2), 183-190.

Cruzat VF, Krause M, Newsholme P (2014) Amino acid supplementation and impact on immune function in the context of exercise. J Int Soc Sports Nutr 11, 61.

Cummins B, Auckland ML, Cummins P (1987) Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. Am Heart J 113, 1333-1344.

Cummins B, Cummins P (1987) Cardiac specific cardiac troponin-I release in canine experimental myocardial infarction: development of a sensitive enzyme-linked immunoassay. J Mol Cell Cardiol 19, 999-1010.

Czerska M, Mikotajewska K, Zielinski M et al. (2015) Today's oxidative stress markers. Medycyna Pracy 66 (3), 939-405.

Dang H, Li J, Liu C, et al. (2017) CGRP attenuates hyperoxia-induced oxidative stress-related injury to alveolar epithelial type II cells via the activation of the Sonic hedgehog pathway. Int J Mol Med 40, 209-216.

Diana A, Guglielmini C, Candini D et al. (2007) Cardiac arrhythmias associated with piroplasmosis in the horse: a case report. Vet J 174, 193-195.

Dobson A, Gleed RD, Meyer RE et al. (1985) Changes in blood flow distribution in equine lungs induced by anaesthesia. Quarterly Journal of Experimental Physiology 70, 283-297.

Donaldson LL, Dunlop GS, Holland MS, et al. (2000) The recovery of horses from inhalant anesthesia: a comparison of halothane and isoflurane. Vet Surgery, 29, 92-101.

Droge W (2000) Free radicals in the physiologic control of cell function. Physiol Rev 82, 47-95.

Dugdale AHA, Obhrai J, Cripps PJ (2016) Twenty years later: a single-centre, repeat retrospective analysis of equine perioperative moratlity and investigation of recovery quality. Vet Anaesth Analg, 43 (2), 171-178.

El-Bahr SM, El-Deeb WM (2016) Acute-phase proteins, oxidative stress biomarkers, proinflammatory cytokines, and cardiac troponin in Arabian mares affected with pyometra. Theriogenology 86, 1132-1136.

FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and other Tools) Resource*. Food and Drug Administration; National Institutes of Health: Silver Springs, MD, USA, 2016.

Fennell L, Church S, Tyrell D et al. (2009) Double-outlet right ventricle in a 10-month-old Friesian filly. Aust Vet J 87, 204-209.

Freyd M. (1923) The graphic rating scale. *Journal of Educational Psychology,* 14, 83–102.

Gladden LB (2004) Lactate metabolism: a new paradigm for the third millennium. J Physiol 558 (1), 5-30.

Gleed RD (1988) Improvement in arterial oxygen tension with change in posture in anesthetized horses. Res Vet Sci 44, 255-259.

Gniwotta C, Morrow JD, Roberts LJ et al. (1997) Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesions. Arterioscler Thromb Vasc Biol 17, 3236-3241.

Gottschalk A, Aken HV, Zenz M at el. (2011) Is anesthesia dangerous? Dtsch Arztebl Int. 108(27), 469-474.

Gyongyosi A, Terraneo L, Bianciardi P, et al. (2018) The impact of moderate chronic hypoxia and hyperoxia on the level of apoptotic and autophagic proteins in myocardial tissue. Oxid Med Cell Longev 16, doi: 10.1155/2018/5786742 (eCollection 2018).

Hayes MHS, Patterson DG. (1921) Experimental development of the graphic rating method. *Psychological Bulletin,*18, 98–99.

Hedenstierna G, Nyman G, Kvart C, et al. (1987) Ventilation-perfusion relationships in the standing horse: An inert gas elimination study. Equine vet J 19 (6), 514-519.

Hill A, Wendt S, Benstoem C, et al. (2018) Vitamin C to improve organ dysfunction in cardiac surgery patients-review and pragmatic approach. Nutrients 10, doi: 10.3390/nu10080974.

Hubbell JAE (1999) Recovery from anesthesia in horses. Equine Vet Educ 11, 160-167.

Hubbell JAE, Aarnes TK, Bednarski RM, Lerche P, Muir WW (2011) Effect of 50% and maximal inspired oxygen concentrations on respiratory variables in isoflurane-anesthetized horses. BMC Vet Res 7 (23), 1-11.

Hubbell JAE, Muir WW (2015) Oxygenation, oxygen delivery and anaesthesia in the horse. Equine Vet J 47, 25-35.

