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Alternative Techniques for Alfaxalone Anesthesia Induction in Dogs and Cats

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To my family, Isabel Carvajal, Vicente Lagos, Andrea Lagos and Leonardo Lagos

I dedicate this victory to all of you who have always been my support and light throughout every pathway in my life. Your love, support, advice and encouragement have allowed me to achieve every goal in life. I always be in debt with you for all your selfless help.

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"The future belongs to those who believe in the beauty of their dreams"

-Eleanor Roosevelt

"I cannot change the direction of the wind, but I can adjust my sails to always reach my destination"

-Jimmy Dean

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Abstract

Alfaxalone is a neuroactive synthetic steroid (Brewster & Bodor, 1990) that produces anesthetic induction with dose- and speed-dependent cardiorespiratory depression in dogs and cats (Chiu et al. 2016; Warne et al., 2015). At clinical doses in unpremedicated dogs (2 mg kg-1) and cats (5 mg kg-1), alfaxalone induces a mild decrease in systemic vascular resistance, systemic blood pressure (Muir et al., 2009; Muir et al., 2008), apnea, hypoventilation, and hypoxemia (Muir et al. 2009; Muir et al., 2008). The cardiorespiratory side effects from alfaxalone induction could be prevented by reducing the total dose of alfaxalone necessary to produce general anesthesia. Therefore, the overall objective of this research dissertation was to investigate the reduction of alfaxalone induction dose by using it in two alternative anesthesia induction techniques, as follows: 1- priming principle of alfaxalone, in dogs and cats, and 2- co-induction of midazolam with a low dose of alfaxalone, in cats. This study also aims to investigate the cardiorespiratory effect of these alternative techniques of induction in dogs and cats.

Priming principle (Djaniani & Ribes-Pastor, 1999) consists on the administration of a pre-calculated low dose of an induction agent, administered prior to the following dose administration of the same induction agent until anesthesia is achieved (Kataria et al., 2010). The present study used priming principle with alfaxalone IV to achieve tracheal intubation in dogs and cats premedicated with dexmedetomidine and methadone. As results, the total dose of alfaxalone was significantly reduced by 27% in dogs and 25% in cats. Cardiorespiratory depression was not observed during the study.

Co-induction is the concomitant administration of two or more drugs with additive or synergistic effect (Sdrales & Miller, 2013). In humans, this induction technique has been well described using midazolam (Liao et al., 2017). The present study investigated the effective dose (ED_{50}) of midazolam to be used as co-induction with alfaxalone in cats. It was determined that the ED_{50} of midazolam is 0.08 ± 0.04 mg kg^{-1} when co-administered with a low dose of alfaxalone (0.25 mg kg^{-1}) in premedicated cats with methadone and dexmedetomidine.

Chapter One
Introduction

Veterinary anesthesia carries a risk for any patient (Brodbelt et al., 2008). Anesthesia-related mortality in dogs and cats was estimated as 1.35% in a large scale study evaluating 3546 small animals anesthetized (Bille et al., 2012). That rate can go as high as 2% in sick dogs and cats (Brodbelt et al., 2008). Clarke and Hall (1990) demonstrated that 22% of dogs and 39% of cats died during induction of anesthesia in a large scale studied developed. This is in agreement with the findings of Brodbelt et al. (2008) who reported the highest anesthetic-related fatalities at induction and also at recovery of anesthesia in dogs and cats (Brodbelt et al., 2008).

Adequate anesthetic monitoring of cardiorespiratory variables, tracheal intubation (TI) and safer anesthetic protocols with drugs that minimally depress the cardiorespiratory function have been linked with lower anesthesia-related fatalities in dogs and cats (Bille et al., 2012; Brodbelt et al., 2008). The most common complications reported in the literature at induction of anesthesia are systemic hypotension, cardiac dysrhythmias, apnea, hypoventilation, hypercapnia, respiratory acidosis, and hypoxemia. (Gaynor et al., 1999; Dyson et al., 1998). Over the last decades, many studies have focused on the development of safer induction techniques of anesthesia, with lower dose requirements that diminish the presentation of the previously mentioned complications.

Balanced anesthesia consists of the administration of two or more drugs that in combination produce better pharmacologic effect at lower total dose of each drug (Grimm et al., 2015). This overall drug decrease will diminish the dose-related side effects of each drug

(Stevens & Ktngston, 1989), leading to safer anesthesia and decreased cardiorespiratory depression (Ilkiw, 1999).   A safe anesthetic induction technique seeks to preserve cardiorespiratory function and therefore, decreases morbidity and mortality (Bille et al., 2012; Brodbelt et al., 2008). New techniques such as priming principle and co-induction are alternative methods of induction of anesthesia associated with lower total dose requirements of the induction agent in humans and veterinary patients (Short & Chui, 1991). Lower total doses of induction agents like propofol or alfaxalone may produce less hemodynamic and respiratory depression (Robinson & Borer-Weir, 2015; Muir et al., 2009; Muir et al., 2008).

Priming principle is a technique of induction reported in human anesthesia (Djaniani & Ribes-Pastor, 1999), where a pre-calculated sub-hypnotic low dose of an induction agent is administered few minutes prior to induction with the same anesthetic drug used for priming. (Karlo et al., 2015; Kataria et al., 2010). The priming dose corresponds to 20 to 25% of the conventional induction dose. (Karlo et al., 2015; Mehta et al., 2015). By utilizing the amnestic, sedative, and anxiolytic properties of propofol at sub-hypnotic dosages, this technique significantly decreased the total dose for induction of this injectable agent and therefore, minimized dose-dependent cardiorespiratory depression at post-induction of anesthesia in humans (Kataria et al., 2010; Djaniani & Ribes-Pastor, 1999).

In contrast, co-induction is the co-administration of two or more drugs, that together potentiate their anesthetic and sedative effects, and consequently minimizes the individual dose requirements for each drug (Kataria et al., 2010). Benzodiazepines are one of the most common groups of drugs used for co-induction in veterinary practice (Liao et al., 2017). Midazolam is a lipid-soluble imidazobenzodiazepine (Schwartz et al., 2012) that possesses a rapid onset of action, produces sedation, and generates centrally mediated muscle

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relaxation (Hopkins et al., 2013). By administering midazolam after propofol in humans and small animals, it has been demonstrated that there is a significant reduction in the total propofol dose needed for anesthetic induction (Robinson & Borer-Weir, 2015; Short & Chiu, 1991). Recently, the successful co-induction of alfaxalone and midazolam was reported for the first time in healthy dogs (Muñoz et al., 2017; Liao et al., 2017).

Alfaxalone is a neuroactive steroid that enhances the inhibitory action of the endogenous gamma aminobutyric acid (GABA) on the central nervous system and binds to the $GABA_A$ receptor to produce its anesthetic effect (Lambert et al., 2003). A clinical study demonstrated that rapid intravenous (IV) administration of alfaxalone, over a 5-second period, caused a decrease in arterial blood pressure and an increase in post-induction apnea in healthy dogs (Amengual et al., 2013). Additionally, alfaxalone has been reported to produce dose-dependent cardiorespiratory depression, most evident at supraclinical doses with systemic hypotension, increased heart rate, apnea, and hypoventilation reported as the most common side effects in dogs and cats (Muir et al., 2009; Muir et al., 2008). Alfaxalone also produces a significant drop in partial pressure of oxygen for arterial blood in a dose and speed dependent manner (Campagna et al. 2015; Suarez et al., 2012; Keates & Whittem, 2012). These cardiorespiratory side-effects support the recommendation of an alfaxalone titration dose whenever administered intravenously (Warne et al., 2015), and it justifies the evaluation of alternative induction techniques, such as priming principle and co-induction with midazolam in dogs and cats.

Chapter Two
Literature Review

2.1 Alfaxalone

In 1941, Seyle described, for the first time, the hypnotic effect of a synthetic water-insoluble progesterone-related steroid in rats (Seyle, 1941). This compound was derived from the pregnane and androstane groups (Seyle, 1941). Research developed in laboratory animals demonstrated that some steroid hormones' metabolites (pregnanolone and pregnanedione) possess anesthetic, analgesic, and muscle relaxation properties (Sear, 1996; Seyle, 1941). Interaction of steroid anesthetics with $GABA_A$ and its chloride channel are described as the mechanism of action for their hypnotic effect (Sear, 1996). The comparison between steroid compounds and barbiturates have demonstrated that steroids anesthetics possess greater therapeutic index, faster hepatic metabolism, and faster elimination than barbiturates (Sear, 1996).

A synthetic water-soluble steroid named hydroxydione was satisfactorily used as an anesthetic agent over ten years in dogs and cats (Taylor & Shearer, 1956). This steroid compound presented high therapeutic index, produced hypnosis, adequate muscle relaxation, quiet recovery, and cardiorespiratory stability (Montmorency et al., 1958; Galley & Rooms, 1956). Nonetheless, pain at injection, thrombophlebitis, and prolonged induction were reported (Montmorency et al., 1958; Galley & Rooms, 1956). Due to these undesirable side effects, in 1956, pharmacologists and chemists started studying new alternative steroid compounds to be used as anesthetic agents (Sear, 1996).

Later, the following key structural features of steroid compounds were reported for making them safer and more potent (Sear, 1996): 1) the presence of an oxygen molecule at both steroid molecule's endings is required for anesthetic activity; 2) substitution inside of the molecule with additional hydroxy groups decreases anesthetic properties; 3) 5α and 5β compounds are highly active, with the 3α-hydroxy-5β molecule presenting the highest anesthetic activity; 4) esters of hydroxyl are less active in the steroid molecule than alcohols (Phillips, 1975).

Based on those structural findings, a new steroid compound, alfaxalone (3α-hydroxy-5-pregnane-11,20-dione) in combination with alfadolone acetate (acetoxy-3-hydroxy-5-pregnane-11,20-dione) and a vehicle (Cremophor EL), was tested for induction of anesthesia in mice, rats, cats, dogs, rabbits and monkeys (Child et al., 1971). This three-in-one water-insoluble drug presentation was denominated as CT 1341 (Child et al., 1971). Both alfadolone and alfaxalone are hydrophobic, therefore, the commercial presentation included polyoxyethylated castor oil-based surfactant (Cremophor EL; 20% W/V, BASF Fine Chemicals, Limburgerhof, Germany) to increase alfaxalone's solubility (Child et al., 1971). Alfaxalone is a synthetic neuroactive steroid that produces a positive allosteric modulation of the GABA$_A$ receptor (Lambert et al., 2003). Its direct binding to this protein receptor potentializes the GABA neurotransmitter effect, which causes an influx of chloride ions into the cell with a secondary hyperpolarization and inhibition of the forthcoming action potential (Lambert et al., 2003), inducing in this way general anesthesia and muscle relaxation (Harrison & Simmonds, 1984). Alfadolone corresponds to an alfaxalone-related steroid that possesses approximately half of alfaxalone's potency (Sear, 1996).

Years later, a new commercial presentation of alfaxalone, free of alfadolone and cremophor EL, was released into the market (Estes et al., 1990; Brewster et al., 1989). The reformulated alfaxalone consisted of large sugar molecules called cyclodextrines (2-hydroxypropyl-β-cyclodextrin; HPCD) that increase the solubility of the steroid compound (Brewster & Bodor, 1990). HPDC are cone-shaped cyclic amylase-derived oligomers that originate from starch degradation, with a hydrophobic center and hydrophilic exterior that offer solubility to the alfaxalone molecule (Brewster & Bodor, 1990). The exterior of these sugar molecules are water-soluble, and the inside has a hydrophobic domain that possesses space for interaction with hydrophobic molecules such as steroids like alfaxalone (Warne et al., 2015). Based on studies in humans and rats, HPCD are excreted unchanged through the kidneys (Gould & Scott, 2005).   Since alfaxalone is a steroid highly insoluble in water (Madder et al., 2010), by adding HPCD to the alfaxalone molecule, its solubility increases 375 times (Rodriguez et al., 2012; Muir et al., 2008). The ratio between alfaxalone and molar HPDC is 1:1, consequently the compound behaves as a sole molecule to create an isotropic solution in water (Warne et al., 2015). The molecule alfaxalone-HPDC must dissociate to permit alfaxalone to obtain its equilibrium between the bound part linked to plasmatic proteins and cell membranes, and the free unbound alfaxalone molecule (Warne et al., 2015).

Alfaxalone-HPDC presentation possesses a high therapeutic index, has not been clinically associated with allergic reactions and is chemically stable (Warne et al., 2015; Ambros et al., 2008). It produces dose-dependent cardiorespiratory depression and anesthetic effect, and has an excellent safety margin with a single lethal dose in dogs as high as 5000 mg kg^{-1} (Muir et al., 2008). Alfaxalone-HPDC is an alternative to propofol and thiopental for fast induction short anesthesia in small animals (Warne et al., 2015). It does not cause

perivascular damage or tissue irritation (Ambros et al., 2008), it does not promote bacterial growth like propofol does, and its injection does not produce pain (Rodriguez et al., 2012). Other advantages of alfaxalone when compared with other induction agents available in dogs and cats are the rapid induction of anesthesia and recovery, good muscle relaxation, quick recovery of consciousness, high tolerance, a wide margin of safety (Ambros et al., 2008), and intramuscular and subcutaneous administration for sedation in cats (Ramo et al., 2013).

2.1.1 Alfaxalone-HPDC in dogs

Alfaxalone-HPDC pharmacokinetic analysis was initially done in beagles (Ferré et al., 2006), and later in Greyhounds (Pasloske et al., 2009). Both studies employed noncompartmental analysis to estimate the pharmacokinetic parameters in this species (Pasloske et al., 2009; Ferré et al., 2006). By administering a clinical 2 mg kg^{-1} dose of alfaxalone-HPDC in 8 healthy beagle dogs the following results were obtained: anesthetic time of 6.4 ± 2.9 minutes, plasma clearance of 59.4 ± 12.9 ml kg^{-1} min^{-1}, harmonic mean plasma terminal half-lives ($t_{1/2}$) of 24.0 ± 1.9 minutes and a volume of distribution (V_d) of 2.4 ± 0.9 L kg^{-1},plasmatic concentration levels were registered up to 2 hours after induction of anesthesia, and the peak plasmatic concentration was 2.3 ± 1.5 mg L^{-1} (Ferré et al., 2006). Sighthound canine breeds, such as greyhound dogs, have been demonstrated to present lower metabolic and clearance rate of some injectable anesthetics (Pasloske et al., 2009). Pharmacokinetic values obtained from un-premedicated greyhounds induced with alfaxalone at 2 mg kg^{-1}, were compared against the alfaxalone pharmacokinetic profile reported by Ferré et al. (2006) in beagles, and it was concluded that on overall the pharmacokinetic

profile of un-premedicated greyhounds (Pasloske et al., 2009) and beagles (Ferré et al., 2006) is fairly similar.

Since alfaxalone-HPCD does not accumulate in plasma or tissues, this drug can be safely administered as a continuous rate infusion for partial or total intravenous anesthesia in dogs (Rodriguez et al., 2012; Suarez et al., 2012; Ambros et al., 2008). A continuous rate infusion of $0.1 \text{ mg kg}^{-1} \text{ min}^{-1}$ of alfaxalone-HPDC has been linked with systemic hypotension and hypoventilation, while a lower dose of $0.07 \text{ mg kg}^{-1} \text{ min}^{-1}$ provides an adequate anesthetic plane without cardiorespiratory depression (Ambros et al., 2008). Alfaxalone-HPDC administered as a continuous rate infusion produces excellent muscle relaxation, as well as rapid recovery of consciousness (Jiménez et al., 2012; Ambros et al., 2008).

Alfaxalone-HPDC dose recommendation varies based on the administration of premedication prior to the induction of anesthesia (Ambrosio et al., 2008). Commonly, premedication is composed of an opioid alone or in combination with another drug such as phenothiazine, a $\propto$-2 agonist, or a benzodiazepine (Kojima et al., 2002). Since premedication produces not only analgesia but also sedation, the requirements of an induction agent are lower (Ambros et al., 2008; Weaver et al., 1990). Two commercial presentations of Alfaxalone-HPCD have been studied and their recommended doses in un-premedicated dogs are: 3 mg kg^{-1} for Alfaxan (Vetoquinol, Spain, 2008) (Rodriguez et al., 2012), and $2 - 3 \text{ mg kg}^{-1}$ for Jurox (Jurox Pty Ltd, Rutherford, Australia) (Maney et al., 2013). Nevertheless, the mean dose required to achieve unconsciousness and tracheal intubation in healthy un-premedicated dogs is reported to be 4.1 ng kg^{-1} (Rodriguez et al., 2012), 2.2 mg kg^{-1} (Ferré et al., 2006), 2.6 mg kg^{-1} (Maney et al., 2013), and 2 mg kg^{-1} (Muir et al., 2008). Then, for premedicated healthy dogs, the reported mean induction dose is 0.8 mg kg^{-1} (Maddern et al.,

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2010), and for premedicated sick patients classified as ASA III to IV dogs, 1 to 2 mg kg^{-1} (Psatha et al., 2011). The induction dose of alfaxalone-HPCD was proven to be decreased by 36% (total dose of 0.7 ± 0.3 mg kg^{-1}) when administered after butorphanol and medetomidine as premedication (Maddern et al., 2010). Additionally, the temperament of the animal and the quality of sedation were directly correlated with a lower induction dose in healthy dogs (Maddern et al., 2010).

Clinically, the quality of induction with alfaxalone-HPCD has been described as good to excellent in premedicated (Maddern et al., 2010; Ambros et al., 2008) and un-premedicated healthy dogs (Rodriguez et al., 2012; Muir et al., 2008, Ferré et al., 2006). In healthy unpremedicated beagle dogs, a clinical or supra-clinical dose of alfaxalone-HPDC (2 and 10 mg kg^{-1}, respectively) allowed rapid and smooth induction of anesthesia and tracheal intubation (Ferré et al., 2006). Administration of clinical (2 mg kg^{-1}) and supraclinical doses (6 and 20 mg kg^{-1}) of alfaxalone-HPDC produced excellent, short-term, smooth, and uneventful induction and recovery of anesthesia in healthy un-premedicated adult dogs (Muir et al., 2008).

Healthy dogs induced with alfaxalone HPDC presented minimal to no response to toe pinch and buccal mucosal stimulation, as well as, great muscle relaxation (Muir et al., 2008). In the same study, the time recorded from lateral to sternal recumbency was approximately 20 minutes after a single dose of 2 mg kg^{-1}, and 80 minutes after a supraclinical single dose of 20 mg kg^{-1} (Muir et al., 2008). Quality of induction between ketamine, propofol, and Alfaxalone-HPCD demonstrated that alfaxalone-HPCD provided a better quality of induction than ketamine, and similar results to propofol (White & Yates,

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2017). However, dogs anesthetized with alfaxalone-HPDC took a longer time to reach sternal recumbency and standing position than dogs induced with propofol (Maney et al., 2013).

Alfaxalone-HPDC induction produces dose- and time-dependent respiratory depression, with a significant decrease in minute ventilation ($\dot{V}E$) (Suarez et al., 2012; Muir et al., 2008). Post-induction apnea has been reported as the most common side effect of alfaxalone-HPDC in healthy dogs (Bigby et al., 2017; Muir et al., 2008). The apnea, recorded by Muir et al., (2008) in healthy dogs lasted between 1 to 3 minutes, and was directly related to the dose of alfaxalone administered (Muir et al., 2008). When compared with propofol, similar levels of hypoventilation have been reported after induction with either alfaxalone or propofol in dogs (Suarez et al., 2012). Ventilatory support and oxygen supplementation, to assist in cases of post induction apnea and short-lived hypoxemia, have been recommended by some authors (Bigby et al., 2017; Rodriguez et al., 2012). Even though hypoventilation and post-induction apnea have been reported in dogs, contradictory studies have demonstrated that doses of alfaxalone-HPDC as high as 4 mg kg^{-1} administered in healthy un-premedicated dogs did not produce the side-effects (Maney et al., 2013). Acepromazine and dexmedetomidine, with or without buprenorphine, administered as a premedication before alfaxalone-HPDC induction, did not seem to affect the incidence or severity of post-induction apnea presentation in healthy dogs (Bigby et al., 2017; Herbert et al., 2013). The speed of injection and not the total dose of alfaxalone-HPDC injected could be the explanation for the variation among the study results.

