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Anesthetic Combination of Alfaxalone, Dexmedetomidine and Hydromorphone for Comparison of Endoscopy-Guided Intubation and Blind Orotracheal Intubation in Domestic Rabbits (Oryctolagus cuniculus)

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Table of Contents

List of Tables

List of Figures

Abstract

Domestic rabbits have a high risk of anesthesia-related mortality compared to other companion animals. Factors that may contribute to increased mortality include drug-induced cardiopulmonary effects (Grint & Murison 2008; Navarrete-Calvo et al. 2013), difficulty performing intubation (Aeschbacher 1995; Flecknell 2016; Wenger et al. 2017) or damage to laryngeal and tracheal tissues following orotracheal intubation (Grint et al. 2006; Phaneuf et al. 2006; Engbers et al. 2017).

Alfaxalone is a neuroactive steroid that produces induction of anesthesia and cardiorespiratory depression in a dose-dependent manner when injected intravenously. The ability to induce anesthesia via an intramuscular (IM) route has several advantages in the rabbit including possible reduction in cardiopulmonary adverse effects and reduced stress of handling for intravenous catheter placement. The dose of alfaxalone needed to produce an adequate plane of anesthesia to allow intubation has not been evaluated in rabbits. Therefore, one objective of this research was to determine the optimal dose of IM alfaxalone in combination with dexmedetomidine and hydromorphone that would allow endoscopic orotracheal intubation of rabbits. For this chapter, three different doses of alfaxalone (2, 5, and 7 mg kg^{-1}) IM were tested in combination with 0.1 mg kg^{-1} hydromorphone and 0.005 mg kg^{-1} dexmedetomidine. Following administration of the drug combination containing 7 mg kg^{-1} alfaxalone, intubation was successful in 80% of rabbits.

The consequences of repeated intubation attempts using different intubation techniques on respiratory tissue damage and the potential increase in mortality are largely unknown and have has not been extensively studied. Therefore, another objective of this research was to compare respiratory tissue damage caused by blind versus endoscopic-guided intubation of rabbits, and to

correlate the number of intubation attempts with the degree of respiratory tissue damage. For this chapter, rabbits were administered 7 mg kg^{-1} alfaxalone in combination with 0.1 mg kg^{-1} hydromorphone and 0.005 mg kg^{-1} dexmedetomidine IM. Intubation was attempted using either the blind or endoscopic-guided technique. The results demonstrated that blind intubation was associated with a higher number of intubation attempts; however, no difference in the degree of tissue damage was observed between the two techniques.

Chapter One. Introduction

The risk of anesthetic-related mortality in companion animals is substantially higher than that reported in humans undergoing general anesthesia. A large-scale, multi-centric study conducted in veterinary species determined the overall risk of anesthetic-related mortality to be 0.17% in dogs, 0.24% in cats and 1.39% in rabbits (Brodbelt et al., 2008). Domestic rabbits appear to be at increased risk of mortality during anesthesia compared to other veterinary species, with healthy rabbits having a risk of 0.73% and sick rabbits having a risk of 7.37% (Brodbelt et al., 2008).

Although specific risk factors have yet to be conclusively determined, many studies have speculated on potential factors that may contribute to this increased risk of mortality seen in rabbits. As a prey species, the easily stressed nature of domestic rabbits may predispose them to increased mortality during handling for catheter placement or induction of anesthesia (Flecknell 2016). In addition, many rabbits present with sub-clinical *Pasteurella multocida* infections which can predispose them to respiratory complications in the perianesthetic period (Johnson-Delaney & Orosz 2011; Flecknell 2016). Similar to other companion animal species, drug-induced cardiorespiratory depression seen with sedatives and anesthetics may also contribute to mortality (Grint & Murison 2008; Navarrete-Calvo et al. 2013; Bradley et al. 2019). Finally, the ability to perform orotracheal intubation of rabbits is challenging, often resulting in failure, especially for personnel with limited experience in this procedure (Grint & Murison 2008; Wenger et al. 2017).