Hubbell JAE, Muir WW. (2009) Considerations for induction, maintenance and recovery *in* Muir WW, Hubbell JAE Equine Anesthesia Monitoring and Emergency Therapy. 2nd ed. Saunders-Elsevier, p.381-396.

Hubbell JAE. (2007) Horses *in* Tranquilli WJ, et al. Lumb & Jones Veterinary Anesthesia and Analgesia 4th ed. Blackwell publishing, p.717-729.

Il'yasova D, Scarbrough P, Spasojevic I (2012) Urinary biomarkers of oxidative status. Clin Chim Acta 413, 1446-1453.

Inoue T, Ku T, Kaneda T, et al. (2002) Cardioprotective effects of lowering oxygen tension after aortic unclamping on cardiopulmonary bypass during coronary artery bypass grafting. Cir J 66, 718-722.

Jacobsen S, Nielsen JV, Kjelgaard-Hansen M et al. (2009) Acute phase response to surgery of varying intensity in horses: a preliminary study. Vet Surgery 38, 762-769.

Janssen LJ (2008) Isoprostanes and lung vascular pathology. Am J Respir Cell Mol Biol 39, 383-389.

Johnston GM, Eastment JK, Wood JL, et al. (2002) The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of phase 1 and 2. Vet Anaesth Analg 29, 159-170.

Johnston GM, Taylor PM, Holmes MA, et al. (1995) Confidential enquiry of perioperative equine fatalities (CEPEF-1): preliminary results. Equine Vet J, 27, 193-200.

Kambayashi Y, Tsuzuki N, Tokushige H, et al. (2018) Comparison of oxidative stress under different propofol administration protocols in Thoroughbred racehorses by bOS and bAP assessment. J Equine Sci 29 (3), 75-78.

Karbing DS, Kjaergaard S, Smith BW et al. (2007) Variation in the PaO2/FiO2 ration with FiO2: mathematical and experimental description, and clinical relevance. Critical Care 11 (6), R118.

Kirschvink N, Art T, de Moffarts B et al. (2002) Relationship between markers of blood oxidant status and physiological variables in healthy and heaves-affected horses after exercise. Equine Vet J Suppl 34, 159-164.[b]

Kirschvink N, Art T, Smith N et al. (1999) Effect of exercise and COPD crisis on isoprostane concentration in plasma and bronchoalveolar lavage fluid in horses. Equine vet J Suppl 30, 88-91.

Kirschvink N, Smith N, Fievez L et al. (2002) Effect of chronic airway inflammation and exercise on pulmonary and systemic antioxidant status of healthy and heaves-affected horses. Equine vet J 34 (6), 563-571.[a]

Kraus MS, Jesty SA, Gelzer AR, et al. (2010) Measurement of plasma cardiac troponin I concentration by use of a point-of-care analyzer in clinically normal horses and horses with experimentally induced cardiac disease. Am J Vet Res 71, 55-59.

Lee JY (2012) Oxidative stress due to anesthesia and surgical trauma and comparison of the effects of propofol and thiopental in dogs. J Vet Med Sci 74 (5), 663-665.

Lee JY (2013) Evaluation of the total oxidant and antioxidant status of the plasma of dogs anaesthetized with isoflurane. Vet Rec 173, 96.

Lee JY, Kim MC (2012) Effect of propofol on oxidative stress status in erythrocytes from dogs under general anaesthesia. Acta Vet Scandinavica, 54, 76.

Lee JY, Kim MC (2014) Comparison of oxidative stress status in dogs undergoing laparoscopic and open ovariectomy. J Vet Med Sci 76 (2), 273-276.

Leece AE, Corletto F, Brearley JC, et al. (2008) A comparison of recovery times and characteristics with sevoflurane and isoflurane anaesthesia in horses undergoing magnetic resonance imaging. Vet Anaesth Analg, 35, 383-391.

Lilliehook I, Brojer J, Nostell K et al. (2019) Hematologic, prostaglandin F2α-metabolite, serum amyloid A, and serum iron changes in horses with experimentally induced endotoxemia. Vet Clin Pathol 49, 319-325.

Lumb AB Applied physiology: Anaesthesia. *In*: Lumb AB (2005) Nunn's Applied Respiratory Physiology. 6th ed. St Louis: Elsevier, 297-326.

Mama KR, Wagner AE, Steffey EP, et al. (2005) Evaluation of xylazine and ketamine for total intravenous anesthesia in horses. Am J Vet Res, 66, 1002-1007.