In healthy un-premedicated dogs, doses of 2 mg kg^{-1} minimally impacted tidal volume, respiratory rate, $PaCO_2$, PaO_2 and pH (Muir et al., 2008), while supraclinical doses (6 and 20 mg kg^{-1}) induced dose-dependent respiratory depression with a significant decrease

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in V̇E, hypercapnia, hypoxemia, and a significantly increase in apnea incidence (Muir et al., 2008). Similar results were reported after alfaxalone-HPCD was used to induce anesthesia in un-premedicated healthy dogs, where significant hypoventilation, with hypercapnia, and a decrease in pH during the post-induction period, were reported (Maney et al., 2013). Rodriguez et al. (2012) demonstrated hypoxemia that lasted for up to 10 minutes after a mean induction dose of alfaxalone-HPDC at 4.15 ± 0.7 mg kg^{-1} in un-premedicated healthy dogs (Rodriguez et al., 2012). Alfaxalone-HPDC related respiratory depression has been linked to depression of the respiratory center, cortex, and brainstem (Warne et al., 2016).

In regards to the cardiovascular function, administration of alfaxalone-HPDC at clinical doses (2 mg kg^{-1}) produced minimal cardiovascular depression in healthy dogs, with a slight decrease in systemic vascular resistance (SVR) as the most remarkable change (Muir et al., 2008). At supraclinical doses (6 and 10 mg kg^{-1}), alfaxalone produced a dose-dependent decrease in cardiac output (CO), arterial blood pressure, SVR, and an increase in heart rate (HR) in healthy un-premedicated dogs (Muir et al., 2008). The normal to higher CO reported by Muir et al. (2008) at clinical dose in face of a slight decrease of SVR could be secondary to the increased HR or a possible increase in myocardial contractility (Muir et al., 2008). Nonetheless, contractibility was not assessed by Muir et al. (2008) (Muir et al., 2008).

Ambros et al. (2008) demonstrated that the cardiac index is maintained at steady levels after induction of anesthesia due to an increase in HR without significant changes in systemic blood pressure and SVR in dogs. Dissimilar findings were reported in 2012, after the administration of alfaxalone-HPCD to effect in healthy unpremedicated dogs, where

significant tachycardia with an increase in cardiac index and a significant decrease in SVR index were reported (Rodriguez et al., 2012).

In healthy dogs, supraclinical doses of alfaxalone-HPDC (20 mg kg^{-1}) produced a significant long-lasting (for the duration of 60 minutes), decrease in systemic blood pressure and CO, probably secondary to decreased myocardial contractility or vasodilation (Muir et al., 2008). The dose required to achieve tracheal intubation in un-premedicated dogs does not change myocardial contractility but decreases SVR significantly during the first few minutes post-induction (Rodriguez et al., 2012). Therefore, the dose-dependent systemic hypotension reported could be mostly related to vasodilation instead of poor contractility (Rodriguez et al., 2012). Also, a significantly decrease in blood pressure, with values returning to normal baseline within 15 to 30 minutes, was associated with a high dose or high administration speed of alfaxalone-HPCD (Muir et al., 2008; Ambros et al., 2008).

Induction of anesthesia and cardiorespiratory impact is comparable between alfaxalone-HPDC and diazepam/fentanyl ± propofol in unhealthy ASA III to IV dogs premedicated with methadone (Psatha et al., 2011). However, alfaxalone-HPCD should be avoided in patients with tachycardia (Rodriguez et al., 2012). It has been postulated that a slower rate of alfaxalone-HPCD injection could minimize or even blunt the short-lived tachycardia (Rodriguez et al., 2012), however, this hypothesis has not been proven.

A smooth and uneventful recovery has been reported after alfaxalone-HPDC induction in dogs (Rodriguez et al., 2012; Psatha et al., 2011; Ambros et al., 2008), however complications such as excitation, agitation, paddling, minor muscle twitching, noise sensitivity, agitation, and even violent movements have been noted (Jiménez et al., 2012; Rodriguez et al., 2012; Madder et al., 2010). These side effects can be exacerbated in cases

of noisy environments during recovery, or poor quality of sedation prior to induction (Jiménez et al., 2012). Healthy un-premedicated dogs, anesthetized with alfaxalone-HPCD, presented excitement when the animals were handled for blood sample collection (Ferré et al., 2006). A quiet environment at recovery was recommended when using alfaxalone-HPDC for induction of anesthesia without premedication or with a minimal level of sedation (Jiménez et al., 2012).

Mean anesthetic duration of anesthesia after a dose of alfaxalone-HPDC at 2 mg kg^{-1} and 10 mg kg^{-1} was 6.4 ± 2.9 minutes and 26.2 ± 7.5 minutes in healthy beagles, respectively (Ferré et al., 2006). Other authors have reported a time of 9.8 ± 2.4 minutes (Muir et al., 2008) and 6.4 ± 2.9 minutes (Ferré et al., 2006) in un-premedicated dogs receiving 2 mg kg^{-1} of alfaxalone IV. Pasloke et al. (2007) also reported 7.1 ± 7 minutes and 35.0 ± 9 minutes in un-premedicated and premedicated healthy Greyhound dogs, respectively, after administration of a bolus of 2 mg kg^{-1} over 60 seconds.

In humans, gender has been shown to have an effect on some drug metabolism and recovery from anesthesia (Ciccone & Holdcroft, 1999). In spite of these findings in humans, no differences were found in the time of recovery between female and male canines after administration of alfaxalone as induction agent (Jiménez et al., 2012).

2.1.2 Alfaxalone-HPDC in cats

Alfaxalone is a suitable drug for total and partial intravenous anesthesia in cats, it presents rapid onset of action, short duration, quick redistribution, and short elimination half-life (Whittem et al., 2008). Additionally, adjustments in infusion rates present a rapid

changes in anesthetic depth (eye position, palpebral reflexes, jaw tone, among others), that allows titration of alfaxalone whenever used as a continuous rate infusion in cats (Campagna et al., 2014). The pharmacokinetics of alfaxalone in cats has been reported as nonlinear (Warne et al., 2015). To date, the therapeutic index of alfaxalone has not been reported yet in cats (Warne et al., 2015).

The recommended dose of alfaxalone in cats is 5 mg kg^{-1} IV (Mathis et al., 2012). Although, the reported alfaxalone induction doses vary based on the study, methodology, and premedication, the range usually falls between 1 to 7 mg kg^{-1} IV (Mathis et al., 2012; Taboada & Murison, 2010). However, induction doses have been reported as high as 11.6 ± 0.3 mg kg^{-1} after induction of premedicated cats with alfaxalone on a continuous rate infusion at the speed of 0.5 mg kg^{-1} min^{-1} (Campagna et al., 2015), and this high dose could possibly be associated with the high speed of administration.

Different dilutions of alfaxalone-HPCD have been assessed in dogs and cats as a possible technique to reduce the total alfaxalone induction dose (Maddern et al., 2010; Zaki et al., 2009). By diluting alfaxalone with saline to 5 mg ml^{-1} concentration, significantly reduced the total induction dose of alfaxalone-HPCD required to achieve tracheal intubation in cats (Zaki et al., 2009), most likely due to an overall slower rate of alfaxalone injection (Maddern et al., 2010). Similar findings have been reported in humans by diluting propofol when compared with the undiluted formulation of propofol (Kazama et al., 2000). The slow rate of injection of alfaxalone has been associated with higher alfaxalone potency and therefore, lower total dose requirement of the drug to achieve hypnosis (Bauquier et al., 2015).

Alfaxalone produces dose and rate-dependent respiratory depression in cats (Muir et al., 2009). Different doses of alfaxalone have been demonstrated to produce variables responses, with respiratory depression reported in some studies, and normal respiratory function reported in another studies. It is possible that the rate of administration of alfaxalone and the premedication used in each study are associated with the different respiratory responses reported in the literature. For example, Schwarz et al. (2014) did not find respiratory depression (apnea, hypoventilation or hypoxemia) after inducing and maintaining cats with alfaxalone. The opposite was found by Muir et al. (2009), where at clinical (5 mg kg-1) and supraclinical doses (15 and 20 mg kg-1) of alfaxalone produced significant decrease in respiratory rate, minute volume, drop in PaO2, and post-induction apnea. Campagna et al. (2015) reported that even when administering a high induction dose of alfaxalone (11.6 ± 0.3 mg kg^{-1}), the respiratory depression produced was moderate, and only 2 out of 10 cats became apneic during that study.

When a continuous rate infusion was compared between alfaxalone and propofol (10 mg kg^{-1} h^{-1} and 12 mg kg^{-1} h^{-1}, respectively), the observed respiratory depression was more profound in the propofol group than in the alfaxalone group, and the need for mechanical ventilation was higher during propofol CRI (Campagna et al., 2015).

Alfaxalone-HPCD administered at supraclinical doses (15 and 50 mg kg^{-1}) in healthy un-premedicated cats caused decrease in heart rate, cardiac output, and systemic pressure in a dose-dependent manner (Muir et al., 2009; Whittem et al., 2008).The administration of a clinical dose of 5 mg kg^{-1} can cause mild vasodilation, mild decrease in SVR, and increase in HR (Muir et al., 2009). However, other studies assessing similar clinical induction dose, reported the occurrence of systemic hypotension (Taboada & Murison, 2010; Whittem et al.,

2008). Due to this dose-dependent hemodynamic depression, the recommendation is that alfaxalone should be titrated to effect whenever administered intravenously in cats (Warne et al., 2015). The significant decrease in cardiac output could be associated with decrease in heart rate and stroke volume (Warne et al., 2015). Based on the results of Muir (2009), the decrease in cardiac output is secondary to decrease in myocardial contractility, once the preload and afterload remained relatively unaffected.

Alfaxalone and propofol used as induction agents in healthy cats produce similar cardiovascular effects with no significant differences between induction techniques (Taboada & Murison, 2010). However, the cardiovascular effect of alfaxalone has not been studied yet in unhealthy cats (Warne et al., 2015).

The effect of alfaxalone on cerebral hemodynamic is unknown and there is no report of hepatic or renal side-effects as well as, hematologic or biochemistry changes in cats (Warne et al., 2015). Alfaxalone does not cause irritation when administered perivascularly, intramuscularly, or subcutaneous (Warne et al., 2015).

Since the commercial preparation of alfaxalone does not contain a microbicidal preservative, its shelf-life is restricted to 6 hours after opening of the vial (Warne et al., 2015). Quality of recovery after induction of anesthesia with alfaxalone in cats premedicated with acepromazine and buprenorphine is overall good, but when compared to propofol, there was a higher number of episodes of paddling and trembling during alfaxalone recovery (Mathis et al., 2012). Similarly, others studies have reported side-effects such as agitation and noise hypersensitivity during recovery time in cats (Zaki et al., 2009; Ferré et al., 2006). One study reported an episode of short-lasting seizure in one out of 47 cats during recovery of anesthesia, where decreasing light and noise stimuli were enough to resolve the episode

(Mathis et al., 2012). Even though these complications have been linked to alfaxalone, the quality of induction and recovery has been defined as clinically acceptable and similar to propofol (Taboada & Murison, 2010). The median time elapsing from induction of anesthesia until standing was 21 minutes in healthy acepromazine and buprenorphine premedicated cats anesthetized for short medical procedures (Mathis et al., 2012).

2.2 Co-induction of Anesthesia

Mono-anesthetic induction technique was the common practice to achieve general anesthesia in the past (Grimm et al., 2015). In order to minimize total drug doses and its cardiorespiratory impact, balanced anesthesia started to take over the mono-anesthetic technique. Balanced anesthesia consists of the co-administration of two or more drugs injected at smaller doses than if administered separately; and pure-mu opioids, alpha-2 agonist, neuromuscular blockers, and benzodiazepines are some of the most common drugs used in this anesthetic induction technique (Grimm et al., 2015; Sanchez et al., 2013). Pure-mu opioids, alfa-2 agonist, neuromuscular blockers, and benzodiazepines are some of the most common drugs used in balanced anesthesia. Balanced anesthesia provides narcosis, muscle relaxation, sedation, and analgesia (Grimm et al., 2015).

The relationship among drugs co-administered at induction of anesthesia could be synergistic or additive (Short & Chui, 1991). An additive effect means that the concomitant injection of two or more drugs produced a pharmacologic effect equal to the addition of the effect of each drug (Sdrales & Miller, 2013). In case of synergistic (supra-additive) effect between two or more drugs, their co-administration will produce a pharmacologic effect

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bigger than the sum of the effect of each drug (Sdrales & Miller, 2013). The most common co-induction drugs administered in veterinary medicine are midazolam, diazepam, ketamine, and lidocaine (Liao et al., 2017).

Propofol and midazolam co-administration at induction of anesthesia (co-induction) demonstrated a synergistic pharmacologic effect in humans (Short & Chui, 1991), dogs (Sanchez et al., 2013), and cats (Robinson & Borer-Weir, 2015) and significantly decreased the total dose of propofol required to achieve general anesthesia. A further advantage of the co-induction technique is the attenuation in the dose-dependent cardiorespiratory depression observed in the mono-anesthetic induction technique (Sanchez et al., 2013).

2.2.1 Midazolam

Midazolam is a short-acting water-soluble imidazobenzodiazepine drug that can be administered subcutaneously, intramuscularly, or intravenously (Schwartz et al., 2012). It possesses a rapid onset of action, produces sedation, and centrally mediated muscle relaxation (Hopkins et al., 2013). Midazolam potentiates the activity of the neurotransmitter GABA at the $GABA_A$ receptor protein to produce sedation and central muscle relaxation (Nordt & Clarck, 1997).

This benzodiazepine has been widely used in human and veterinary anesthesia as a co-induction agent (Hopkins et al., 2014; Short & Chui, 1991). A significant reduction in the total dose of propofol necessary to produce general anesthesia was observed when co-administered with midazolam in humans (Adachi et al., 2001; Short & Chui, 1991). In veterinary anesthesia, midazolam satisfactorily spared the total induction dose of propofol in

dogs and cats (Robinson & Borer-Weir, 2015; Robinson & Borer-Weir, 2013), although, the sparing effect here was not dose-dependent (Robinson & Borer-Weir, 2015; Robinson & Borer-Weir, 2013).

In humans, midazolam has an anxiolytic effect (Riker et al., 2009), whereas, in healthy dogs and cats, when midazolam was given intravenously alone, it produced excitement and increased motor activity (Covey-Crump & Murison, 2008; Stegmann & Bester, 2001). This effect could possibly be associated with disinhibition of suppressed behavior or a reaction of a decreased muscle tone (Robinson & Borer-Weir, 2013). Other midazolam side-effects reported in the literature are ataxia, profound weakness, and hyperresponsiveness to noise in small animals (Hopkins et al., 2013; Stegmann & Bester, 2001). The administration of midazolam prior to propofol has been associated with myoclonic twitching and paddling in dogs (Hopkins et al., 2014).

Midazolam sparing effect of propofol dose depends on the order of the administration of both agents (Covey-Crump & Murison, 2008). If midazolam is administered in sedated dogs prior to the induction of propofol, it does not reduce propofol dose (Covey-Crump & Murison, 2008). However, if midazolam is injected after the induction of propofol, it satisfactorily reduces the total propofol dose needed for tracheal intubation and general anesthesia in both, dogs and cats (Robinson & Borer-Weir, 2015; Robinson & Borer-Weir 2013; Sánchez et al., 2013).

In small animals, the cardiovascular depression produced by midazolam is negligible (Robinson & Borer-Weir 2013), although there are studies demonstrating a decrease in systolic arterial pressure after its administration (Hopkins et al., 2014; Adams et al., 1985). In a study performed in humans with cardiomyopathy, anesthetized for a cardiac surgery,

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midazolam did not affect the hemodynamic function (Samuelson et al., 1981). The hemodynamic safety and stability effects of midazolam were demonstrated at clinical doses in dogs (0.25 mg kg^{-1}) but not at a supraclinical dose (10 mg kg^{-1}) (Jones et al., 1979). However, even with the high doses of midazolam administered in the study done by Jones (1979), no changes in coronary blood flow, systemic or coronary vascular resistance, stroke volume, or stroke work were observed (Jones et al., 1979).

Recently, the sparing effect of midazolam as co-induction with alfaxalone on the total required alfaxalone dose for induction in healthy dogs was investigated. (Liao et al., 2017; Muñoz et al., 2017). Midazolam has a significant sparing effect when administered after a low initial dose of alfaxalone at 0.5 mg kg^{-1} (Liao et al., 2017) and 0.25 mg kg^{-1} (Muñoz et al., 2017). A possible synergistic or additive effect on the GABA$_A$ receptor between alfaxalone and midazolam was hypothesized as an explanation for the significant sparing effect of this co-induction technique (Muñoz et al., 2017). The combination of alfaxalone and midazolam produced a better quality of induction when compared with sole alfaxalone induction technique in fentanyl-sedated dogs (Liao et al., 2017). To date, there are no studies evaluating the influence of midazolam administration's order, before or after alfaxalone, on the sparing effect of the total dose of alfaxalone.

2.3 Priming principle

The priming principle was first described in human anesthesia to facilitate tracheal intubation with non-depolarizing neuromuscular blockers (NDNB) (Foldes, 1984). These drugs are used in humans to facilitate tracheal intubation (TI) in a fast sequence of induction

(Taboada et al., 1986). Since some of the NDNB have a slow onset of action (2 to 3 minutes), a faster paralysis could be attained by administering a low sub-paralyzing dose of a NDNB, prior to the larger intubating dose of the same drug (Taboada et al., 1986). This technique was named priming principle, and the sub-paralyzing dose was denominated priming dose (Foldes, 1984). The priming dose should be large enough to develop a moderate level of the block in the neuromuscular transmission (Foldes, 1984). That level of blockage has been correlated with the occupancy of 75% of the acetylcholine endplate receptors by the NDNB (Foldes, 1984). By testing different small doses of vecuronium it was determined that the priming dose should be 15 to 20% of the dose needed to achieve TI (50 to 60% of effective dose 95; Taboada et al., 1986) (Foldes, 1984). The technique consisted of first administering the priming dose of the NDNB, and then immediately after a large intubating dose of the NDNB (Foldes, 1984). The time elapsed between priming dose and the large dose was 2 to 4 minutes (Kumar et al., 2006). By administering the neuromuscular blocker in this way, at the end of the second larger dose, a profound neuromuscular paralysis was obtained (Foldes, 1984). This effect can be explained by the occupancy of 90% of the acetylcholine receptors after the large dose injection (Foldes, 1984; Paton & Waud, 1967). Apart from speeding and facilitating TI, the priming principle also reduced the total dose of the NDNB by 20 to 35% (Foldes, 1984), and also the duration of the clinical effect of the priming dose; it diminished the presentation and severity of dose-dependent NDNB side-effects, and allowed the rapid identification of hypersensitivity that prevented the administration of the second larger dose (Foldes, 1984).

Years later, the priming principle was extended to be used with propofol during induction of anesthesia in humans (Djaiani & Ribes-Pastor, 1999; Maroof & Khan, 1996).

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Since propofol is one of the most common injectable agents used in human anesthesia (Djaiani & Ribes-Pastor, 1999) and its cardiorespiratory depression is dose- and speed-related, new induction techniques, that minimize the total required dose to achieve general anesthesia, are desirable (Karlo et al., 2015). The priming principle, also denominated as auto-co-induction technique, consisted on the administration of a low sub-hypnotic pre-calculated dose of an injectable agent (priming dose) administered few minutes prior to the following larger dose administration of the same induction drug until general anesthesia is achieved (Mehta et al., 2015).The priming dose corresponds to 20 to 25% of the regular induction dose of an injectable anesthetic (Kataria et al., 2010; Kumar et al., 2006). By producing anxiolytic, sedative and amnestic effects, the priming dose aims to reduce the sympathetic drive (Anderson & Robb, 1998), and to significantly reduce the total dose needed to produce hypnosis (general anesthesia) allowing TI (Kataria et al., 2010; Kumar et al., 2006).

The use of priming principle with propofol, in healthy humans, is reported to significantly reduce the total dose of propofol by 10.2% (Karlo et al., 2015) and 31.9% (Kataria et al., 2010). However, in geriatric humans (>70 years old), ASA I to III,   no significant propofol dose reduction was registered with the priming technique (Jones et al., 2002). The sparing effect of the technique over thr total propofol dose has been associated with significantly less cardiorespiratory depression at induction of anesthesia, in a dose-dependent manner (Mehta et al. 2015; Kataria et al. 2010). Significant lower drop in systemic blood pressure at post-induction was also registered when compared to the traditional technique of induction with propofol (Kataria et al., 2010). Less hemodynamic depression was described in the studies carried out by Djaiani and Ribes-Pastor (1999), Kumar et al.

(2006) and Mehta et al., (2015) (Mehta et al. 2015; Kumar et al, 2006; Djaiani & Ribes-Pastor, 1999). Similarly, the respiratory function was preserved by applying the priming principle with propofol (Kumar et al, 2006). Post-induction apnea was registered in 66.6% of the human patients receiving the traditional technique of propofol, versus 8.3% of patients receiving propofol priming principle (Djaiani & Ribes-Pastor, 1999).

Anderson and Robb (1998) postulated that the effective sparing effect of priming propofol was secondary to a possible anxiolytic effect produced by the sub-hypnotic dose of propofol (priming dose) injected prior to the full induction dose of the same agent (Anderson & Robb, 1998). Differences in ranges of total dose reduction could be in part associated with the different criteria used to determine the end of the induction time, and therefore, the end of the administration of the larger propofol dose in each study. The most common criteria used by the investigators was: loss of eyelashes reflex (Mehta et al, 2015; Pratap et al., 2015; Kumar et al, 2006), bispectral value lower than 45 (Kataria et al., 2010), loss of verbal contact (Karlo et al., 2015; Pratap et al, 2015; Jones et al., 2002; Anderson & Robb, 1998), or response to placement of a facemask (Anderson and Robb, 1998). None of these criteria are used in veterinary anesthesia.

When comparing midazolam-propofol co-induction *versus* priming propofol in adult humans ASA I and II, the drug sparing effect of midazolam co-induction was higher than priming propofol (40% *versus* 23%, respectively) (Djaiani & Ribes-Pastor, 1999). Similar results were reported in a comparable study assessing the same co-induction technique and priming propofol in a similar patient population (adult human ASA I and II) (Kataria et al., 2010). In that study, it was found that midazolam-propofol co-induction reduces propofol

requirements by 45.4%, while the priming technique reduced the total propofol dose to a lesser extent (31.9%) (Kataria et al., 2010).

The time interval between the priming dose and the larger induction dose has not been established yet. Most of the studies with propofol employed a 2-minute time interval (Karlo et al., 2015; Mehta et al., 2015; Pratap et al., 2015; Kataria et al., 2010; Jones et al., 2002; Djaiani & Ribes-Pastor, 1999). Only one study used 1-minute time interval (Pratap et al., 2015), or time was not reported in the methodology of the study (Kumar et al., 2006). The speed of injection of the priming propofol dose is variable, with no rate standardized in human anesthesia and ranging over a few seconds as a bolus (Anderson & Robb, 1998), or over 30 seconds (Pratap et al., 2015), or at continuous rate infusion of 3 mg min^{-1} (Karlo et al., 2015), or the information was not provided in the study (Mehta et al., 2015; Kataria et al., 2010; Jones et al., 2002; Djaiani & Ribes-Pastor, 1999).

Studies in humans (Stoelting 1977; King et al. 1951) and in dogs (Riccó & Henao, 2014), have reported a reflex sympathetic stimulation during the tracheal intubation after the induction of anesthesia. The sympathetic reflex consisted of increased in HR and systemic blood pressure, and it was produced by the used of the laryngoscope and the action of tracheal intubation. This sympathetic reflex response at intubation was significantly attenuated with the use of propofol priming and midazolam-propofol co-induction in adult healthy humans (Kataria et al., 2010).

2.4 Up-and-down Method

In 1948 Dixon and Mood reported for the first time the up-and-down method (UDM) for binary response variables (yes or no outcome) (Dixon & Mood, 1948). The UDM is used for estimating the median of a distribution (Vágerö & Sundber, 1999), which corresponds to the point in which 50% of the subjects evaluated present a positive response to a treatment in the dose-response curve (Vágerö & Sundber, 1999). This curve is a graphical representation in the Cartesian plane of the increasing dose plotted on the X-axis and the corresponding response plotted on the Y-axis (Pace & Stylianou, 2007). The dose-response curve has a probabilistic meaning in binary responses (positive or negative outcomes) (Pace & Stylianou, 2007). The dose in which 50% of the subjects evaluated present the same outcome corresponds to the median effective concentration (EC_{50}) or effective dose 50 (ED_{50}) (Pace & Stylianou, 2007).

The UDM is applied to experiments in which there is only two possible outcomes (yes or not, positive or negative, etc.) (Dixon & Mood, 1948). This method was initially described to be used in experiments with explosives (Dixon & Mood, 1948) however, years later, it was extended to be used in bio-assays for toxicological experiments (Davis, 1971) Examples of the bio-essays determined by the UDM are: the determination of the EC_{50} or minimum alveolar concentration (MAC) of inhalant anesthetics (Pace & Stylianou, 2007), the determination of the minimum EC_{50} of lidocaine and bupivacaine required for analgesia during labor in women (Columb & Lyons, 1995), studies to determine the EC_{50} or ED_{50} of multiple drugs such as intrathecal and intravenous opioids (Sztark et al., 2005, Stocks et al., 2001), intravenous $\propto$-2 agonist (Stapelfeldt et al., 2005), intrathecal local anesthetics

(Ginosar et al., 2004), intravenous local anesthetics (DiGeronimo et al., 2014), among other applications in humans and veterinary species (Pace & Stylianou, 2007).

The UDM methodology consists on the administration of an initial dose (X) of a specific treatment. The X could be decided by aleatory determination according with researcher experience, the lowest dose of the treatment, or the dose believed to be closer to the effective dose (Pace & Stylianou, 2007; Dixon & Mood, 1948). It has not be demonstrated that any of the previous alternative modalities to decide X is more beneficial than the others. All subjects are used only once. The final outcome for each subject must be a quantal response (yes or no). According with the outcome of the X tested on the first subject (n_1), the following subject (n_2) receives either an increased or a decreased dose interval (Y). The dose interval between X and Y are also predetermined before the beginning of the experiment and must remain unchangeable during the experiment (Pace & Stylianou, 2007). Therefore, the n_2 always receives a higher or lower dose based on the response of n_1 (X+Y or X-Y, respectively) (Figure 2.1) (Pace & Stylianou, 2007; Davis, 1971; Dixon & Mood, 1948). Accordingly, the third subject (n_3) will receive either an increased or decreased dose based on the response obtained from the second subject (n_2) and so forth so on as described on figure 2.1 (Pace & Stylianou, 2007; Dixon & Mood, 1948). Therefore, every trial cannot be started until the final outcome of the previous evaluated subject is known, with exception of the initial trial (Brownlee et al., 1953).

Sequential methods must have an ending rule (Pace & Stylianou, 2007). The UDM can be stopped either when a determined total sample (N) size is reached or when at least four independent crossovers are recorded (Dixon & Mood, 1948; Dixon, 1965). A crossover

is defined as a positive or negative outcome of two subjects evaluated in a sequence (Dixon & Mood, 1948) (Figure 2.2).
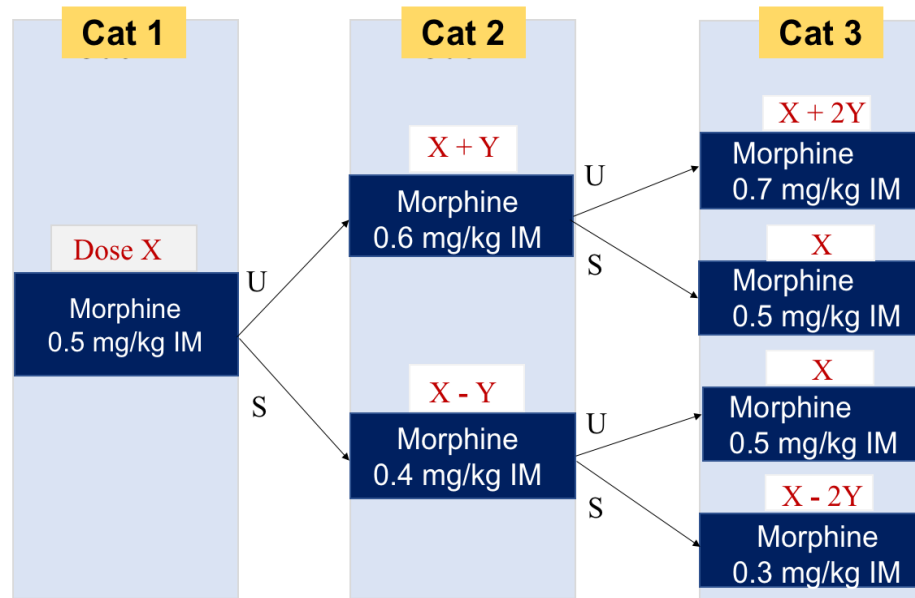
Figure 2.1. Example of an up and down design. X: Initial morphine dose (0.5 mg kg^{-1}) intramuscularly, Y: Morphine dose interval of $\pm$ 0.1 mg kg^{-1}, S: Successful sedation, U: Unsuccessful sedation. Example modified from Brandão, 2014.

Graphical display of the outcome of every trial is presented in most of the studies using UDM (Figure 2.2) (Pace & Stylianou, 2007). This illustration was first presented by Dixon and Mood (Dixon & Mood, 1948). Usually, the Y-axis presents the dose or concentration with the fix dose interval, and on the X-axis represents the subjects evaluated in a sequence (Figure 2.2). The positive or negative outcome are visualized in sequential order to facilitate the crossover identification (Pace & Stylianou, 2007) (Figure 2.2).

Figure 2.2. Case example of the graphical display of the results of the up-and-down method applied in 9 healthy cats tested for sedation (successful or unsuccessful sedation), after administration of morphine intramuscularly.
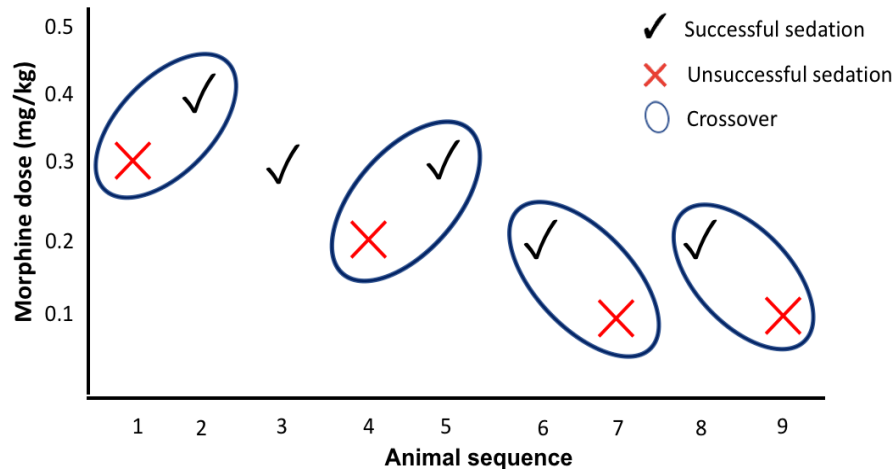
"Rejects" are described as subjects excluded in the UDM sequence due to failure of the inclusion criteria fulfillment, unexpected adverse reaction to drugs, protocol violation, uncertainty in drugs and treatments administered, or treatment administration failure (Pace & Stylianou, 2007). Once a subject is excluded, the next one in the sequence will receive the dose indicated for the subject excluded to avoid altering the sequence trial dose (Pace & Stylianou, 2007).The subjects or individuals evaluated with the UDM must be a random representative samples of the population studied (Vágerö & Sundber, 1999).

The UDM is used to reduce the number of subjects necessary for an experiment (N). Back in 1948, a sample size larger than 40 or 50 experimental subjects was required to obtain reliability results (Dixon & Mood, 1948). The UDM was designed to reduce the sample size by 30 to 40% (Dixon & Mood, 1948). In 1953, Brownlee et al. (1953) demonstrated UDM was a reliable technique for sample sizes with less than 10 subjects. Years later, Dixon (1965) reviewed its UDM by applying some statistical modifications to reduce furthermore the N

and evaluated to N as low as 6. In spite of the possibility to use the modified UDM with N of 6 subjects, most of the anesthetic researches have performed their studies with 20 to 40 subjects as sample size (Pace & Stylianou, 2007). Based on the results of some simulation studies using the UDM, it was confirmed that sample size from 20 to 40, will be enough to estimate the ED_{50} or EC_{50} in most circumstances (Pace & Stylianou, 2007).

In cases in which the N is as low as 6 subjects, it is important to start the bio-essays with the first trial testing X as close as possible to ED_{50} or EC_{50} (Dixon, 1965). Also, in this scenario the experiment is going to be performed until the N to be evaluated is assessed (Dixon, 1965). For determination of the ED_{50} or EC_{50}, various statistical models have been postulated and used. Pace and Stylianou (2007) after a literature review and analysis of 16 human anesthesia studies using UDM between 2000 to 2006, concluded that the use of isotonic regression with confidence interval derived by bootstrapping was an adequate technique for the estimator calculation. The isotonic regression is an adequate technique of analysis since the estimation of the effective dose minimally employs unverifiable assumptions such as normality (symmetry) of the distribution of the data in the dose-response curve (Pace & Stylianou, 2007). It is also suggested to avoid the probit and logit regression analysis (Pace & Stylianou, 2007). These two methods are used to extrapolate EC_{50} or ED_{50} to determine EC_{95} or ED_{95}, or other different quantiles (Pace & Stylianou, 2007). This extrapolation is not recommended since it cannot be assumed that the sigmoidal distribution of the dose-response curve are fitted by a symmetric logistic curve (Pace & Stylianou, 2007). That is an unverifiable assumption that should be avoided (Pace & Stylianou, 2007).

Advantages of the UDM are as follows: 1) reasonably simple study performance (Pace & Stylianou, 2007); 2) minimizes ethical concerns in toxicology studies (Pace &

Stylianou, 2007); 3) the statistical analysis is simpler than the regular methods of evaluation for dichotomy studies (Dixon & Mood, 1948); 4). This design concentrates the test close to the median, which minimizes the N evaluated when compared with regular methodologies in which various groups with larger same sample size are receiving different experimental dosages (Dixon & Mood, 1948).

In regards to the disadvantages of the method, it has been postulated as follows:

1) N is not necessarily reduced under some specific conditions, like evaluation of chemical insecticides in insects (Dixon & Mood, 1948). In this type of study, the subject of evaluation is a colony of insects instead of an individual insect, therefore the N is not reduced (Dixon & Mood, 1948);

2) The development of every sequential trial depends on the final outcome of the previous trial (Brownlee et al., 1953). This limitation in some type of studies can be time-consuming, especially in models in which the outcome could take days or even weeks (Brownlee et al., 1953). For this reason, UDM cannot be used if an outcome of a treatment takes longer than 48 hours (Pace & Stylianou, 2007);

3) As with other methodologies, the UDM's results could potentially present bias on the data collected or the way in which it is analyzed (Vágerö & Sundberg, 1999).

Maximum effort should be done to minimize bias and variability when developing clinical trials that target to find the ED_{50} (Pace & Stylianou, 2007). Bias in the UDM is defined as the difference existing between the real ED_{50} and the value estimated by the method (Pace & Stylianou, 2007). Some important requirements are needed in order to use the UDM. First, the variables evaluated should be normally distributed or should be transformed to become normally distributed (Dixon & Mood, 1948). Secondly, this method

is suitable to determine values that are close to the median like ED_{50} and not extreme percentages such as effective dose 99 (ED_{99}), or effective dose 95 (ED_{95}) (Dixon & Mood, 1948). The UDM has a high sensitivity estimating the median but not the smaller or larger extreme values (Dixon & Mood, 1948). The EC_{50} or ED_{50} estimated with the UDM does not offer reliable information of the upper tail of the sigmoidal dose-response curve, especially when the symmetric logistic curve cannot be verified due to the small samples used in this sequential methodology (Pace & Stylianou, 2007). Therefore, extrapolation from ED_{50} to find ED_{95} or ED_{99} must not be performed (Pace & Stylianou, 2007). Only in exceptional cases in which the normality of the distribution of the data can be completely guaranteed, the effective dose of extreme values (ED_{95} and ED_{99}, for example) could be calculated (Dixon & Mood, 1948). In cases in which other quantiles different from 50 are the aim of a study such as ED_{95} or ED_{99}, the biased coin design (BCD) should be applied instead of the UDM to avoid unverifiable extrapolations coming from EC_{50} or ED_{50} (Pace & Stylianou, 2007; Durham et al., 1997). The BCD has a similar sequential methodology as UDM, but it differs in a variable instead of fixed Y dose (Pace & Stylianou, 2007; Durham et al., 1997).

Chapter Three
Determination of Midazolam Dose for Co-induction with Low Dose of Alfaxalone in Cats

3.1 Introduction

Alfaxalone (3a-hydroxy-5a-pregnane-11, 20-dione) is a neuroactive synthetic steroid with positive allosteric modulation of the gamma-aminobutyric acid subunit A (GABA$_A$) protein receptor (Lambert et al., 2003). This effect inhibits neuronal excitability (Albertson et al., 1992), produces hyperpolarization of the neuron and prevents propagation of the action potential leading to general anesthesia (Lambert et al., 2003). Alfaxalone has been reported to cause dose-dependent hemodynamic depression in un-premedicated cats (Warne et al., 2015; Muir et al., 2009), with mild decrease in systolic blood pressure and systemic vascular resistance at clinical doses of 3 - 5 mg kg^{-1} (Taboada & Murrison, 2010; Muir et al., 2009; Whittem et al., 2008). Further decreases in cardiac output, systemic blood pressure, systemic vascular resistance, and heart rate (HR) have been reported at supraclinical doses (15 and 50 mg kg^{-1}) in cats (Muir et al., 2009). Also, alfaxalone has been described to negatively impact the respiratory function in a dose-dependent manner at clinical (Warne et al., 2015; Taboada & Murrison, 2010; Whittem et al., 2008) and supraclinical (Muir et al., 2009) intravenous (IV) doses in cats. Apnea, hypoventilation, and decreased partial pressure of oxygen are reported as the most common adverse effects in this species (Warne et al., 2015; Muir et al., 2009).

Midazolam is a water-soluble benzodiazepine that potentiates the activity of the neurotransmitter GABA over the GABA$_A$ receptor to produce sedation and central muscle

relaxation (Nordt & Clarck, 1997) without depressing the cardiorespiratory function when administered in dogs and cats (Robinson & Borer-Weir, 2015; Seddighi et al., 2011; Ilkiw et al., 1996). The technique of concomitant co-administration of two or more drugs with additive or synergistic effects for the induction of anesthesia has been described as co-induction (Hopkins et al., 2014; Covey-Crump & Murison, 2008) with the goal to reduce the total dose of each drug used (Robinson & Borer-Weir, 2013; Anderson & Robb, 1998). A lower total dose of alfaxalone has been associated with less cardiorespiratory depression in dogs and cats (Chiu et al., 2016; Warne et al., 2015; Muir et al., 2009; Muir et al., 2008). Midazolam is one of the most common co-induction agents employed in veterinary medicine (Liao et al. 2017). The alfaxalone-midazolam co-induction technique has been recently reported as an alternative technique of induction to induce general anesthesia and allows TI in dogs (Muñoz et al., 2017).

In 1948, Dixon and Mood reported the up-and-down method (UDM) for binary response variables (yes or no outcome), to determine the dose or concentration related to positive outcome in 50% of the sample evaluated in the dose-response curve (Pace & Stylianou, 2007; Dixon & Mood, 1948). The UDM was modified to be used for estimating the median of a distribution of small samples (Vågerö & Sundber, 1999), which corresponds to the point at which 50% of the subjects evaluated present a positive response to the treatment (Vågerö & Sundber, 1999). The dose-response curve has a probabilistic meaning in binary responses (quantal variables), where the dose at which 50% of the subjects evaluated present the same response corresponds to the median effective dose or effective dose 50 (ED_{50}) (Pace & Stylianou, 2007). This sequential methodology was initially used in human anesthesia to determine the effective concentration 50 (EC_{50}) or minimum alveolar

concentration (MAC) of inhalant anesthetics (Pace & Stylianou, 2007). Nowadays, the UDM is used in a wide number of studies evaluating the EC_{50} or ED_{50} of multiple drugs (Pace & Stylianou, 2007).

The aim of this study was to investigate the ED_{50} of midazolam required for TI when used as a co-induction with a low dose of alfaxalone (0.25 mg kg^{-1}) in cats. It was hypothesized that a low dose of alfaxalone (0.25 mg kg^{-1} IV) followed by midazolam co-induction technique would successfully induce general anesthesia and allow TI in cats.

3.2 Materials and Methods

3.2.1 Animals

Fourteen mixed-breed adult cats, 6 females and 8 males, between 5 and 12 years old, and weighing between 4.4 to 6.8 kg, were used. All cats were part of the LSU research feline colony. The night before the study, food was withheld for eight hours, but water was offered *ad libitum*. Animals were considered healthy based on a complete physical exam and blood work (complete blood count and serum biochemistry profile). The study was approved by the Louisiana State University Institutional Animal Care and Use Committee (2016/16100).