Manohar M, Gustafson R, Nganwa D (1987) Skeletal muscle perfusion during prolonged 2.03% end-tidal isoflurane-O_2 anesthesia in isocapnic ponies. Am J Vet Res 48, 6, 946-951.

Marntell S, Nyman G, Hedenstierna G (2005) High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses. Vet Anesth Analg 32, 338-347.

Mason DE, Muir WW, Wade A (1987) Arterial blood gas tensions in the horse during recovery from anesthesia. J Am Vet Res 190 (8), 989-994.

Matthews NS, Hartsfield SM, Mercer D, et al. (1998) Recovery from sevoflurane anesthesia in horses: comparison to isoflurane and effect of postmedication with xylazine. Vet Surg, 27 (5), 480-485.

Matthews NS, Miller SM, Hartsfield SM, et al. (1992) Comparison of recoveries from halothane VS isoflurane anesthesia on horses. J Am Vet Med Assoc, 201 (4), 559-563.

Matthews NS, Mohn TJ, Yang M, et al. (2017) Factors associated with anesthetic-related death in dogs and cats in primary care veterinary hospitals. JAVMA, 250 (6), 655-665.

McGurrin MK, Physick-Sheard PW, Kenney DG (2008) Transvenous electrical cardioversion of equine atrial fibrillation: patient factors and clinical results in 72 treatments episodes. J Vet Intern Med 22, 609-615.

Mee AM, Cripps PJ, Jones RS (1998). A retrospective study of mortality associated with general anesthesia in horses: elective procedures. Vet Rec 14, 275-276.

Meephansan J, Rungjang A, Yingmema W, et al. (2017) Effect of astaxanthin on cutaneous wound healing. Clin Cosmet Investig Dermatol 10, 259-265.

Milne, G.L., Yin, H. and Morrow, J.D. (2008) Human biochemistry of the isoprostane pathway. J Biol Chem 283, 15533-15537.

Mitchell B. (1969) Equine anesthesia: an assessment of techniques used in clinical practice. Equine Vet J 1, 261-275.

Montuschi P, Barnes PJ, Roberts II LJ (2004) Isoprostanes: markers and mediators of oxidative stress. The FASEB Journal 18, 1791-1800.

Morrow JD, Harris TM, Roberts LJ (1990) Noncyclooxygenase oxidative formation of a series of novel prostaglandins: analytical ramifications for measurement of eicosanoids. Anal Bioch 184, 1-10.

Morrow JD, Hill KE, Burk RF et al. (1990) A series of prostaglandin F2-like compounds are produced in vivo in humans by a noncyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 87, 9383- 9387.

Morrow JD, Minton T, Mukundam CR et al. (1994) Free-radical induced generation of isoprostanes in vivo. Evidence for the formation of D-ring and E-ring isoprostanes. J Biol Chem 269, 4317-4326.

Morrow JD, Roberts LJ (2002) The Isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. Am J Respir Crit Care Med 166, S25-S30.

Mosen M, Waldmann AD, MacFarlane P, et al. (2016) Horses auto-recruit their lungs by inspiratory breath holding following recovery from general anesthesia. Plos One 11 (6), 1-12.

Nath LC, Anderson GA, Hinchcliff KW et al. (2012) Clinicopathologic evidence of myocardial injury in horses with acute abdominal disease. J AM Vet Med Ass 241 (9), 1202-1208.

Nath LC, Anderson GA, Hinchcliff Kw et al. (2012) Serum cardiac troponin I concentrations in horses with cardiac disease. Aust Vet J 90, 351-357.[a]

Niki E. (2009) Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med 47, 469-484.

Niki, E. and Yoshida, Y. (2005) Biomarkers for oxidative stress: measurement, validation, and application. J Med Invest 52 Suppl, 228-230.

Noschka E, Were SR, Crisman MV et al. (2011) Implications of urine F2-isoprostane metabolite concentration in horses with colic and its potential use as predictor for surgical intervention. Equine vet J 43 (39), 34-41.

Nourooz-Zadeh J, Halliwell B, Anggard E et al. (1997) Evidence for the formation of F3-isoprostanes during peroxidation of eicosapentaenoic acid. Biochem Biophys Res Commun 236, 467-472.

Nourooz-Zadeh J, Liu EH, Anggard E et al. (1998) F4-Isoprostanes: a novel class of prostanoids formed during peroxidation of docosahexaenoic acid (DHA). Biochem Biophys Res Commun 242, 338-344.