3.2.2 Study design

On the day of the study, cats were randomly assigned to a sequential allocation from 1 to 14 using a randomization software (www.randomization.com). Cats were premedicated with dexmedetomidine at 3 µg kg^{-1} (Dexdomitor, Zoetis Inc., United States) and methadone at 0.3 mg kg^{-1} (Methadone hydrochloride injection USP, Mylan, United States) administered intramuscularly in the semimembranosus or semitendinosus muscles. Quality of sedation was subjectively evaluated using a numeric rating scale from one to five (Appendix A.3) 20 minutes after premedication, by the same evaluator (AL). In sequence, a 22-gauge 2.5 cm (1-inch) catheter (Sur-Vet® Surflo ETFE, Terumo, United States) was aseptically placed into the medial saphenous vein of the left hind limb. All cats were instrumented to receive a multifunction monitor (VetTrendsV, SystemVet, United States) for the following measurements: HR and cardiac rhythm via a lead II electrocardiogram; systolic, mean, and diastolic blood pressures (SAP, MAP and DAP, respectively) via oscillometric technique using a cuff (width of 40% of forelimb circumference) placed on the mid-length of the radius bone; and also systolic blood pressure (SAPd) was measured via Doppler ultrasonic flow detector (Parks medical electronics Inc., United States) using the contralateral limb.

Cats were pre-oxygenated for 5 minutes with 100% oxygen (2 L/min) administered via face mask. Induction of anesthesia was performed by IV administration of alfaxalone at 0.25 mg kg^{-1} (Alfaxan; Jurox Pty Ltd, Australia) over 60 seconds using a calibrated precision syringe pump (CareFusion, Alaris PC, United States). Sixty seconds later, a predetermined midazolam dose, based on the UDM, was injected over 5 seconds and then TI intubation was attempted. The criteria for TI were the loss of lateral and medial palpebral reflexes, and jaw

tone, along with easy extrusion of the tongue out of the mouth. Lidocaine (0.01 mg kg^{-1}; Vetone®, United States) was splashed over the arytenoids (right after midazolam administration and 30 seconds prior to TI). All reflexes prior to the TI, and the TI itself, were qualitatively evaluated using a scoring system from 1 to 3 (Appendix A.2). Only one attempt of TI was performed per animal. A cuffed-endotracheal tube (4.5 outer diameter) and a laryngoscope were used in all cats. Syringe pump settings and drug administrations were performed by one investigator (PQW) while the assessment of reflexes, tracheal intubation and its scoring, were performed by a second investigator (AL) who was blind to the treatment used. If TI was not successful, the study with that cat was over. With a successful TI, the tracheal tube cuff was inflated to an intra-cuff pressure of 20 cmH$_2$O, measured with a manual manometer (Exactus II BMS, Tennessee, United States). In sequence, all intubated cats received additional monitoring: capnography for expired end-tidal carbon dioxide (PE$^{´}$CO$_2$) and respiratory rate (f_R), and pulse oximetry (placed on the tongue) for measurement of hemoglobin oxygen saturation (SpO$_2$) (VetTrends^{TM}V, SystemVet^{TM}, United States). Animals were allowed to spontaneously breathe room air (FIO$_2$=0.21) after intubation. In case of apnea (absence of spontaneous respiratory effort during 60 seconds) or SpO2 < 90% immediately after induction or at any point of the study, cats would receive intermittent positive-pressure ventilation via a reservoir bag of a Bain breathing circuit with 100% O$_2$ (2 L/min) and the event would be recorded.

Based on the UDM, a sequential model for midazolam dose was designed with the initial dose of 0.3 mg kg^{-1} and the variation dose of 0.1 mg kg^{-1}. Dosages were established prior to the beginning of the study and variation dose was maintained the same throughout the entire study (Dixon 1965; Stylianou & Fluornoy, 2002). The first cat out of the 14

received the initial midazolam 0.3 mg kg^{-1} IV (Midazolam injection USP, Akorn Inc., United States). Based on the response of this first cat, successful or unsuccessful TI, the second and following cats received the adjusted dose of midazolam ($\pm$ 0.1 mg kg^{-1}) (Figure 2.1). Therefore, if TI could not be performed on the first cat, the experiment with that animal would be over and the cardiorespiratory variables would be excluded from the analysis. Then, the midazolam dose would increase by 0.1 mg kg^{-1} for the second cat (0.4 mg kg^{-1}). In the opposite scenario, with a successful TI, midazolam dose would be decreased by 0.1 mg kg^{-1} for the second cat (0.2 mg kg^{-1}). In this way, the third and following cats would receive an adjusted midazolam dose ($\pm$ 0.1 mg kg^{-1}) according to the response to TI of the previous cat (successful or unsuccessful TI). From this UDM, a crossover was defined as a positive (successful TI) or negative (unsuccessful TI) effect observed between two sequential cats. In the present study, the sequential trials were followed until there were at least 4 sequential or non-sequential crossovers. Each cat was used only once.

3.2.3 Data collection

Cardiorespiratory variables (HR, f_R, SAP, DAP, MAP, and SAPd) were collected at baseline (defined as the experimental time after instrumentation and right before induction of anesthesia) and after alfaxalone (0.25 mg kg^{-1}) administration (IP$_1$). The same cardiorespiratory variables along with SpO_2 and $PE'CO_2$ were measured and collected immediately after TI (end of IP$_2$) and every two minutes after TI (TI$_2$, TI$_4$, TI$_6$, TI$_8$, etc) until cats were extubated. Quality of induction of anesthesia (1 to 3; Appendix A.3), TI (1 to 3; Appendix A.2), and recovery from anesthesia (1 to 3; Appendix A.4) were assessed and

scored (numerical scales) in all cats by the same blinded investigator (AL). Cats were extubated and allowed to recover as soon as they presented palpebral reflexes, strong jaw tone, and swallowing movements. Time under anesthesia was recorded in minutes from the successful TI until extubation. After extubation, cats were under investigators supervision (PQ and AL) until the cephalic catheter was removed, and cats were placed back into their cages. In case of dysphoria (excessive vocalization, anxiety, struggling) during recovery from anesthesia, acepromazine (0.01 mg kg^{-1}) (Acepromazine Maleate, VetOne, United States) IV was administered and the occurrence was recorded.

3.3 Statistical Analysis

Data analysis was performed using R 3.4.0 (R Core Team, Austria, 2017). The estimator and 95% confidence interval were obtained from six independent crossovers and an isotonic regression with bootstrapping simulation with R codes was employed for the determination of midazolam ED_{50} (Stylianou & Fluornoy, 2002; Pace & Stylianou, 2007). Logistic regression was used to analyze the correlation between sedation score and TI. A p-value <0.05 was considered statistically significant.

3.4 Results

All animals completed the study. Eight of 14 cats were successfully intubated (8/14) and six were not intubated (6/14) (Graph 3.1). After applying UDM in the 14 animals, six

independent crossovers were identified (Graph 3.1). The ED_{50} of midazolam was $0.081 \pm$ 0.045 mg kg^{-1} when administered in co-induction with a low dose of alfaxalone (Graph 1). Sedation score and successful TI presented a strong positive correlation with the logistic regression (p=0.02). All cardiorespiratory variables collected from the intubated and not intubated cats were within reference range, and none of the animals presented post-induction apnea or SpO_2 lower than 90%. The induction of anesthesia and recovery was uneventful in all cats. Smooth TI (one out of three; Appendix A.2) was recorded in seven of the eight cats, and fair TI (two out of three; Appendix A.2) was recorded in one of eight cats. Cats were anesthetized for 17.3 ± 7.5 minutes. None of the animals received acepromazine at recovery.
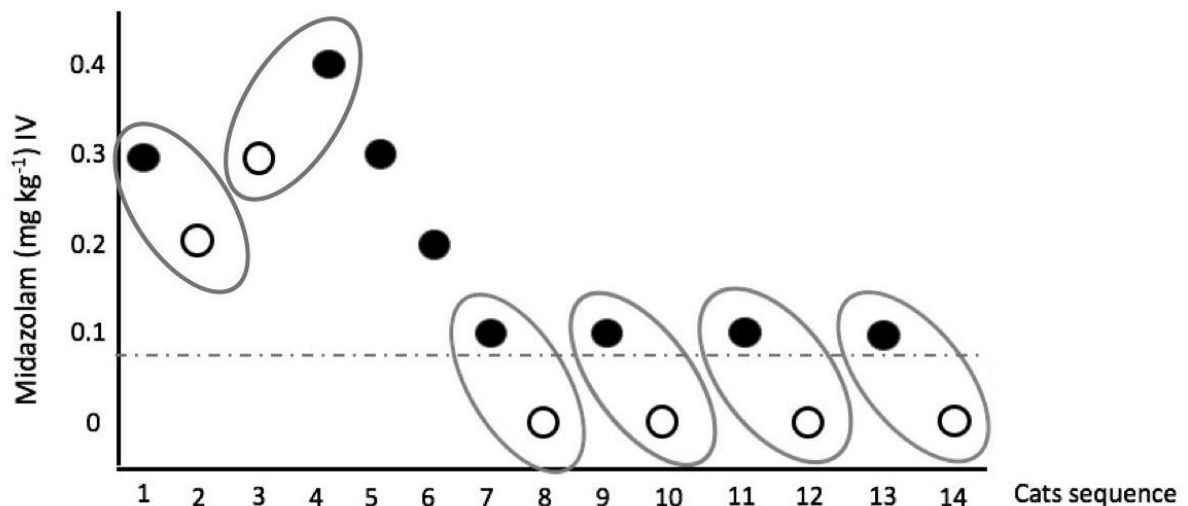
Graph 3.1. Graphical distribution of the results obtained in 14 cats after receiving a low dose of alfaxalone followed by a variable dose of midazolam determined by the up-and-down method. Initial midazolam dose was set at 0.3 mg kg^{-1}, and following midazolam doses were increased or decreased by equal dose spacing (± 0.1 mg kg^{-1}) based on the endotracheal intubation outcome of the previous cat. Crossover events are defined as two opposite outcomes (successful and unsuccessful endotracheal intubation) in two sequential animals. Solid circle: successful endotracheal intubation, open circle: unsuccessful endotracheal intubation, oval: crossover in two sequential cats, dashed line: ED_{50} of midazolam when co-administered with alfaxalone.

3.5 Discussion

The effective dose of midazolam needed to achieve TI, when used as co-induction with 0.25 mg kg^{-1} of alfaxalone in sedated cats, was 0.08 ± 0.04 mg kg^{-1}. This dose was calculated from six independent crossovers by applying the UDM (Pace & Stylianou, 2007) and it corresponds to the lowest midazolam co-induction dose with an injectable anesthetic described in the literature of cats (Robinson & Borer-Weir, 2015; Gorayeb et al., 2015; Bley et al., 2007).

The administration of a sub-hypnotic dose of propofol, ranging between 20 to 25% of the induction dose, (Mehta et al., 2015; Djaiani & Ribes-Pastor, 1999), has been reported to possess sedative, anxiolytic and amnestic properties in healthy adult humans (Mehta et al., 2015; Karlo et al., 2015; Djaiani & Ribes-Pastor, 1999). In a preliminary study developed by the same authors of this research, it was found that by administering a quarter of alfaxalone lower dose (1 to 3 mg kg^{-1}), one minute prior to the induction of anesthesia with the same induction drug (alfaxalone), it was significantly reduced the total mg of alfaxalone required to achieve hypnosis and TI in healthy cats (Lagos-Carvajal et al., 2017). Based on the result of that study, this research aimed to use the possible sedative effect of the same sub-hypnotic dose of alfaxalone (0.25 mg kg^{-1}) injected one minute prior to the midazolam co-induction.

This study corresponds to the first research assessing the alfaxalone-midazolam co-induction technique in cats. The same co-induction method has been recently reported in healthy dogs (Muñoz et al., 2017; Liao et al., 2017), and it has been explained by the possible agonistic effect of both drugs over the GABA$_A$ receptor (Muñoz et al., 2017). While alfaxalone causes allosteric modulation and binds directly to the GABA$_A$ receptor (Lambert

et al., 2003; Albertson et al., 1992), midazolam instead, enhances the affinity of the $GABA_A$ receptor for the neurotransmitter GABA (Mohler & Richards, 1988). In this way, the co-administration of these two drugs could potentially enhance the sedative, anesthetic and muscle relaxant effect of both drugs.

The midazolam dose interval ($\pm$ 0.1 mg kg^{-1}) was established prior to the beginning of the research project and was maintained the same throughout the study. (Pace & Stylianou, 2007; Stylianou & Flournoy, 2002; Dixon, 1965). According to the UDM, the seventh cat was successfully intubated at the dose of 0.1 mg kg^{-1} and the following cat in the sequence (eighth subject) needed to receive saline instead of midazolam. This same scenario was observed for cats 10, 12 and 14.

The lowest midazolam co-induction dose demonstrated in dogs when co-administered with alfaxalone at 0.25 mg kg^{-1} (Muñoz et al., 2017) or 0.5 mg kg^{-1} (Liao et al., 2017) was 0.3 mg kg^{-1}. In the current study, a much lower dose of midazolam (0.081 $\pm$ 0.045 mg kg^{-1}) in co-induction with alfaxalone was sufficient to allow TI. The lower requirements of midazolam found in this study could be explained by: 1) the sedative effects from premedication combined with the possible sedative or anxiolytic effect of the sub-hypnotic dose of alfaxalone; or 2) the possible synergistic effect of alfaxalone and midazolam over the $GABA_A$ receptor (as described by Munoz et al., 2017).

Overall, sedation score and successful TI presented a strong positive correlation as it was observed by the second and third cats, which had the poorest sedation score (one out of five; Appendix A.1). Those cats were not successfully intubated, even when they received doses of midazolam at 0.2 and 0.3 mg kg^{-1}, respectively (Graph 1). Nonetheless, independent of the level of sedation, TI was only successful when alfaxalone and midazolam were co-

administered. This was demonstrated in cats 8, 10, 12 and 14 that did not receive midazolam and were not successfully intubated (Table 1), even for cat 14 which received the best sedation score possible (score five out of five; Appendix A.1). This finding was unexpected by the authors since the alfaxalone dose administered corresponds to only a quarter of the lowest induction dose indicated for premedicated cats (1 to 3 mg kg^{-1}) (Zaki et al., 2009).

An important limitation of this study corresponds to the wide midazolam dose interval used, $\pm$ 0.1 mg kg^{-1}. Since the calculated ED$_{50}$ of midazolam described here was 0.08 $\pm$ 0.04 mg kg^{-1}, a narrower dose interval (0.02 or 0.03 mg kg^{-1}) could had prevented the no administration of midazolam in some of the experimental subjects.

Chapter Four
The Use of Priming Alfaxalone as an Induction Technique in Healthy Cats

4.1 Introduction

Alfaxalone is a neuroactive synthetic steroid approved in 2012 by the Food and Drug Administration (FDA) for use as an induction agent in dogs and cats in the United States (Warne et al. 2015). This induction agent enhances the inhibitory action of the endogenous gamma (γ) aminobutyric acid (GABA) on the central nervous system and binds to the $GABA_A$ receptor to produce its anesthetic effect (Lambert et al., 2003). Alfaxalone has been reported to produce rapid onset of action, short duration, quick redistribution, and short elimination in cats (Whittem et al., 2008). It also produces dose dependent depression of the respiratory function, manifested by decrease in minute ventilation and significant decrease in partial pressure of oxygen in the arterial blood of cats (Muir et al. 2009; Muir et al. 2008). Alfaxalone has been shown to promote cardiorespiratory depression in a dose and speed related manner (Muir et al., 2009; Whittem et al., 2008). When used in clinical doses (3 to 5 mg kg^{-1}) it induced mild decrease in systemic vascular resistance (Muir et al., 2009), systemic hypotension (Taboada & Murison, 2010; Whittem et al. 2008), and increase in heart rate (Muir et al., 2009) in healthy cats. Due to the dose-dependent cardiorespiratory depression, it is recommended to titrate the alfaxalone dose during anesthesia induction in cats (Warne et al., 2015).

Priming a drug for anesthesia induction is an alternative technique reported in human anesthesia (Kumar et al., 2006). This technique consists of the administration of a pre-

calculated sub-hypnotic low dose of an induction agent injected few minutes prior to induction with the same anesthetic drug until a state of general anesthesia is achieved (Karlo et al., 2015; Kataria et al., 2010). Usually, a priming dose corresponds to 20% of the conventional anesthetic induction dose (Mehta et al. 2015), although 25% has been also reported (Karlo et al., 2015). The priming technique is well described for propofol (Mehta et al., 2015; Kataria et al., 2010; Kumar et al., 2006). At sub-hypnotic doses, propofol has amnestic, sedative, and anxiolytic properties (Djaiani & Ribes-Pastor, 1999). In humans, priming of propofol significantly decreased the total dose of propofol, and therefore, minimized the cardiorespiratory depression immediately after induction, in a dose dependent manner (Mehta et al., 2015; Kataria et al., 2010).

The objective of this study was to investigate the effect of priming alfaxalone in the total induction dose of alfaxalone to achieve TI and its related respiratory effects in cats. It was hypothesized that the priming technique would significantly decrease the total dose (mg kg^{-1}) of alfaxalone required to achieve general anesthesia and allow TI. It was also hypothesized that the reduced total dose of alfaxalone would prevent the dose-dependent respiratory side effects of alfaxalone in cats.

4.2 Materials and Methods

Eight spay/neuter mixed-breed cats (4 females, 4 males), 5 to 12 years old and weighing 4.7 to 6.4 kg, were used in a crossover design. Health status was determined based on a complete physical examination, complete blood cell count, and serum biochemistry

panel. The study was approved by the Louisiana State University Institutional Animal Care and Use Committee (2016/16100).

Food was withheld for 8 hours prior to the study, but water was available until premedication. On the day of the study, all cats were premedicated with methadone (0.3 mg kg^{-1}; Mylan□, Rockford, Illinois, United States) and dexmedetomidine (3 mcg kg^{-1}; Dexdomitor, Zoetis Inc., United States) mixed in the same syringe and injected intramuscularly in the semitendinosus or semimembranosus muscles. Fifteen minutes after injection, the quality of sedation was assessed by the same evaluator (AL) using a numeric rating scale from 1 to 5 (Appendix B.1). Later, a 22-gauge 2.5 cm (1-inch) catheter (Sur-Vet Surflo ETFE, Terumo, United States) was placed aseptically into the medial saphenous vein. After IV catheterization, cats started to be monitored with an ECG lead II for heart rate (HR) and rhythm, and non-invasive blood pressure using an Oscillometric device (VetTrends, SystemVet, United States) for systolic, diastolic and mean blood pressure monitoring (SAP, MAP and DAP, respectively). The blood pressure cuff (sized 40% of the limb's circumference) was placed on the right forelimb at the mid third of the antebrachium.

After instrumentation, cats were pre-oxygenated for 5 minutes with 100% oxygen at 2 L min^{-1} via face mask. Cats were randomly assigned using a randomization software (www.randomization.com) to one of two anesthetic induction treatment groups in a crossover design with an 8-day washout period between treatments groups. The goal of the induction of anesthesia was to achieve TI. Anesthetic induction was divided into two phases: injection of saline or alfaxalone priming over 60 seconds (IP$_1$) then administration of alfaxalone as continuous infusion till TI was achieved (IP$_2$). A period of 60 seconds was allowed between IP$_1$ and IP$_2$ to observe any respiratory changes (decreased respiratory rate

or apnea) caused by the injections at IP1, priming alfaxalone and saline Treatment groups were as follows: Control (CG) using saline 0.025 mL kg^{-1} (same volume as it would be used for alfaxalone) over 60 seconds (IP$_1$) followed by 0.5 mg kg^{-1} min^{-1} of alfaxalone (Alfaxan, Jurox Inc., Australia) administered until TI was achieved (IP$_2$); and Priming (PG) using 0.25 mg kg^{-1} alfaxalone injected over 60 seconds (IP$_1$) followed by 0.5 mg kg^{-1} min^{-1} alfaxalone administered until TI. Alfaxalone and saline administrations were done with a precision syringe pump (CareFusion, Alaris PC, United States). A blinded investigator, AL, performed the TI based on the loss of palpebral reflexes (lateral and medial), jaw tone, and the ability to easily extrude the tongue out of the mouth. Lidocaine was splashed (0.01 mg kg^{-1}; Vetone, United States) over the vocal folds 30 seconds before TI, which was performed using a 4.5 mm outer diameter cuffed-endotracheal tube with the aid of a laryngoscope. While TI was performed, alfaxalone administration was stopped and the total milligrams of alfaxalone used were recorded for each cat for both treatment groups.

After the endotracheal tube was in place, the cuff was inflated at an intra-cuff pressure of 20 cmH$_2$O verified by a manual manometer (Exactus II BMS, Tennessee, United States). Induction quality and endotracheal intubation quality were assessed by the same investigator (AL) using scores from 1 to 3 (Appendix B.3 and B.2, respectively). Immediately after TI, a capnography adaptor for expired end-tidal partial pressure of carbon dioxide (PE$^\cdot$CO2) and respiratory rate (f_R) measurements, and a pediatric respirometer for tidal volume (Vt) measurements were attached to the proximal end of the endotracheal tube. Minute ventilation (V̇E) was calculated by the product of f_R and Vt. A pulse oximetry probe was placed on the tip of the tongue for determination of arterial hemoglobin oxygen saturation (SpO$_2$). A

multiparametric monitor (General Electric, United States) was used to display and record the above mentioned parameters.

Following hemodynamic parameters were assessed: HR, f_R, SAP, MAP and DAP were recorded at baseline (B), right after IP_1, and immediately after TI (IP_2). After TI, the same parameters (HR, f_R, SAP, MAP and DAP), along with the addition of $PE'CO2$, Vt and SpO_2, were recorded every two-minutes (TI_2, TI_4, TI_6, TI_8, etc.) until the animals were extubated. Throughout the entire experimental time, cats were monitored for apnea (absence of respiratory movements for 60 seconds), SpO_2 <90%, and systemic hypotension (MAP < 60 mmHg). In case of apnea, cats would have received intermittent positive-pressure ventilation with the reservoir bag of a Bain breathing circuit with 100% O_2 (2 L/min) and the event would have been recorded. In case of hypotension, affected cat(s) would have received a bolus of ephedrine (0.1 mg kg-1 IV).

Cats were extubated as soon as they presented lateral and medial palpebral reflexes and a strong jaw tone with head and/or body movements. All the monitoring equipment, and the intravenous catheter, were removed after extubation. The animals were then placed in their individual carriers and remained under observation for one hour. Quality of recovery was evaluated using a scale ranging from 1 to 3 (Appendix B.4). Total anesthesia time was recorded in minutes. In case of dysphoria during recovery, cats received acepromazine (0.01 mg kg^{-1}; Acepromazine Maleate, VetOne, United States) administered IV over the needle.

4.3 Statistical Analysis

Data analyses were performed using SAS (9.4 software, SAS Institute Inc., United States). Data were analyzed at B, IP_1, IP_2, TI_2, TI_4, TI_6 and TI_8. A one-way mixed analysis of variance (ANOVA) model was used to analyze the variables measured without any time point (sedation, induction, TI and recovery score and total mg of alfaxalone) with a drug used (treatment) as the fixed effect and each animal as the random effect. The mean cardiorespiratory variables collected over time in each group were analyzed with two-way ANOVA with mixed effects among experimental groups. Drug used (treatment), time points and their interactions were entered as the fixed effect and each animal was entered as the random effect. All data was reported as mean values $\pm$ SE. Pearson correlation was performed between total alfaxalone dose and $\dot{V}E$. A $p<0.05$ was considered statistically significant.

4.4 Results

All cats completed the study. There was no statistical difference in sedation, induction and recovery score between groups. The total dose of alfaxalone administered to CG (1.41 $\pm$ 0.17 mg kg^{-1}) was significantly higher (p=0.04) than that for PG (1.06 $\pm$ 0.2 mg kg^{-1}) (Graph 4.1).

Total Alfaxalone Dose
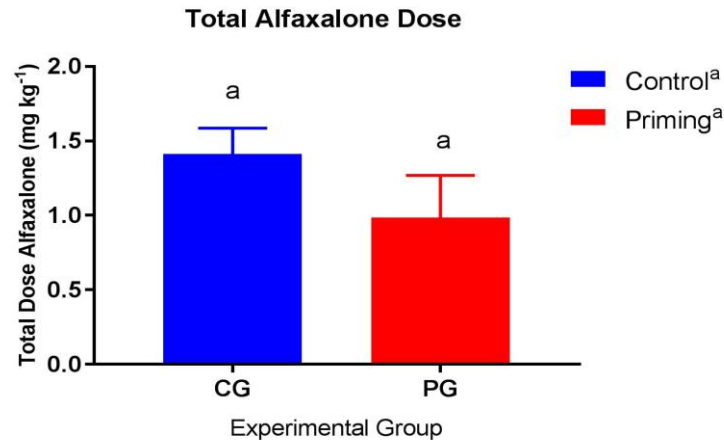
Graph 4.1. The total dose of alfaxalone, in mg kg^{-1}, required to achieve endotracheal intubation in eight healthy adult cats receiving two induction techniques: control group using saline (0.025 mL kg^{-1}) followed by alfaxalone (0.5 mg kg^{-1} min^{-1}) and priming group (0.25 mg kg^{-1}) followed by alfaxalone (0.5 mg kg^{-1} min^{-1}). Data are presented as least squares means estimates ± SE. CG: Control group, PG: Priming group. [a] Significant difference between groups.

Overall RR was not significantly different between groups. None of the animals presented apnea or SpO$_2$ lower than 90% throughout the study. Overall, Vt was significantly higher (p<0.01) in the CG when compared to the PG, although there were no statistically significant differences in the V̇E between groups (Graph 4.2). None of the cats presented dysphoria at recovery.

Tidal Volume
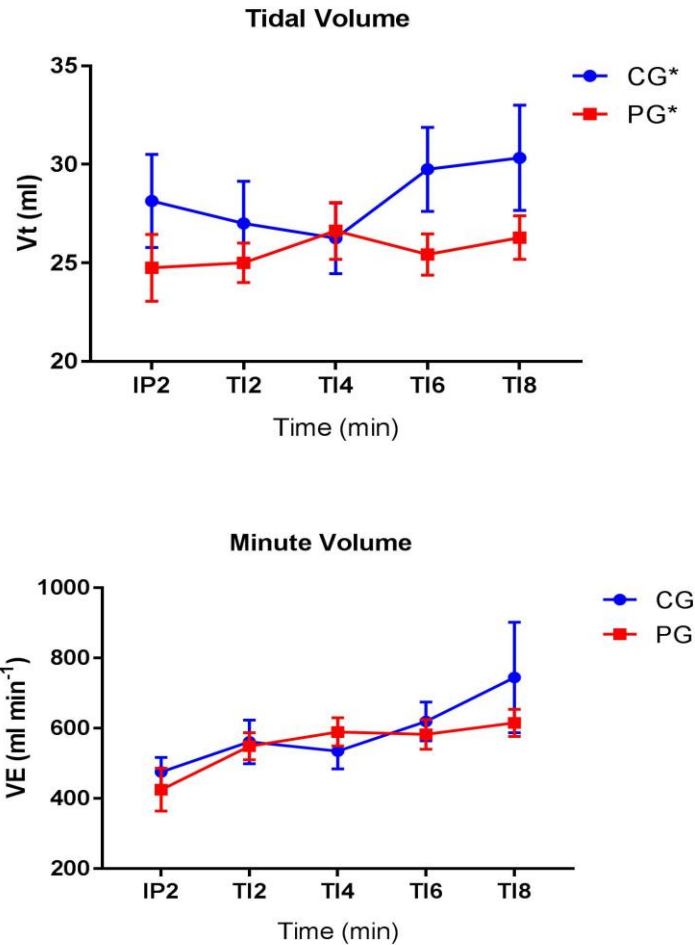
Minute Volume

Graph 4.2. Mean tidal volume (Vt) and minute volume (VE) from B to TI8, recorded in 8 healthy adult cats receiving two experimental induction treatments (control (CG) and priming (PG) alfaxalone; B: baseline, IP_1: induction phase 1, IP_2: induction phase 2, TI_2: 2 minutes after tracheal intubation, TI_4: 4 minutes after tracheal intubation, TI_6: 6 minutes after tracheal intubation, TI_8: 8 minutes after tracheal intubation. *Significant difference between groups.

Overall HR of the CG was significantly higher ($p<0.01$) than the PG. The SAP, MAP and DAP were significantly higher in the PG than in the CG ($p<0.01$) (Graph 4.3). None of the animals presented systemic hypotension (MAP<60 mmHg). The MAP and total alfaxalone dose presented a significant negative correlation at IP_2, TI_2, TI_4 and TI_8 (<0.04).

Mean Arterial Blood Pressure
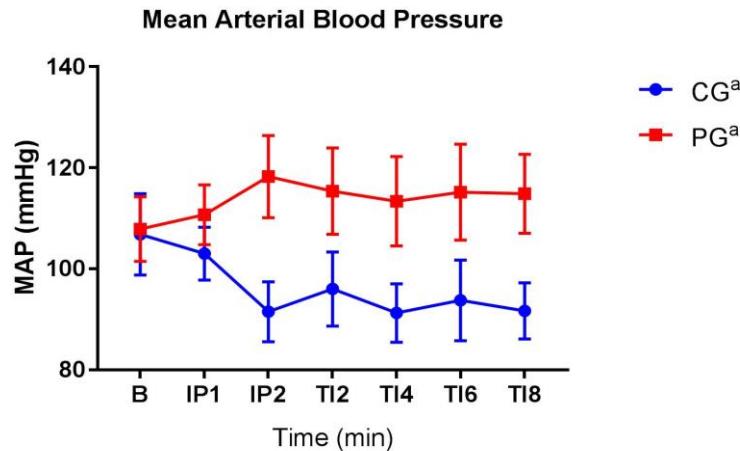
Graph 4.3. Mean arterial blood pressure from B to TI8 recorded in 8 healthy adult cats receiving two experimental induction treatments (control and priming alfaxalone; n=8 per group) in a crossover manner. B: baseline, IP_1: induction phase 1, IP_2: induction phase 2, TI_2: 2 minutes after tracheal intubation, TI_4: 4 minutes after tracheal intubation, TI_6: 6 minutes after tracheal intubation, TI_8: 8 minutes after tracheal intubation. *Significant difference between groups.

4.5 Discussion

The present study demonstrated that priming technique using alfaxalone for induction of anesthesia in cats satisfactorily induced anesthesia to allow TI, and significantly reduced the total dose of alfaxalone by 24.6%. Furthermore, this technique attenuated the after induction reduction in MAP, and did not present a significant negative impact on V̇E.

Similar percentages of alfaxalone dose reduction reported here (24.6%) has been demonstrated by applying priming principle with propofol in humans (23% Djaiani & Ribes-Pastor, 1999 and 32% Kataria et al., 2010, respectively). The significant alfaxalone sparing demonstrated in PG could be explained by the possible sedative effect produced by the sub-hypnotic dose of alfaxalone, as it has been demonstrated for sub-hypnotic doses of propofol

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in humans (Roberson & Robb, 1998). A sub-hypnotic effect has been described for propofol in humans (Kataria et al., 2010; Kumar et al., 2006). Sub-hypnotic doses of propofol have been reported to decrease the sympathetic drive and to significantly reduce the total milligrams required to produce complete hypnosis in humans (Roberson & Robb, 1998). Nonetheless, the level of sedation produced by sub-hypnotic doses of alfaxalone has not been evaluated in cats.

The speed of the rate of alfaxalone priming injection, over 60 seconds, could have further contributed to the alfaxalone sparing effect found in the PG. A slow rate of alfaxalone administration has been reported to increase its relative potency, and therefore, to decrease the total alfaxalone dose required to achieve TI in cats (Bauquier et al., 2015). Also, the premedication with methadone and dexmedetomidine, could have further contributed to the alfaxalone sparing effect on the total dose of alfaxalone observed. However, the crossover nature of this research and the lack of significant correlation between total dose of alfaxalone and sedation score could have minimized the impact of the premedication on the total dose of alfaxalone using the priming technique.

The Vt of the PG was significantly lower than the CG, while V̇E, although higher in PG when compared to CG, did not reach statistically significant levels. Studies with priming principle with propofol in humans have demonstrated less respiratory depression when compared with the conventional technique of induction with the same drug (Djaiani & Ribes-Pastor, 1999; Kumar et al., 2006; Pratap et al., 2015). Post induction apnea has been reported as a side effect of alfaxalone induction (Bauquier et al., 2015) mostly when administered at supraclinical doses (15 and 50 mg kg^{-1}) (Muir et al., 2009) or at fast speed of administration (Bauquier et al., 2015). None of the animals in the present study presented post-induction

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apnea possibly due to the slow rates of alfaxalone administration as priming as a continuous rate infusion. In the current study, using clinical doses of alfaxalone, none of the animals presented this complication. Another study evaluating the same low rate of alfaxalone injection used in this study (0.5 mg kg^{-1} min^{-1}) reported a post-induction apnea in 2 out of 6 cats (Bauquier et al., 2015). This difference could be explained by the higher total alfaxalone dose administration in Bauquier's study, as a premedication (3 mg kg^{-1}) and as an induction agent (2.1 mg kg-1).

The significant negative correlation between total alfaxalone dose and MAP was observed in the CG that presented the highest alfaxalone dose requirement and the lowest MAP after induction of anesthesia (from IP$_2$ to TI$_8$). Although none of the animals in the CG presented systemic hypotension, the significant decrease in MAP after induction of anesthesia could be a concern in patients with higher anesthetic risk. Nonetheless, the evaluation of the cardiovascular function was not the objective of the study, and the non-invasive blood pressure was recorded with the oscillometric device. The evaluation of different oscillometric devices have demonstrated poor accuracy when compared to the invasive blood pressure (Acierno et al., 2010; Branson et al., 1997). Furthermore, the monitor VeT Trends used in this study has not been validate in cats yet.

The overall HR of the CG was significantly higher than the PG. However, heart rate recordings were very similar between groups with exception of IP1. Cats in the CG presented a spike in the HR during IP1 (Graph 2), that corresponds to the time that saline IV was injected. At that experimental time, cats in the PG received priming alfaxalone dose. The higher HR observed in the CG could be secondary to the cat manipulation during the saline injection t or the mechanical stimulus of the injection of the crystalloid itself. Therefore, the

significant heart rate difference between CG and PG seems not associated with the alfaxalone cardiac side effects.

This study had some limitations: 1, the low number of subjects evaluated. However, the type-II error was minimized in this study by the previous sample size calculation for two experimental groups with a statistical power of 80%; 2, The possible influence of the sympathetic system over the cardiovascular variables collected at IP_2 produced by the TI and the use of a laryngoscope (King et al., 1951; Stoelting, 1977; Ricco & Henao-Guerrero, 2014). Nonetheless, any possible sympathetic influence produced by the TI at IP_2 was recorded in all experimental groups, making data comparable.

This is the first study evaluating the priming principle in veterinary anesthesia. Further studies are required to evaluate the benefits of this technique in non-healthy animals and using other induction agents such as propofol.

Chapter Five
Priming Alfaxalone and Alfaxalone-midazolam Co-induction in Healthy Dogs

5.1 Introduction

Alfaxalone-HPDC (2-hydroxypropyl-beta-cyclodextrin) is a neurosynthetic steroid reintroduced into the veterinary market as a molecule compacted in large sugar molecules (cyclodextrins), which increase the solubility of the drug (Brewster & Bodor, 1990). Alfaxalone produces unconsciousness and muscle relaxation due to its effect on the $GABA_A$ receptor in the central nervous system (Ferré et al., 2006). It also produces smooth induction of anesthesia with dose- and speed-dependent cardiorespiratory depression in dogs and cats (Chiu et al., 2016). At clinical doses (2 mg kg^{-1}), alfaxalone induces mild decrease in both, systemic vascular resistance and systemic blood pressure, in un-premedicated dogs (Muir et al., 2008); while at supraclinical doses (6 and 20 mg kg^{-1}) it induces significant decrease in cardiac output, increase in heart rate (HR), and further decrease in systemic blood pressure (Tamura et al., 2015; Muir et al., 2008). However, respiratory depression is the most remarkable side effect of alfaxalone (Chiu et al., 2016; Muir et al., 2008).

Apnea, hypoventilation, and hypoxemia are reported as the most common side-effects of alfaxalone in dogs and cats (Muir et al., 2009, Muir et al., 2008). The rapid speed of alfaxalone administration, over 5 seconds, has been associated with a reduction in systemic blood pressure, hypotension, and post-induction apnea in dogs (Bigby et al., 2017; Amengual et al., 2013). A slow injection rate of alfaxalone over 40 to 60 seconds has been demonstrated to prevent post induction apnea (Maddern et al., 2010; Muir et al., 2008).

Priming principle (Karlo et al., 2015), also called auto-co-induction technique (Djaniani & Ribes-Pastor, 1999) is an anesthesia induction method first described first in humans (Djaniani & Ribes-Pastor, 1999; Kataria et al., 2010). This technique consists of the administration of a low pre-calculated dose of an induction agent, known as priming dose, administered a few minutes prior to the following dose administration of the same induction agent, until general anesthesia is achieved (Kataria et al., 2010). The priming dose of propofol causes sedative and anxiolytic effects (Roberson & Robb, 1998), and it ranges between 20% (Methta et al., 2015) to 25% (Jones et al., 2002; Karlo et al., 2015) of the induction dose. It has been demonstrated in humans that the use of the priming principle significantly reduced the total dose of propofol required to achieve hypnosis (Djaniani & Ribes-Pastor, 1999; Kataria et al., 2010). The use of the priming principle, and its consequent reduction in total dose of a given anesthetic agent, has not yet been assessed for alfaxalone.

The objective of this study was to investigate the priming induction of alfaxalone, and its cardiorespiratory effects, at two different speed of administrations, to decrease the total dose of alfaxalone required to induce general anesthesia and allow TI in dogs. It was hypothesized that 1, a slow administration of priming alfaxalone, over 60 seconds, would successfully induce general anesthesia to allow TI; 2, the slow administration of priming alfaxalone would reduce the total dose of alfaxalone and consequently prevent the post-induction cardiorespiratory depression; 3, the fast administration, over 5 seconds, of priming alfaxalone would successfully induce general anesthesia to allow TI; 4, the fast administration of priming alfaxalone would reduce the total dose of alfaxalone but it would not prevent the cardiorespiratory depression due to the speed of administration.

5.2 Materials and Methods

5.2.1 Animals

The present study was approved by the Louisiana State University Institutional Animal Care and Use Committee. Ten intact female Mongrel dogs, nine months old, weighing between 10.2 and 13.5 Kg, from the LSU research canine colony, were enrolled in this study. All dogs were determined to be healthy based on the results of a complete physical examination and blood work analysis (complete blood count and serum biochemistry profile). Animals were housed and maintained in accordance with the LSU Division of Laboratory Animal Medicine. Food was withheld for eight hours, but water was offered *ad libitum*, prior to the beginning of the study.

5.2.2 Study design

Three anesthetic induction treatments were randomly assigned in a crossover design with an 8 day-washout period between treatments groups. On the day of the study, dogs were mask-induced with sevoflurane at 5% (Sevoflurane, Vetone®, United States) in oxygen (2 L min^{-1}) then adjusted between 2 to 3% to maintain a light anesthetic plane (ventromedial rotated eyes, absence of palpebral reflexes and some jaw tone). Dogs were positioned in left lateral recumbency and monitored with ECG lead II for heart rate and rhythm, arterial hemoglobin oxygen saturation (SpO$_2$), and non-invasive blood pressure with the

oscillometric technique. A 30 to 40% leg circumference size cuff was placed proximal to the left foreleg carpus. The cuff was connected to a multifunction monitor (VetTrends™V, SystemVet™, United States) for measurement of non-invasive systolic, diastolic and mean arterial blood pressure (SAP, DAP, and MAP, respectively). After clipping and sterile disinfection, a 20-gauge 3.2 cm (11/4-inch) catheter (Sur-Vet® Surflo ETFE, Terumo, United States) was aseptically placed into the right cephalic vein, and a 22-gauge 2.5 cm (1-inch) catheter (Sur-Vet® Surflo ETFE, Terumo, United States) was aseptically placed into the dorsal pedal artery of the left hind limb. With catheters in place, dogs were allowed to recover from anesthesia. A minimum of a 60-minute washout period was allowed between recovery from the sevoflurane anesthesia and beginning of the alfaxalone study.