Nyman G, Hedenstierna G (1989) Ventilation-perfusion relationships in the anaesthetized horse. Equine vet J 21 (4), 274-281.

Parchmann S, Mueller MJ (1998) Evidence for the formation of Dinor isoprostanes E1 from α-linolenic acid in plants. J Biol Chem 273, 3265-32655.

Pascoe PJ, McDonell WN, Trim CM, et al. (1983) Mortality rates and associated factors in equine colic operations – a retrospective study of 341 operations. Can Vet J, 24 (3), 76-85.

Pepys MB, Baltz ML, Tennent GA (1989) Serum amyloid A (SAA) in horses: objective measurement of the acute phase response. Equine Vet J 21, 106-109.

Platt JP, Simon BT, Coleman M et al. (2018) The effects of multiple anaesthetic episodes on equine recovery quality. Equine Vet J 50, 111-116.

Portier K, Crouzier D, Guichardant M, et al. (2009) Effects of high and low inspired fractions of oxygen on horse erythrocyte membrane properties, blood viscosity and muscle oxygenation during anaesthesia. Vet Anesth Analg 36, 287-298.

Preiser JC (2012) Oxidative Stress. Journal of Parenteral and Enteral Nutrition 36 (3), 147-154.

Proudfoot J, Barden A, Mori T et al. (1999) Measurement of urinary F(2)-isoprostanes as markers of in vivo lipid peroxidation – a comparison of enzyme immunoassay with gas chromatography/mass spectrometry. Anal Biochem 272, 209-215.

Pryor WA, Stanley JP (1975) Suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymic production of prostaglandin endoperoxides during autoxidation. J Org Chem 40, 3615-3617.

Putman AK, Contreras GA, Sordillo LM (2021) Isoprostanes in Veterinary Medicine: Beyond a Biomarker. Antioxidants 10, 145, 1-16.

Radcliffe RM, Divers TJ, Fletcher DJ et al. (2012) Evaluation of L-lactate and cardiac troponin I in horses undergoing emergency abdominal surgery. Journal of Vet Emergency and Critical Care 22 (3), 313-319.

Raijmakers MT, Burton GJ, Jauniaux E et al. (2006) Placental NAD(P)H oxidase mediated superoxide generation in early pregnancy. Placenta 27, 158-163.

Rishniw M, Simpson KW (2005) Cloning and sequencing of equine cardiac troponin I and confirmation of its usefulness as a target analyte for commercial troponin I analyzers. J Vet Diagn Invest 17, 582-584.

Roberts LJ, Morrow JD (2000) Measurement of F2-isoprostanes as index of oxidative stress in vivo. Free Radic Biol Med 28, 505-513.

Rossi TM, Pyle WG, Maxie MG et al. (2014) Troponin assays in the assessment of the equine myocardium. Equine Veterinary Journal 46, 270-275.

Santos M, Fuente M, Garcia-Iturralde P, et al. (2003) Effects of alpha-2 adrenoceptor agonists during recovery from isoflurane anaesthesia in horses. Equine Vet J, 35 (2), 170-175.

Schneider C, Pratt DA, Porter NA et al. (2007) Control of oxygenation in lipoxygenase and cyclooxygenase catalysis. Chem Biol 14, 473-488.

Schreier T, Kedes L, Gahlmann R (1990) Cloning, structural analysis, and expression of the human slow twitch skeletal muscle/ cardiac troponin C gene. J Biol Chem 265, 21247-21253.

Seco-Diaz OM, Durando MM, Birks EK et al. (2014) Cardiac troponin I concentrations in horses with colic. J Am Vet Med Ass 245 (1), 118-125.

Sies H. (1985) Oxidative Stress, 1st ed, Academic Press, New York

Slack J, Boston R, Driessen B et al. (2011) Effect of general anesthesia on plasma cardiac troponin I concentrations in healthy horses. Journal of Vet Cardiology 13, 163-169.

Smith KA, Sheperd J, Wakil A et al. (2011) A comparison of methods for the measurement of 8-iso$PGF_{2\alpha}$: a marker of oxidative stress. Ann Clin Biochem 48, 147-154.

Soffler C, Campbell VL, Hassel DM (2010) Measurement of urinary F2-isoprostanes as markers of in vivo lipid peroxidation: a comparison of enzyme immunoassays with gas chromatography-mass spectrometry in domestic animal species. J Vet Diagn Invest 22, 200-209.