At the end of the washout period, dogs received intravenous methadone (0.2 mg kg⁻¹; Mylan®, Rockford, United States) and dexmedetomidine (1 ug kg^{-1}; Dexdomitor, Zoetis Inc., United States) as premedication. Ten minutes after, the quality of sedation was always assessed by the same investigator (AL), using a numeric rating scale from 0 (no sedation) to 3 (profound sedation) (Appendix C.1). In sequence, dogs were placed in sternal recumbency and HR and rhythm were monitored with an ECG lead II. The arterial catheter was connected to a multifunction monitor (B650, General Electric, United States) via a disposable pressure transducer system (Truewave 3.6M/12FT, Edwards Lifesciences, Germany). The transducer was attached at one side to a 500-ml saline bag (0.9% Sodium Chloride Injection USP, Hospira Inc., United States) pressurized at 300 mmHg, and to the other side to a non-distensible saline solution-filled extension line attached to the dorsal pedal artery catheter. Before the beginning of the study, the electronic disposable transducer was leveled at the right atrium (scapulohumeral joint) and zeroed to atmospheric pressure. Previous to the data

collection, the extension line was inspected for air bubbles zeroed, checked and calibrated prior to the beginning of each study. A measure of systolic, mean and diastolic arterial blood pressure variables (SAP, MAP, and DAP, respectively) was performed during the study.

Anesthetic induction was divided into two phases: phase 1 (IP$_1$) and phase 2 (IP$_2$). The objective of the anesthetic induction treatment was to achieve TI in each experimental group. Dogs received one of the three experimental intravenous (IV) induction treatments administered by an evaluator (PQ): 1) Control Group (CG), saline 0.025 mL kg^{-1} (based on equivalent alfaxalone volume injected for the three other experimental groups) over 60 seconds (IP$_1$) administered by a precision syringe pump (CareFusion, Alaris PC, United States) followed by alfaxalone 0.5 mg kg^{-1} min^{-1} (Alfaxan; Jurox Pty Ltd, Australia) administered via syringe pump until TI was achieved (IP$_2$); 2) Slow Priming Group (SPG), alfaxalone 0.25 mg kg^{-1} over 60 seconds (IP$_1$) administered by a precision syringe pump followed by alfaxalone 0.5 mg kg^{-1} min^{-1} administered via a precision syringe pump until TI was achieved (IP$_2$); and 3) Fast Priming Group (FPG), alfaxalone 0.25 mg kg^{-1} over 5 seconds (IP$_1$), followed by alfaxalone 0.5 mg kg^{-1} min^{-1} administered by a precision syringe pump until TI was achieved (IP$_2$) (Figure 5.1).
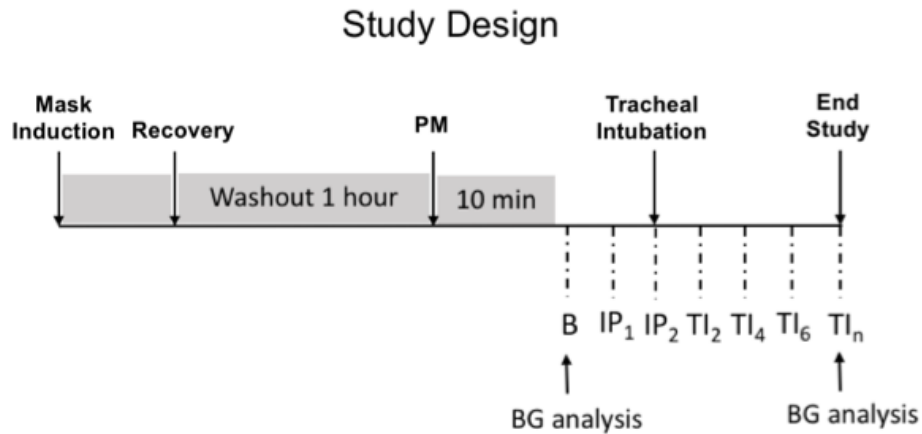
Study Design

Figure 5.1. Experimental design employed to evaluate three induction techniques (1-priming alfaxalone, 2-fast priming alfaxalone, and 3-control) in 10 healthy dogs randomly assigned in a crossover manner with 7-days washout period among groups. (n=10 animals per group). B: baseline, IP_1: induction phase 1, IP_2: induction phase 2, TI_2: 2 minutes after endotracheal intubation, TI_4: 4 minutes after endotracheal intubation, TI_6: 6 minutes after endotracheal intubation, TIn: last recording before tracheal extubation, BG: blood gas analysis

During IP_2 (CG, SPG, and FPG), the same blinded researcher (AL) attempted to perform TI based on loss of lateral and medial palpebral reflexes, loss of jaw tone and easy extrusion of the tongue out of the mouth without resistance. The TI was performed with a cuffed-endotracheal tube of adequate size using a laryngoscope. Quality of TI was qualitatively scored from 1 to 5 by an evaluator (AL) (Appendix C.2). Once the endotracheal tube was successfully placed into the trachea, the continuous rate infusion of alfaxalone was stopped and the total milligrams of alfaxalone administered were recorded.

Quality of induction from 1 to 4 was scored by the same blinded evaluator that performed the TI (AL) (Appendix C.3). Immediately after TI, the endotracheal tube's cuff was inflated until an intra-cuff pressure between 20 to 25 cmH2O measured with a manual manometer (Exactus II, BMS, United States)., was reached A capnography and pediatric

respirometer (pediatric respirometer, B650, General Electric, United States) attached to a multi-parametric monitor (B650, General Electric, United States) were connected to the endotracheal tube for the expired end-tidal partial pressure of carbon dioxide (PE´CO2), respiratory rate (f_R) and tidal volume (Vt) measurement. Minute ventilation ($\dot{V}E$) was calculated by the product of f_R and Vt. A pulse oximeter probe was placed on the tongue for measurement of hemoglobin oxygen saturation (SpO_2).

5.2.3 Data collection

Baseline (B) corresponded to the experimental time after instrumentation and right before induction of anesthesia (IP_1 and IP_2). Cardiorespiratory variables (HR, SAP, MAP, DAP, f_R) were recorded at B and at the end of IP_1. After IP_2 injection, additional variables were collected immediately after successful TI (HR, SAP, MAP, DAP, PE´CO2, f_R, Vt, $\dot{V}E$, and SpO_2), and every 2 minutes after TI (TI_2, TI_4, TI_6, TI_8, etc.) until the animals were extubated (TE) (Figure 5.1). Dogs were spontaneously breathing room air (FiO_2=0.21). After IP_1, and during the remaining of the study, if post-induction apnea (absence of respiratory movements for 30 seconds) was observed or if SpO_2 was <90%, two ml of arterial blood were withdrawn from the arterial catheter for blood gas analysis. Immediately after, the dogs were connected to a circle breathing circuit with medical oxygen (FiO_2=100%). Positive pressure ventilation (4 to 6 breaths per minute) was done till spontaneous ventilation was restored and SpO_2 values were within normal ranges (SpO_2 > 97%).

At B and TE time points, 2-ml of arterial blood were collected into a heparinized syringe (PICO50, Radiometer, Denmark) for blood gas analysis using a bed-side monitor

ISTAT (VetScan i-STAT, Abaxis, Canada) with the cartridge CG4+ (i-STAT CG4+, Abbott Point of Care Inc., United States) with correction of temperature at 37°C. Immediately after collection, samples were analyzed for pH, PaO_2, $PaCO_2$, TCO_2, HCO_3, and lactate recording.

Dogs were extubated when they were breathing spontaneously, regained positive palpebral reflexes (lateral and medial), and adequate jaw tone and were swallowing. At recovery, the arterial and venous catheters were removed from the dorsal pedal artery and cephalic vein, respectively. Quality of recovery was scored by an evaluator (AL) from 1 to 6 (Appendix C.4). Time (minutes) from TI until TE was also recorded in each group. If a dog presented dysphoria at recovery, acepromazine at 0.01 mg kg^{-1} (Acepromazine Maleate, VetOne, United States) was administered IV and the event was recorded.

5.3 Statistical Analysis

Data analyses were performed using SAS (9.4 software, SAS Institute Inc.,, United States). Data were analyzed at B, IP_1, IP_2, TI_2, TI_4, TI_6 and TI_8. A one-way mixed analysis of variance (ANOVA) model was used to analyze the variables measured without any time point (sedation, induction, TI and recovery score and total mg of alfaxalone) with a drug used (treatment) as the fixed effect and each animal as the random effect. The mean cardiorespiratory variables collected over time (B to TI_8) in each group were analyzed with two-way ANOVA with mixed effects among experimental groups. Drug used (treatment), time points and their interactions were entered as the fixed effect and each animal was entered as the random effect. Arterial blood gases values recorded at B and TE were analyzed with mixed ANOVA with drug used and time as the fixed effects and each animal as the random

effect. The residuals from all ANOVA models were checked for normality with the Shapiro-Wilk test. When a fixed effect was detected, Tukey post-hoc comparisons were performed with least square means for the effect. Significance was set at $p < 0.05$.

5.4 Results

All dogs completed the study. There were no significant differences in any cardiovascular, respiratory and blood gas values at B among groups. There was no significant difference in sedation score among groups and there was no significant correlation between sedation score and total alfaxalone dose administered in each experimental group.

There was no significant difference in induction quality among groups. The total alfaxalone required to achieved TI was significantly lower in SPG (1.1 ± 0.4 mg kg^{-1}) than in CG (1.5 ± 0.3 mg kg^{-1}) and FPG (1.5 ± 0.2 mg kg^{-1}) (Graph 5.1).

Total Alfaxalone Dose
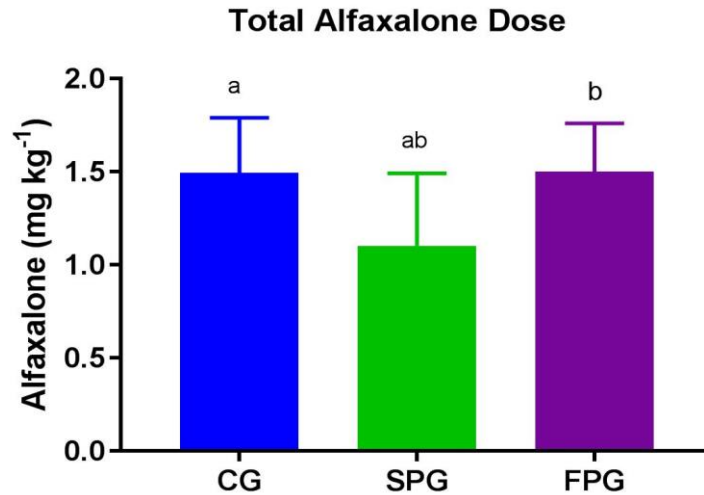
Graph 5.1. Mean ± standard deviation of total alfaxalone dose (mg kg^{-1}) required to achieve endotracheal intubation in ten adult female dogs that received three different induction treatments (control, slow priming and fast priming) in a crossover design. [a,b] Significantly different between two groups. CG: control group, SPG: Slow priming group, FPG: Fast priming group.

During this experiment, the mean HR, SAP, MAP, and DAP between B to TI_8 was compared among groups. The FPG presented a significantly lower HR and SAP, MAP, and DAP when compared with CG and SPG. There was no differences in this hemodynamic variables between CG and SPG (Graph 5.2).
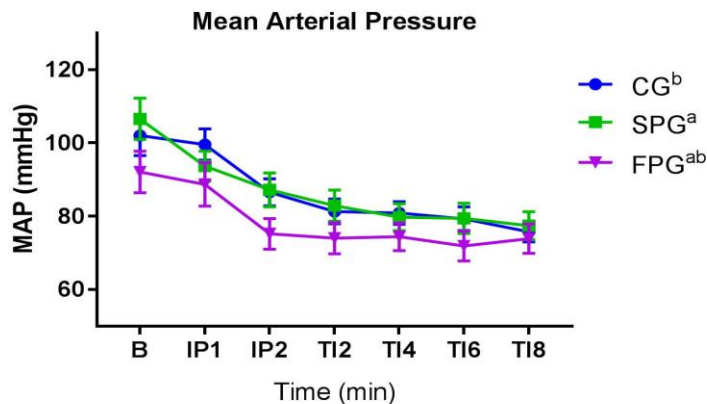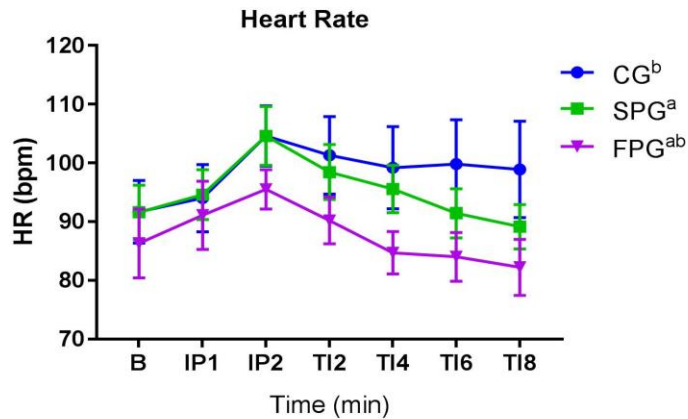
Graph 5.2. Mean heart rate and invasive mean arterial blood pressure (B to TI_8) recorded from 10 female dogs receiving saline (control group), alfaxalone low-dose at fast speed (fast priming group) or at slow speed (slow priming group) at induction phase 1, followed by 0.5 mg kg^{-1} min^{-1} of alfaxalone as induction phase 2 until achieve endotracheal intubation. Data are presented as a mean $\pm$ SE. B: baseline, IP_1: induction phase 1, IP_2: induction phase 2, TI_2: 2 minutes after endotracheal intubation, TI_4: 4 minutes after endotracheal intubation, TI_6: 6 minutes after endotracheal intubation, TI_8: 8 minutes after endotracheal intubation. [a,b,c] Significantly different between two groups.

None of the animals presented post-induction apnea in any of the groups. The f_R was significantly decreased after IP_1 in all treatment groups. There were no statistically significant differences on mean f_R among groups. The mean Vt was significantly higher in

the FPG (125.4 ± 1.6 ml) than in the CG (107.7 ± 1.5 ml). There was no significant difference in $\dot{V}E$ among groups.

The mean PaO_2 was significantly lower in SPG in comparison to the CG (80.5 ± 2.0 mmHg and 90 ± 2.1 mmHg, respectively). None of the groups presented hypoxemia at baseline or at TE ($PaO_2 < 60$ mmHg; Gaynor et al., 1999). There were no significant differences on $PaCO_2$ among groups. The pH was significantly lower in FPG (7.29 ± 0.01) than in SPG (7.33 ± 0.01). The mean TCO_2 was significantly lower in FPG than in SPG (21.0 ± 0.6 and 22.2 ± 0.6 mmol L^{-1}, respectively). Likewise, the base deficit was significantly lower in FPG than in SPG (-7.0 ± 0.6 and -4.7 ± 0.6 mmol L^{-1}, respectively).

There was no significant difference in time under anesthesia and recovery score among groups. One dog of the CG and one in the FPG presented dysphoria at recovery that was successfully managed with acepromazine IV.

5.5 Discussion

Our results indicate, that fast and slow priming alfaxalone (FPG and SPG), as alternative induction techniques satisfactorily induced general anesthesia to allow TI in healthy dogs premedicated with dexmedetomidine and methadone. In the SPG the total alfaxalone dose was significantly reduced by 26.6% while FPG required a similar dose than the conventional alfaxalone induction technique (CG). Among the induction techniques evaluated in this study, the CG and SPG presented the best cardiorespiratory stability.

Similar to the technique of priming propofol reported in humans (Mehta et al., 2015; Kataria et al., 2010), priming alfaxalone with a slow alfaxalone rate of injection (SPG), the total dose of alfaxalone required to produce hypnosis was significantly reduced. Although the alfaxalone total dose was significantly lower in the SPG when compared to the CG, no clinical cardiorespiratory benefits were detected with this dose reduction. The lower total dose in the slow priming denotes a possible clinical benefit in sick dogs ASA III or IV in which lower total induction dose could be associated with less cardiorespiratory depression and better outcome. Future studies are recommended to address this issue.

The significant sparing effect found in the SPG could be explained by a possible sedative or anxiolytic effect produced by the sub-hypnotic dose of alfaxalone. Such an effect might minimize the subsequent alfaxalone dose required to achieve general anesthesia, as it has been postulated for propofol in human anesthesia (Pratap et al., 2015; Kumar et al., 2006; Anderson & Robb, 1998). Comparable percentages of diminution have been reported with priming propofol in healthy adult human with ranges between 10.2% (Karlo et al., 2015) to 31.9% (Kataria et al., 2010). Further studies evaluating the possible sedative effect of sub-hypnotic doses of alfaxalone in dogs are required.

Although the same priming dose of alfaxalone (0.25 mg kg^{-1}) was injected in both priming groups (SPG and FPG), only the SPG produced a significant sparing effect. This finding suggests a possible initial sedation caused by a sub-hypnotic dose of alfaxalone required for the slow administration speed to satisfactorily spare the alfaxalone dose. The rate of administration of a drug impacts the initial stages of the drug distribution to the target tissues (Stokes & Hutton, 1991). Studies developed with in humans, when propofol was administered in different speed of injection demonstrated that slower rates of administration

produced lower total propofol dose requirement (Larsson & Wahlstrom, 1994; Stokes & Hutton, 1991).

According with Hull (1979), after injection of an induction agent, the time elapsed until unconsciousness is achieved depends on circulation and the biophase kinetics. Due to the physicochemical properties and biophase kinetics of propofol, its transport to the target tissue depends on the rate of infusion to reach effective concentrations, due to the slow equilibrium between brain and blood (Stokes & Hutton, 1991). Lower rates of propofol infusion can reach adequate concentration in the biophase to produce hypnosis (Larsson & Wahlstrom, 1994; Stokes & Hutton, 1991). Therefore, administering propofol to produce unconsciousness at a higher rates than its equilibrium between blood and brain would lead an overdose (Bigby et al., 2017; Stokes & Hutton, 1991). Although there are no studies of alfaxalone biophase kinetics, based on the results of this study, it is possible that similar finite transport time to reach adequate biophase concentration happens with alfaxalone as with propofol. In this way, the slow priming injection allowed depression at the CNS that possibly spared further alfaxalone dose at IP_2. Similar results of sparing alfaxalone at low rates of administration have been demonstrated in healthy dogs (Bigby et al., 2017) and cats (Bauquier et al., 2015).

A potential limitation of this study is the low number of dogs evaluated. Although the sample size was calculated with 20% type II error (statistical power of 80%), we cannot exclude that with an increase in sample size   a significant clinical cardiorespiratory benefit of SPG compared to CG could have been observed. Another limitation corresponds to not collecting cardiorespiratory variables immediately after IP_2 and right before TI. The variables collected at IP_2 were registered after successful TI, Riccó and Henao (2014) described the

sympathetic response (increase in HR and systemic blood pressure) related to TI. Therefore, the variables collected could have been influenced by the sympathetic response produced by the TI. However, this fact does not affect the results of this study, since in all experimental groups the variables were collected with the same design in a crossover manner. A third limitation corresponds to the no blood gas analysis immediately after IP_1 and IP_2 since dogs during that time could have been hypoxemic.

This study determined that by applying the priming principle at a slow rate of administration of the priming dose, the total induction dose required to induce general anesthesia to allow TI in healthy dogs premedicated with methadone and dexmedetomidine is reduced by 26.6%. Although there was a significant dose reduction of alfaxalone, that reduction was not associated with any cardiorespiratory benefit when compared with the CG. This finding could be explained by the conservative rate of alfaxalone administration (0.5 mg kg^{-1} min^{-1}) that possibly prevented the alfaxalone cardiorespiratory depression even in the CG that received the highest alfaxalone dose.

After reviewing the literature, this study corresponds to the first research evaluating the priming principle in dogs. Further studies are required to evaluate this induction technique in other species and with other induction agents such as propofol.

Chapter Six
Conclusions

Alfaxalone is a neuroactive synthetic steroid, widely used as induction agent in veterinary anesthesia, mostly in dogs and cats (Muir et al., 2009; Muir et al., 2008). The main advantages of this agent are the rapid and smooth induction of anesthesia, quick redistribution of the drug, short effect, rapid elimination, no accumulation and good quality of recovery (Warne et al., 2015; Muir et al., 2008). However, alfaxalone produces dose and speed related cardiorespiratory depression, manifested by increasing or decreasing HR, decreasing systemic blood pressure, reduction in CO, hypoventilation, post-induction apnea and transitory hypoxemia (Warne et al., 2015; Muir et al., 2008). All these side-effects justify the titration of alfaxalone whenever administered intravenously in dogs and cats.