Sorenson PR, Robinson NE (1980) Postural effects in lung volumes and asynchronous ventilation in anaesthetized horses. J Apply Physiol 48, 97-103.

Staffieri F, Bauquier SH, Moate PJ, et al. (2009) Pulmonary gas exchange in anaesthetized horses mechanically ventilated with oxygen or a helium/oxygen mixture. Equine Vet J 41, 747-752.

Staffieri F, de Monte V, de Marzo C, et al. (2010) Effects of two fractions of inspired oxygen on lung aeration and gas exchange in cats under inhalant anaesthesia. Vet Anaesth Analg 37, 483-490.

Staffieri F, Franchini D, Carella GL, et al. (2007) A computed tomographic analysis of the effects of two inspired oxygen concentrations on pulmonary aeration in anesthetized and mechanically ventilated dogs. Am J Vet Res 68, 925-931.

Steffey EP, Wheat JD, Meager DM, et al. (1977) Body position and mode of ventilation influences arterial pH, oxygen, and carbon dioxide tensions in halothane-anesthetized horses. Am J Vet Res 38 (3), 379-382.

Tomsic K, Nemec SA, Nemec A et al. (2018) Influence of sevoflurane or propofol anaesthesia on oxidative stress parameters in dogs with early-stage myxomatous mitral valve degeneration. A preliminary study. Acta Veterinaru-Beograd 68 (1), 32-42.

Tsuzuki N, Sasaki N, Kusano K et al. (2016) Oxidative stress markers in Thoroughbred horses after castration surgery under inhalation anesthesia. J Equine Sci 27 (2), 77-79.

Tu BP, Weissman JS (2004) Oxidative protein folding in eukaryotes: mechanisms and consequences. J Cell Biol 164, 341-346.

Valverde A, Gunkel C, Doherty TJ, et al. (2005) Effect of a constant rate of infusion of lidocaine on the quality of recovery from sevoflurane or isoflurane general anaesthesia in horses. Equine Vet J, 37 (6), 559-564.

Vasiljevic M, Krstic V, Stankovic S et al. (2018) Cardiac troponin I in dogs anaesthetized with propofol and sevoflurane: the influence of medetomidine premedication and inspired oxygen fraction. Vet Anaesth Analg 45, 745-753.

Vettorato E, Chase-Topping ME, Clutton RE (2010) A comparison of four systems for scoring recovery quality after general anaesthesia in horses. Equine Vet J, 42 (5), 400-406.

Wagner AE, Mama KR, Steffey EP, et al. (2002) Behavioral responses following eight anesthetic induction protocols in horses. Vet Anesth Analg, 29 (4), 207-211.

Wagner AE, Mama KR, Steffey EP, et al. (2008) A comparison of equine recovery characteristics after isoflurane or isoflurane followed by xylazine-ketamine infusion. Vet Anaesth Anal, 35, 154-160.

Wendelborn DF, Seibert K, Roberts LJ (1988) Isomeric prostaglandin F2 compounds arising from prostaglandin D2: a family of eicosanoids produced in vivo in humans. Proc Natl Acad Sci USA 85, 304-308.

West JB (2008) Gas Exchange. In: Pulmonary Pathophysiology, the essentials, 7th ed, West JB. Wolters Kluwer Lippincott Williams and Wilkins, Philadelphia, Pennsylvania, pp.17-36.

West JD. and Marnett, L.J. (2006) Endogenous reactive intermediates as modulators of cell signaling and cell death. Chem Res Toxicol 19, 173-194.

Whitehair KJ, Steffey EP, Willits NH, et al. (1993) Recovery of horses from inhalation anesthesia. Am J of Vet Res, 54 (10), 1693-1702.

Young SS, Taylor PM (1993). Factors influencing the outcome of equine anesthesia: a review of 1314 cases. Equine Vet J, 25, 147-151.

Zhang J, Gao J, Guo G, et al. (2018) Anesthesia and surgery induce delirium-like behavior in susceptible mice: the role of oxidative stress. Am J Transl Res 10, 2435-2444[a].

Zhang XY, Xu ZP, Wang W, et al. (2018) Vitamine C alleviates LPS-induced cognitive impairment in mice by suppressing neuroinflammation and oxidative stress. Int Immunopharmacol 65, 438-447[b].

Zhang Y (2018) Cell toxicity mechanism and biomarker. Clin Tranl Med 29, 34.