A safe anesthesia induction technique seeks to preserve cardiorespiratory function and therefore, decreases morbidity and mortality during intubation. In order to improve the safety of anesthesia and aiming to reduce the total induction dose, the technique of co-induction was developed (Maddern et al., 2010). This induction technique is described as the combination of two or more drugs, acting synergistically or additively between them (Short & Chui, 1991), in order to enhance their effect and to reduce the total dose of each drug. Due to the sparing properties of the technique, co-induction is usually associated with fewer side-effects (Kataria et al., 2010; Jones et al., 2002). One of the most common co-induction drugs administered in veterinary medicine are benzodiazepines (Liao et al., 2017). Recently, the alfaxalone-midazolam co-induction technique was successfully demonstrated in healthy dogs (Munoz et al., 2017), but the technique has not been assessed in cats yet.

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Another alternative to reduce the total induction dose described in the literature is the priming principle (Kataria et al., 2010; Kumar et al., 2006). This technique is an anesthetic induction technique that uses an induction agent in a low dose (defined as ¼ of the conventional dose) administered few minutes prior to the subsequent administration of the same induction agent until general anesthesia and TI is achieved (Kumar et al., 2006; Taboada et al., 1986). This technique has only been tested with propofol in humans (Djaniani & Ribes-Pastor, 1999), and it has never been assessed in veterinary anesthesia.

Based on these facts and the lack of studies testing these induction techniques suing alfaxalone in dogs and cats, the objective of this dissertation was to investigate the reduction of alfaxalone induction total dose (mg kg^{-1}) by administering it in two alternative anesthesia induction techniques, as follows: 1) Priming principle of alfaxalone, in healthy dogs and cats; and 2) Co-induction of midazolam with low dose of alfaxalone, in healthy cats. This study also aimed to assess the cardiorespiratory function associated with these alternative techniques of induction, in order to determine if they offer any hemodynamic or respiratory benefit when administered to healthy dogs and cats.

The three studies were successfully developed and all the animals studied completed the researches. The first study developed in cats was conducted to determine the ED$_{50}$ of midazolam required for co-induction with alfaxalone to achieve general anesthesia and TI. From six independent crossovers it was estimated that the ED$_{50}$ of midazolam was 0.08 ± 0.04 mg kg^{-1} when co-administered, one minute after the injection of a low dose of alfaxalone (0.25 mg kg^{-1}) in methadone and dexmedetomidine-sedated healthy cats. This technique co-induction technique successfully induced general anesthesia and allowed smooth TI. No cardiorespiratory side-effects were reported in the cats evaluated. Future studies are required

to clinically evaluate this dose in healthy and sick cats. Finding the ED_{50} of midazolam combined with a low dose of alfaxalone would be clinically relevant for sick patients, since both alfaxalone and midazolam are co-administered in very low doses with the technique described in this study.

The successful alfaxalone and midazolam co-induction technique could be explained by a possible synergistic or additive effect between both drugs over the $GABA_A$ receptor. Pharmacokinetic studies evaluating the dose-response curves of each drug separately and both drugs in combination, as well as, calculation of the coefficient of synergism of the alfaxalone-midazolam co-induction are needed to determine if the combination produces an additive or a supra additive (synergistic) effect.

In the second and third researchers, the priming principle was assessed for the first in cats and dogs. Priming principle with alfaxalone successfully induced general anesthesia and allowed TI in all cats and dogs. By applying this principle it was significantly reduced the total alfaxalone dose required to achieve TI by 24.6% in cats and 26.6% in dogs when compared with the control group. The sparing effect was only observed when the priming dose (¼ of alfaxalone induction dose) was administered slowly over 60 seconds. Although in both studies it was demonstrated a significant sparing effect of alfaxalone dose, only in cats that dose reduction was correlated with better respiratory function when compared with the control group.

Although the current studies had power analysis 80% prior to the beginning of the study, it is possible that an error type II could not allow determining significant cardiorespiratory differences when comparing the priming principle with the control group. It is unknown if with a higher sample size the alfaxalone dose reduction would be correlated

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with a better cardiorespiratory function in dogs and cats. It is possible that the lower alfaxalone dose requirements found in these studies denotes a possible clinical benefit in sick cats and dogs. This issue needs to be addressed in future clinical studies.

It has been postulated that the satisfactory sparing effect of the priming principle applied with propofol in humans is secondary to the sedative and anxiolytic effect of the sub hypnotic dose (priming dose) of this drug in humans (Roberson and Robb, 1998). In this dissertation due to the significant alfaxalone sparing effect found in dogs and cats, it was hypothesized that a similar sedative effect is produced by sub hypnotic doses of alfaxalone (priming dose) in dogs and cats. Nonetheless, after reviewing the veterinary literature, there are no studies evaluating the possible sedative effect of sub hypnotic doses of this induction agent in these species. Further studies are required to address this issue, to better explain the successful sparing effect of the priming technique with alfaxalone in dogs and cats.

The slow administration of the priming dose in the dogs' research was associated with alfaxalone sparing effect and no cardiovascular depression. It is possible that similar to propofol, alfaxalone has a slow equilibrium between circulation (blood) and the CNS (brain) (Stokes & Hutton, 1991), which limits the rate of uptake of the anesthetic into the brain, due to the finite transport time required to reach adequate biophase drug concentration. Future studies evaluating the alfaxalone biophase kinetics are needed to better understand the relationship between rate of injection of alfaxalone and biophase alfaxalone concentration. Understanding the time that takes reach an equilibrium between blood and brain based on the physicochemical properties of alfaxalone could prevent to overdose this drug at induction of anesthesia.

The alfaxalone induction dose in premedicated dogs ranges between 1 to 2 mg kg^{-1} (Muir et al., 2008). The fast injection of only a quarter of the lowest alfaxalone induction dose injected over 5 seconds, significantly dropped the systemic blood pressure and heart rate in dogs. Alfaxalone presents both, dose and speed related cardiovascular side-effects, and even though, a very low dose of alfaxalone was injected (0.25 mg kg^{-1}), the cardiovascular depression was still observed probably not because of the dose administered, but because of the speed of injection. Based on the results of this research, although FPG induces general anesthesia and allowed smooth TI in dogs, this technique is not advised to be used since it does not offer any alfaxalone total dose benefit when compared with the conventional technique of induction with this agent, and also because even though patients did not present bradycardia or hypotension, the drop in these variables recorded in this study is undesirable, mostly in sick animals.

In conclusion, this dissertation demonstrated the importance of midazolam as co-induction agent, since with a very low dose of alfaxalone (0.25 mg kg-1), midazolam in co-induction allowed the TI in cats. This study estimated the ED$_{50}$ of midazolam (0.08 ± 0.04 mg kg^{-1}) required to achieve TI in 50% of the cats premedicated with methadone (0.3 mg kg^{-1}) and dexmedetomidine (3 ug kg^{-1}) intramuscularly, and induced with a quarter of alfaxalone dose (0.25 mg kg^{-1}) administered slowly, 60 seconds prior to midazolam administration. Additionally, this research demonstrated that by injecting alfaxalone in a different way of administration of two induction phases according with the priming principle, it is possible to minimize the total dose requirements needed to achieve TI in dogs and cats. This technique guaranteed an adequate respiratory function in cats, and cardiovascular function in dogs. After reviewing the literature, this dissertation corresponds to the first research evaluating

alfaxalone-midazolam co-induction in feline, and this research assessed for the first time the priming principle in veterinary anesthesia, in dogs and cats. Further studies assessing this priming technique in other species and with other induction agents such as propofol are needed.

References

Adachi, U., Watanabe, K., Higuchi, H., & Satoh, T. (2001). A small dose of midazolam decreases the time to achieve hypnosis without delaying emergence during short-term propofol anesthesia. *Journal of Clinical Anesthesia*, 13(4),277-80.

Albertson, T., Walby, W., & Joy, R. (1992). Modification of GABA-mediated inhibition by various injectable anesthetics. *Anesthesiology*, 77(3),488-499.

Ambros, B., Duke-Novakovski, T., & Pasloske, K. (2008). Comparison of the anesthetic efficacy and cardiopulmonary effects of continuous rate infusions of alfaxalone-2-hydroxypropyl-b-cyclodextrin and propofol in dogs. *American Journal of Veterinary Research,* 69(11),1391–1398.

Amengual, M., Flaherty, D., Auckburally, A., Bell, A., Scott, E., & Pawson, P. (2013). An evaluation of anaesthetic induction in healthy dogs using rapid intravenous injection of propofol or alfaxalone. *Veterinary Anaesthesia and Analgesia*, 40(2),115–123.

Anderson, L., & Robb, H. (1998). Comparison of midazolam co-induction with propofol predosing for induction of anaesthesia. *Anaesthesia*, 53(11),117-120.

Bauquier, S., Warne, L., Carter, J., Whittem, T., & Beths, T. (2017). Influence of two administration rates of alfaxalone at induction on its relative potency in cats: a pilot study. *Journal of Feline Medicine and Surgery,* 19(2), 231-234.

Bigby, S., Beths, T., Bauquier, S., & Carter, J. (2017). Effect of rate of administration of propofol or alfaxalone on induction dose requirements and occurrence of apnea in dogs. *Veterinary Anaesthesia and Analgesia*, 44(6), 1267-1275.

Bille, C., Auvigne, V., Libermann, S., Bomassi, E., Durieux, P., & Rattez, E. (2012). Risk of anaesthetic mortality in dogs and cats: an observational cohort study of 3546 cases. *Veterinary Anaesthesia and Analgesia*, 39(1), 59–68.

Bley, C., Roos, M., Price, J., Ruess-Melzer, K., Buchholz, J., Poirier, V., & Kaser-Hotz, B. (2007). Clinical assessment of repeated propofol-associated anesthesia in cats. *Journal of the American Veterinary Medical Association*, 231(9),1347–1353.

Brandão, J. (2014). Cardiovascular tolerance and safety of intravenous lidocaine in the broiler chicken (*Gallus gallus domesticus*). MS thesis, Louisiana State University. 35-39.

Brewster, M., Estes, K., & Bodor, N. (1989). Development of a non-surfactant formulation for alfaxalone through the use of chemically-modified cyclodextrins. *Journal of Parental Science and Technology,* 43(6),262-265.

Brodbelt, D., Blissitt, K., Hammond, R., Neath, P., Young, L., Pfeiffer, D., & Wood, J. (2008). The risk of death: the Confidential Enquiry into Perioperative Small Animal Fatalities. *Veterinary Anaesthesia and Analgesia,* 35(5),365-373.

Brownlee, K., Hodges, Jr., & Rosenblatt, M. (1953). The Up-and-Down Method with Small Samples. *Journal of the American Statistical Association.* 48(262),262-277.

Campagna, I., Schwarz, A., Keller, S., Bettschart-Wolfensberger, R., & Mosing, M. (2015). Comparison of the effects of propofol or alfaxalone for anaesthesia induction and maintenance on respiration in cats. *Veterinary Anesthesia and Analgesia,* 42(5),484-492.

Casoni, D., Spadavecchia, C., & Adami, C. (2015). S-ketamine versus racemic ketamine in dogs: their relative potency as induction agents. *Veterinary Anaesthesia and Analgesia,* 42(3),250-259.

Child, K., Currie, J., & Dis, B. (1971). The pharmacological properties in animals of CT1341 – a new steroid anaesthetic agent. *British Journal of Anaesthesia,* 43(1),2–13.

Chiu, K., Robson, S., Devi, J., Woodward, A., & Whittem, T. (2016). The cardiopulmonary effects and quality of anesthesia after induction with alfaxalone in 2-hydroxypropyl-

β-cyclodextrin in dogs and cats: a systematic review. *Journal of Veterinary Pharmacology Therapy,* 39(6),525-538.

Ciccone, G., & Holdcroft, A. (1999). Drugs and sex differences: a review of drugs relating to anaesthesia. *British Journal of Anaesthesia,* 82(2),255-265.

Clarke, K., & Hall, L. (1990). A survey of anaesthesia in small animal practice: AVA/BSAVA report. *Journal of Veterinary Anaesthesia,* 17(1),4-10.

Columb, M., & Lyons, G. (1995). Determination of the minimum local analgesic concentrations of epidural bupivacaine and lidocaine in labor. *Anesthesia and Analgesia*, 81(4),833-837.

Covey-Crump, G., & Murison, P. (2008). Fentanyl or midazolam for co-induction of anaesthesia with propofol in dogs. *Veterinary Anaesthesia and Analgesia,* 35(6),463-472.

Cressey, D., Claydon, P., Bhaskaran, N., & Reilly, C. (2001). Effect of midazolam pretreatment on induction dose requirements of propofol in combination with fentanyl in younger and older adults. *Anaesthesia,* 56(2),108-113.

DiGeronimo, P., da Cunha, A., Pypendop, B., Brandão, J., Stout, R., Rinaldi, M., & Tully, T. (2017). Cardiovascular tolerance of intravenous bupivacaine in broiler chickens (*Gallus gallus domesticus*) anesthetized with isoflurane. *Veterinary Anaesthesia and Analgesia,* 44(2),287-294.

Dixon, W. (1965). The Up-and-Down Method for Small Samples. *Journal of the American Statistical Association,* 60(312), 967-978.

Dixon, W., & Mood, A. (1948). A Method for Obtaining and Analyzing Sensitivity Data. *Journal of the American Statistical Association,* 43(241),109-126.

Djaniani, G., & Ribes-Pastor, M. (1999). Propofol auto-co-induction as an alternative to midazolam co-induction for ambulatory surgery. *Anaeshesia,* 54(1),63-67.

Driver, I., Wiltshire, S., Mills, P., Lillywhite, N., & Howard-Griffin, R. (1996). Midazolam co-induction and laryngeal mask insertion. *Anaesthesia,* 51(8),782-784.

Durham, S., Flournoy, N., & Rosenberger, W. (1997). A random walk rule for phase I clinical trials. *Biometrics,* 53(2),745-760.

Dyson, D., Maxie, M., & Schnurr, D. (1998). Morbidity and mortality associated with anesthetic management in small animal veterinary practice in Ontario. *Journal of the American Animal Hospital Association*, 34(4),325-335.

Estes, K., Brewster, M., Webb, A., & Bodor, N. (1990). A non- surfactant formulation for alfaxalone based on an amorphous cyclodextrin: Activity studies in rats and dogs. *International Journal of Pharmaceutics,* 65(1),101-107.

Ferré, P., Pasloske, K., Whittem, T., Ranasinghe, M., Li, Q., Lefebvre, H. (2006). Plasma pharmacokinetics of alfaxalone in dogs after an intravenous bolus of Alfaxan-CD RTU. *Veterinary Anaesthesia and Analgesia.* 33(4):229–236.

Foldes, F. (1984). Rapid tracheal intubation with non-depolarizing neuromuscular blocking drugs: the priming principle. *British Journal of Anesthesia*, 56(6),663.

Galley, A., & Rooms, M. (1956). An intravenous steroid anaesthetic. Experiences with Viadril. *Lancet,* 23(270):990-994.

Gaynor, J., Dunlop, C, Wagner, A., Wertz, E., Golden, A., & Demme, W. (1999). Complications and mortality associated with anesthesia in dogs and cats. *Journal of the American Animal Hospital Association,* 35(1),13-17.

Ginosar, Y., Mirikatani, E., Drover, D., Cohen, S., & Riley, E. (2004). ED$_{50}$ and ED$_{95}$ of intrathecal hyperbaric bupivacaine coadministered with opioids for cesarean delivery. Anesthesiology, 100(3),676–682.

Gorayeb, D., de Araújo, J., Corrêa, F., Rios, S., & Gaido, R. (2015). Effects of combined midazolam and propofol in anesthesia induction and recovery of cats undergoing ovariohysterectomy. *Ciências Agrarias,* 36(6),4269-4276.

Gould, S., & Scott, R. (2005). 2-Hydroxypropyl-b-cyclodextrin (HP-b-CD): a toxicology review. *Food and Chemical Toxicology,* 43(10),1451-1459.

Griffenhagen, G., Rezende, M., Gustafson, D., Hansen, R., Lunghofer, P., & Mama, K. (2015) Pharmacokinetics and pharmacodynamics of propofol with or without 2% benzyl alcohol following a single induction dose administered intravenously in cats. *Veterinary Anaesthesia and Analgesia,* 42(5),472-483.

Grimm. K., Lamont, A., Tranquilli, W., Greene, S., & Robertson S. (2015). Veterinary anesthesia and analgesia. Fifth edition of Lumb and Jones. pp.196-277.

Hansen, L., & Bertelsen, M. (2013). Assessment of the effects of intramuscular administration of alfaxalone with and without medetomidine in Horsfield's tortoises (Agrionemys horsfieldii). *Veterinary Anaesthesia and Analgesia*, 40(6),68-75.

Herbert, G., Bowlt, K., Ford-Fennah, V., Covey-Crump, G., & Murrell, J. (2013). Alfaxalone for total intravenous anaesthesia in dogs undergoing ovariohysterectomy: a comparison of premedication with acepromazine or dexmedetomidine. *Veterinary Anaesthesia and Analgesia,* 40(2),124-133.

Hopkins, A., Giuffrida, M., & Larenza, M. (2014). Midazolam, as a co-induction agent, has propofol sparing effects but also decreases systolic blood pressure in healthy dogs. *Veterinary Anaesthesia and Analgesia,* 41(1),64-72.

Hull, C., (1979). Pharmacokinetics and pharmacodynamics. *British Journal of Anaesthesia,* 51,579-594.

Ilkiw, J. (1999). Balanced anesthetic techniques in dogs and cats. *Clinical Techniques in Small Animal Practice,* 14(1),27-37.

Ilkiw, J., Suter, C., Farver, T., McNeal, D., & Steffey, E. (1996). The behaviour of healthy awake cats following intravenous and intramuscular administration of midazolam. *Journal of Veterinary Pharmacology Therapy,* 19(3),205-216.

Jiménez, C., Mathis, A., Mora, S., Brodbelt, D., & Alibhai, H. (2012). Evaluation of the quality of the recovery after administration of propofol or alfaxalone for induction of anaesthesia in dogs anaesthetized for magnetic resonance imaging. *Veterinary Anaesthesia and Analgesia.* 39:151-159.

Jones, D., Stehling, L., & Zauder, H. (1979). Cardiovascular responses to diazepam and midazolam maleate in the dog. *Anesthesiology,* 51(5),430-434.

Jones, N., Elliot, S., & Knight, J. (2002). A comparison between midazolam co-induction and propofol predosing for the induction of anaesthesia in the elderly. *Anesthesia*, 57(7),649-653.

Karlo, R., Singh, N., Singh, K., Singh, T., Devi, N., & Devi, M. (2015). Priming effects of propofol during induction of anesthesia. *Journal of Medical Society,* 29(2),92-95.

Kataria, R., Singhal, A., Prakash, S., & Singh, I. (2010). A comparative study of efficacy of propofol auto-co-induction versus midazolam propofol co-induction using the priming principle. *Indian Journal of Anaesthesia,* 54(6),558-561.

Kazama, T., Ikeda, K., Morita, K., Kikura, M., Ikeda, T., Kurita, T., & Sato, S. (2000). Investigation of effective anesthesia induction doses using a wide range of infusion rates with undiluted and diluted propofol. *Anesthesiology*, 92(4),1017-28.

King, B., Harris, L., Greifenstein, F., Elder, J., & Dripps, R. (1951). Reflex circulatory responses to direct laryngoscopy and tracheal intubation performed during general anesthesia. *Anesthesiology*, 12(5),556–566.

Kojima, K., Nishimura, R., Mutoh, T., Hong, S., Mochizuki, M., & Sasaki, N. (2002). Effects of medetomidine-midazolam, acepromazine-butorphanol, and midazolam-butorphanol on induction dose of thiopental and propofol and on cardiopulmonary changes in dogs. *American Journal of Veterinary Research*, 63(12),1671-1679.

Kumar, A., Sanikop, C., & Kotur, P. (2006). Effect of priming principle on the induction dose requirements of propofol- A randomized clinical trial. *Indian Journal of Anaesthesia,* 50(4),283-287.

Lagos-Carvajal, A., Queiroz-Williams, P., Ricco, C., Nevarez, J., Cremer, da Cunha A, & Liu, C. (2017). The use of priming alfaxalone as an induction technique in cats. *Veterinary Anaesthesia and Analgesia,* 44(5),1262.e15.

Lambert, J., Belelli, D., Peden, D., Vardy, A., Peters, J. (2003). Neurosteroid modulation of GABA receptors. *Progress in Neurobiology,* 71(1):67–80.

Larsson, J., & Wahlstrom, G. (1994). Optimum rate of administration of propofol for induction of anaesthesia in rats. *British Journal of Anaesthesia,* 73,692-694.

Liao, P., Sinclair, M., Valverde, A., Mosley, C., Chalmers, H., Mackenzie, S., Hanna, B. (2017). Induction dose and recovery quality of propofol and alfaxalone with or without midazolam coinduction followed by total intravenous anesthesia in dogs. *Veterinary Anaesthesia and Analgesia,* 44(5),1016-1026.

Lienhart, A., Auroy, Y., Péquignot, F., Benhamou, D., Warszawski, J., Bovet, M., & Jougla, E. (2006). Survey of Anesthesia-related Mortality in France. *Anesthesiology,* 105(6),1087–1897.

Maddern, K., Adams, V., Hill, N., & Leece, E. (2010). Alfaxalone induction dose following administration of medetomidine and butorphanol in the dog. *Veterinary Anaesthesia and Analgesia,* 37(1),7-13.

Maney, J., Shepard, M., Braun, C., Cremer, J., & Hofmeister, E. (2013). A comparison of cardiopulmonary and anesthetic effects of an induction dose of alfaxalone or propofol in dogs. *Veterinary Anaesthesia and Analgesia,* 40(3),237-244.

Marcus, E., Brewster, E., Estes, K., & Bodor, N. (1990). An intravenous toxicity study of 2-hydroxypropyl-β-cyclodextrin, a useful drug solubilizer, in rats and monkeys. *International Journal of Pharmaceutics,* 59(3):231-243.

Mathis, A., Pinelas, R., Brodbelt, D., & Alibhai, H. (2012). Comparison of quality of recovery from anaesthesia in cats induced with propofol or alfaxalone. *Veterinary Anaesthesia and Analgesia,* 39(3),282-290.

Mehta, S., Dabhi, P., Golwala, M., & Upadhyay, R. (2015). Effect of priming principle on the induction dose requirement of Propofol. *International Journal of Research in Medical Sciences,* 4(1):69-73.

Miles, Davis. (1971). Comparison of Sequential Bioassays in Small Samples. *Journal of the Royal Statistical Society,* 33(1):78-87.

Mohler, H., & Richards, J. (1988). The benzodiazepine receptor: a pharmacological control element of brain function. *European Journal of Anaesthesiology*, 2,15-24.

Montmorency, F., Chen, A., Rudel, H., Glas, W., & Lee, L. (1958). Evaluation of cardiovascular, respiratory and general pharmacologic properties of hydroxydione. *Anesthesiology*, 19(4),450-456.

Muir, W., Lerche, P., Wiese, A., Nelson, L., Pasloske, K., & Whittem, T. (2008). Cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in dogs. *Veterinary Anaesthesia and Analgesia,* 35(6),451-462.

Muir, W., Lerche, P., Wiese, A., Nelson, L., Pasloske, K., & Whittem, T. (2009). The cardiorespiratory and anesthetic effects of clinical and supraclinical doses of alfaxalone in cats. *Veterinary Anaesthesia and Analgesia,* 36(1):42-54.

Muñoz, K., Robertson, S., & Wilson, D. (2017). Alfaxalone alone or combined with midazolam or ketamine in dogs: intubation dose and select physiologic effects." *Veterinary Anaesthesia and Analgesia,* 44(4),766-744.

Nadeson, R., & Goodchild, C. (2000). Antinociceptive properties of neurosteroids II. Experiments with Saffan and its components alphaxalone and alphadalone to reveal separation of anesthetic and antinociceptive effects and the involvement of spinal cord GABA$_A$ receptors. *Pain,* 88(1),31–39.

Nightingale, J., & Lewis, I. (1992). Recovery from day-case anaesthesia: comparison of total i.v. anaesthesia using propofol with an inhalation technique. *British Journal of Anaesthesia*, 68(4):356-359.

Nordt, S., & Clark, R. (1997). Midazolam: a review of therapeutic uses and toxicity. *The Journal of Emergency Medicine,* 15(3),357-365.

Pace, N., & Stylianou, M. (2007). Advances in and limitations of up-and-down methodology: a précis of clinical use, study design, and dose estimation in anesthesia research. *Anesthesiology,* 107(1),144-152.

Pasloske, K., Sauer, B., Perkins, N., & Whittem, T. (2009). Plasma pharmacokinetics of alfaxalone in both premedicated and unpremedicated Greyhound dogs after single, intravenous administration of Alfaxan® at a clinical dose. *Journal of Veterinary Pharmacology and Therapeutics,* 32(5):510-513.

Paton, W., & Waud, D. (1967). The margin of safety of neuromuscular transmission. *Journal of Physiology*, 191(1),59-90.

Pratap, S., Shareena, T., Mahmood, S., & Padmanabha, S. (2015). Hemodynamic study during induction of general anaesthesia with propofol using priming principle. *Journal of Evolution of Medicine and Dentistry Sciences*, 63(4),10914-10920.

Psatha, E., Alibhai, H., Jimenez-Lozano, A., Armitage-Chan, E., Brodbelt, D. (2011). Clinical efficacy and cardiorespiratory effects of alfaxalone, or diazepam/fentanyl for induction of anaesthesia in dogs that are a poor anaesthetic risk. *Veterinary Anaesthesia and Analgesia.* 38(1):24-36.

Ramoo, S., Bradbury, L., Anderson, G., & Abraham, L. (2013). Sedation of hyperthyroid cats with subcutaneous administration of a combination of alfaxalone and butorphanol. *Australian Veterinary Journal*, 91(4),131-136.

Riccó, C., & Henao-Guerrero, N. (2014). Cardiovascular effects of orotracheal intubation following anesthetic induction with propofol, ketamine-propofol, or ketamine-diazepam in premedicated dogs. *Journal of Animal Veterinary Medical Association,* 244(8), 934-939.

Riker, R., Shehabi, Y., Bokesch, P., Ceraso, D., Wisemandle, W., Koura, F., Whitten, P., Margolis, B., Byrne, D., Ely, E., & Rocha, M. (2009). Dexmedetomidine vs Midazolam for Sedation of Critically Ill PatientsA Randomized Trial. *Journal of the American Medical Association,* 301(5):489-499.

Robinson, R., & Borer-Weir, K. (2015). The effects of diazepam or midazolam on the dose of propofol required to induce anaesthesia in cats. *Veterinary Anaesthesia and Analgesia*, 42(5),493-501.

Robinson, R., & Borer-Weir, K. (2015). The effects of diazepam or midazolam on the dose of propofol required to induce anaesthesia in cats. *Veterinary Anaesthesia and Analgesia,* 42(5):493-501.

Robinson, R., & Borer-Weir, K. (2013). A dose titration study into the effects of diazepam or midazolam on the propofol dose requirements for induction of general anaesthesia in client owned dogs, premedicated with methadone and acepromazine. *Veterinary Anaesthesia and Analgesia,* 40(5):455-463.

Rodriguez, M., Muñoz-Rascón, P., Navarrete-Calvo, R., Goméz-Villamandos, R., Domínguez, J., Fernández J., Quirós, C., & Granados, M. (2012). Comparison of the cardiopulmonary parameters after induction of anaesthesia with alphaxalone or etomidate in dogs. *Veterinary Anaesthesia and Analgesia*, 39(4),357–365.

Samuelson, P., Reves, J., Kouchoukos, N., Smith, L., & Dole, K. (1981). Hemodynamic responses to anesthetic induction with midazolam or diazepam in patients with ischemic heart disease. Anesthesia and Analgesia, 60(11):802-809.

Sánchez, A., Belda, E., Escobar, M., Agut, A., Soler, M., & Laredo, F. (2013). Effects of altering the sequence of midazolam and propofol during co-induction of anaesthesia. *Veterinary Anaesthesia and Analgesia.* 40(4):359-366.

Schwartz, M., Muñana, K., Nettifee-Osborne, J., Messenger, K., & Papich, M. (2013). The pharmacokinetics of midazolam after intravenous, intramuscular, and rectal administration in healthy dogs. *Journal of Veterinary Pharmacology Therapy*, 36(5),471-437.

Schwarz, A., Kalchofner, K., Palm, J., Picek, S., Hartnack, S., Bettschart-Wolfensberger, R. (2014). Minimum infusion rate of alfaxalone for total intravenous anaesthesia after sedation with acepromazine or medetomidine in cats undergoing ovariohysterectomy. *Veterinary Anaesthesia and Analgesia. 41*:480-90.

Sdrales, L., & Miller, R. (2013). Second. Edition. pp.9-14.

Sear, J. (1996). Steroid anesthetics: old compounds, new drugs. Journal of Clinical Anesthesia, 8(3),91S-98S.

Seddighi, R., Egger, C., Rohrbachà, B., Cox, S., & Doherty, T. (2011). The effect of midazolam on the end-tidal concentration of isoflurane necessary to prevent movement in dogs. *Veterinary Anaesthesia and Analgesia*, 38(3),195–202.

Selye, H. (1941). Studies concerning the anesthetic action of steroid hormones. *Journal of Pharmacology and Experimental Therapeutics,* 73(2),116–121.

Short, T., & Chui, P. (1991). Propofol and midazolam act synergistically in combination. *British Journal of Anaesthesia*, 67(5):539-545.

Stapelfeldt, C., Lobo, E., Brown, R,. & Talke, P. (2005). Intraoperative clonidine administration to neurosurgical patients. *Anesthesia and Analgesia*, 100(1),226-232.

Stegmann, G., & Bester, L. (2001). Some clinical effects of midazolam premedication in propofol-induced and isoflurane-maintained anaesthesia in dogs during ovariohysterectomy. *Journal of the South African Veterinary Association*, 72(4):214-216.

Stella, V., & He, Q. (2008). Cyclodextrins. *Toxicologic Phatology,* 36(1),30-42.

Stevens, W., & Ktngston, H. (1989). Inhalation anesthesia. In. Barash PG Cullen BF, Stoelting RK (eds): Clinical Anesthesia (Ed 1). Philadelphia PA, J B Lippncott, 1989, pp 293-321.

Stocks, G., Hallworth, S., Fernando, R., England, A., Columb, M., & Lyons, G. (2001). Minimum local analgesic dose of intrathecal bupivacaine in labor and the effect of intrathecal fentanyl. Anesthesiology, 94(4):593–598.

Stoelting, R. (1977) Circulatory changes during direct laryngoscopy and tracheal intubation: influence of duration of laryngoscopy with or without prior lidocaine. *Anesthesiology* 47(4), 381–383.

Stokes, D., & Hutton, P. (1991). Rate-dependent induction phenomena with propofol: implications for the relative potency of intravenous anesthetics. *Anesthesia and Analgesia,* 72(5), 578–583.

Stylianou, M., & Flournoy, N. (2002). Dose finding using the biased coin up-and-down design and isotonic regression. *Biometrics,* 58(1),171-177.

Suarez, M., Dzikiti, B., Stegmann, F., & Hartman, M. (2012). Comparison of alfaxalone and propofol administered as total intravenous anaesthesia for ovariohysterectomy in dogs. *Veterinary Anaesthesia and Analgesia,* 39(3),236-44.

Sztark, F., Chopin, F., Bonnet, A., & Cros, A. (2005). Concentration of remifentanil needed for tracheal intubation with sevoflurane at 1 MAC in adult patients. *European Journal of Anaesthesioly*, 22(12),919-924.

Taboada, F., & Murison, P. (2010). Induction of anaesthesia with alfaxalone or propofol before isoflurane maintenance in cats. *Veterinary Record*, 167(3):85-89.

Taboada, J., Rupp, S., & Miller, R. (1986). Refining the priming principle for vecuronium during rapid-sequence induction of anesthesia. *Anesthesiology*, 64(2),243-247.

Tamura, J., Ishizuka, T., Fukui, S., Oyama, N., Kawase, K., Miyoshi, K., Sano, T., Pasloske, K., & Yamashita, K. (2015). The pharmacological effects of the anesthetic alfaxalone after intramuscular administration to dogs. *Journal of Veterinary Medicine Science*, 77(3),289-96.

Taylor, N., & Shearer, W. (1956). The anaesthetic properties 0f 21 hydroxypregnanedione sodium hemisuccinate (hydroxydione); a pharmacological and clinical study of 130 cases. *British Journal of Anaesthesia,* 28(2),67-76.

Thompson, E., & Greenhough, S. (1982). Adverse responses to iv anaesthetics. *British Journal of Anaesthesia,* 54(10),55-58.

Vågerö, M., & Sundberg, R. (1999). The distribution of the maximum likelihood estimator in up-and-down experiments for quantaldose-response data. *Journal of Biopharmaceutical Statistics*, 9(3),499-519.

Warne, L., Beths, T., Whittem, T., Carter, J., & Bauquier, S. (2015). A review of the pharmacology and clinical application of alfaxalone in cats. *Veterinary Journal*, 203(2):141-148.

Weaver, B., & Raptopoulos, D. (1990). Induction of anaesthesia in dogs and cats with propofol. *Veterinary Record,* 126(25):617-620.

White, K., & Yates, D. (2017). Clinical comparison of alfaxalone, ketamine and propofol following medetomidine and methadone in dogs. *Veterinary Anaesthesia and Analgesia,* 44(5):1027-1034.

Whittem, T., Pasloske, K., Heit, M., & Ranasinghe, M. (2008). The pharmacokinetics and pharmacodynamics of alfaxalone in cats after single and multiple intravenous administration of Alfaxan at clinical and supraclinical doses. *Journal of Veterinary Pharmacology Therapy.* 31:571-9.

Zaki, S., Ticehurst, K., & Miyaki, Y. (2009). Clinical evaluation of Alfaxan-CD® as an intravenous anaesthetic in young cats. *Australian Veterinary Journal,* 87(3),82–87.

APPENDIX A. Numerical Score System for Quality of Sedation, Endotracheal Intubation, Induction and Recovery in Cats

Appendix A.1. Description of the numerical scoring system employed to evaluate the quality of sedation after pre-anesthetic medication in 14 healthy cats (Robinson & Borer-Weir 2015)

Score	Description
1	Bright, alert, no discernible sedation
2	Mild sedation, appears sleepy or quiet
3	Moderate sedation, very sleepy; may be recumbent but can be roused
4	Heavy sedation, recumbent, difficult to rouse
5	Profound sedation, lateral recumbence, cannot be roused

Appendix A.2. Description of the numerical scale employed to score the quality of endotracheal intubation observed in 14 cats induced with alfaxalone-midazolam co-induction (Griffenhagen et al. 2015)

Score	Description
1	Smooth. No swallowing, coughing, tongue or jaw movement, intubated first attempt.
2	Fair. Some tongue movement, slight coughing, 1-3 attempts to intubate.
3	Poor. Marked tongue/jaw movement and swallowing or coughing, unable to intubate or more than three attempts at intubation.

Appendix A.3. Description of the numerical scale of quality of induction employed in 14 cats induced with alfaxalone-midazolam co-induction (Griffenhagen et al. 2015)

Score	Description
1	Smooth. No outward sign of excitement, rapidly assumes lateral recumbence, good muscular relaxation
2	Fair. Mild signs of excitement, some struggling.
3	Poor. Hyperkinesis, obvious signs of excitement, vocalization, defecation or urination

Appendix A.4. Description of the numerical recovery scale employed in 14 cats induced with alfaxalone-midazolam co-induction (Griffenhagen et al. 2015)

Score	Description
1	Smooth. Assumes sternal recumbence with little or no struggling, and may attempt to stand and walk with little or no difficulty.
2	Fair. Some struggling, requires assistance to achieve sternal recumbence or to stand, responsive to external stimuli, becomes quiet in sternal recumbence.
3	Poor. Prolonged struggling, unable to assume sternal recumbence or difficulty in maintaining sternal or standing position, becomes hyperkinetic when assisted, prolonged paddling or swimming motion.

APPENDIX B. Numerical Score System for Sedation, Endotracheal Intubation, Induction and Recovery in Cats

Appendix B.1. Numerical score system employed to assess quality of sedation in 8 healthy

adult mix-breed cats (Robinson & Borer-Weir 2015)

Score	Description
1	Bright, alert, no discernible sedation
2	Mild sedation, appears sleepy or quiet
3	Moderate sedation, very sleepy; may be recumbent but can be roused
4	Heavy sedation, recumbent, difficult to rouse
5	Profound sedation, lateral recumbency, cannot be roused

Appendix B.2. Descripting scale employed to numerically score the quality of endotracheal

intubation in 8 healthy adult cats (Griffenhagen et al. 2015)

Score	Description
1	Smooth. No swallowing, coughing, tongue or jaw movement, intubated first attempt.
2	Fair. Some tongue movement, slight coughing, 1-3 attempts to intubate.
3	Poor. Marked tongue/jaw movement and swallowing or coughing, unable to intubate or more than three attempts at intubation.

Appendix B.3. Quality of induction score employed in 8 healthy adult cats (Griffenhagen et al. 2015)

Score	Description
1	Smooth. No outward sign of excitement, rapidly assumes lateral recumbency, good muscular relaxation
2	Fair. Mild signs of excitement, some struggling.
3	Poor. Hyperkinesis, obvious signs of excitement, vocalization, defecation or urination

Appendix B.4. Numerical recovery score used to evaluate the quality of recovery in 8 healthy adult cats (Griffenhagen et al. 2015)

Score	Description
1	Smooth. Assumes sternal recumbency with little or no struggling, and may attempt to stand and walk with little or no difficulty.
2	Fair. Some struggling, requires assistance to achieve sternal recumbency or to stand, responsive to external stimuli, becomes quiet in sternal recumbency.
3	Poor. Prolonged struggling, unable to assume sternal recumbency or difficulty in maintaining sternal or standing position, becomes hyperkinetic when assisted, prolonged paddling or swimming motion.

APPENDIX C. Numerical Score System for Sedation, Endotracheal Intubation, Induction and Recovery in Dogs

Appendix C.1. Scoring system employed to evaluated the quality of sedation in 10 adult dogs ten minutes after dexmedetomindine (1 mcg kg^{-1}) and methadone (0.2 mg kg^{-1}) intravenous sedation. (Amengual et al. 2013)

Score	Description
0	No sedation
1	Mild sedation (i.e. quieter but still bright and active)
2	Moderate sedation (i.e. quiet, reluctant to move, ataxic but still able to walk)
3	Profound sedation (i.e. unable to walk)

Appendix C.2. Descripting scale employed to numerically score the quality of endotracheal intubation in 10 healthy female adult dogs. (Casoni et al. 2015)

Score	Description
1	Smooth intubation without reaction
2	Mild coughing during or immediately after intubation
3	Pronounced coughing during or immediately after intubation
4	Swallowing, gagging, head movements during or immediately after intubation
5	Failed attempt

Appendix C.3. Numerical score system employed for quality of induction assessment in 10
female healthy dogs (Casoni et al. 2015)

Score	Description
1	Ideal: smooth uneventful induction
2	Good: mild twitching or excitement, head movements
3	Unsatisfactory: pronounced twitching, or excitement, backwards movements, presence of paddling
4	Induction not reached

Appendix C.4. Qualitative scoring system employed to evaluate quality of recovery after
anesthesia in 10 healthy dogs (Jiménez et al. 2012)

Score	Description
1	Early-extubated, easy transition to alertness, coordinated movement. Late-alert, coordinated movement
2	Early- fairly easy transition, holds head up, no body movement attempted Late-holds head up, no body movement
3	Some incoordination, does not startle, generally quiet
4	Limited muscle control, startles, may paddle or whine
5	Uncoordinated whole-body movements, startles, vocalizes
6	Emergence delirium, thrashing, cannot easily restrain

VITA

Angie Lagos Carvajal was born in Bogotá, Colombia on October 1984. She graduated from Veterinary Medicine at the National University of Colombia in 2011. Later, she worked as a Veterinarian at the Large Animal Teaching Clinic of the National University of Colombia, from January 2011 to June 2012. In 2012, Angie Lagos was accepted to do an externship in veterinary anesthesia at Sao Paulo State University (UNESP) in Botucatu, Brazil. In March of 2013, she started a Master in Anesthesiology that successfully completed in February 2015. During that period, Angie Lagos participate as a teaching assistant in a Veterinary Anesthesiology specialization program in Sao Paulo, Brazil, at the Institute Bioethicus during one year (2014-2015). In 2015, she started a three-year residency in veterinary anesthesia and analgesia at the Veterinary School of Louisiana State University, in United States. In the same year, she was accepted as a graduate student in the Department of Veterinary Clinical Sciences at the School of Veterinary Medicine of the Louisiana State University. Currently, Dr. Lagos anticipates graduation in Biomedical and Veterinary Medical Sciences at Louisiana State University in May 2018.