Because of the inherent challenges in orotracheal intubation of rabbits, it is common practice to perform anesthetic procedures in non-intubated rabbits (Brodbelt 2006; Lee et al. 2018). Hence, the consequences of drug-induced cardiorespiratory depression may be of greater importance in rabbits, because they may become more susceptible to, hypoventilation, bradypnea,

apnea and hypoxemia associated with specific drug combinations (Hellebrekers et al. 1997; Grint & Murison 2008; Huynh et al. 2014). Thus, anesthetic drug selection may affect mortality rate, especially in non-intubated rabbits. Many different anesthetic protocols have been described in rabbits, each offering specific advantages, but also producing variable and potentially severe adverse effects. The most commonly utilized anesthetic protocols in rabbits include a combination of a dissociative anesthetic, α-2 agonist, opioid and/or benzodiazepine (Brodbelt 2006). More recently, alfaxalone has become licenced for use in cats and dogs in the United States among other countries. Although alfaxalone use in rabbits is still off-label, several studies have emerged investigating its clinical use and potential adverse effects in rabbits.

Alfaxalone is a synthetic neuroactive steroid that binds to $GABA_A$ receptors and facilitates the inhibitory action of gamma-aminobutyric acid (GABA) resulting in central nervous system depression (Lambert et al. 2003). Similar to dissociative anesthetics, such as ketamine, alfaxalone can be administered via the intramuscular (IM) route allowing induction of anesthesia (Marsh et al. 2009). Many studies have investigated the clinical effects of alfaxalone administered intramuscularly and have demonstrated that it can provide reliable sedation progressing to anesthesia with increasing doses in rabbits (Marsh et al. 2009; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019). However, dose-dependent respiratory depression, including apnea, may also occur and are more profound when alfaxalone is combined with other sedative agents (Navarrete-Calvo et al. 2013; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019).

Rabbits are less commonly intubated compared to other companion animals as a result of the technical challenges associated with intubation and the risk of causing trauma to the fragile tissues of the upper respiratory tract (Grint et al. 2006; Phaneuf et al. 2006; Engbers et al. 2017). The challenge in intubating rabbits is largely a result of the unique oropharyngeal anatomy which

makes it difficult to visualize the larynx and appropriately place an endotracheal tube (Eatwell 2014). As such, several different methods to establish a patent airway in the rabbit have been described, including variations in blind orotracheal intubation (Krüger et al. 1994; Falcão et al. 2001), endoscopic intubation (Worthley et al. 2000; Tran et al. 2001; Johnson 2010), nasotracheal intubation (Stephens DeValle 2019), laryngeal mask airway (Smith et al. 2004; Kazakos et al. 2007) and supraglottic airway devices (Crotaz 2010; Uzun et al. 2015).

Few studies have investigated the association between repeated intubation attempts in rabbits and the occurrence of respiratory tissue damage. Current information is largely based on anecdotal evidence and case reports. Tracheobronchial injury following blind orotracheal intubation has been documented; however, a definitive cause of tissue damage could not be determined, and the number of intubation attempts was not reported in these studies (Grint et al. 2006: Phaneuf et al. 2006). In a prospective randomized experimental study, blind orotracheal intubation was found to cause more damage to tracheal tissue compared to a supraglottic airway device (Engbers et al. 2017). In this study, the number of intubation attempts was recorded, however the degree of tissue damage in relation to histopathology score was not statistically analyzed. The lack of controlled clinical trials warrants further investigation of the incidence, etiology and clinical consequences of repeated intubation attempts in rabbits.

Chapter Two. Literature Review

2.1. Induction of Anesthesia

Rabbits are a commonly anesthetized species and may undergo general anesthesia for a variety of reasons including diagnostic imaging, surgical procedures, and for research-related purposes (Brodbelt et al. 2008). However, they also present with one of the highest perianesthetic mortality rates reported in veterinary species (Brodbelt et al. 2008). Drug-induced cardiopulmonary depression may be one contributing factor to this increased risk of mortality; thus, an induction protocol that provides immobility, sedation and anxiolysis while minimizing cardiovascular depression is ideal. In addition, some authors have speculated that drug dose and clinical effects may vary depending on the breed, strain, age and health status of the rabbit; thus, making it difficult to form comparisons among studies or extrapolate data to clinical settings (Aeschbacher 1995; Cantwell 2001). This phenomenon has been demonstrated. One study has demonstrated strain-specific and sex-specific differences in response to some anesthetic drugs (Avsaroglu et al. 2003).

2.1.1. Dissociative Anesthetics

Ketamine is a dissociative anesthetic widely used in veterinary medicine (Akkerdaas et al. 2001; Ferreira et al. 2015). As with other species, ketamine produces dose-dependent sedation and anesthesia in rabbits; however, it does not provide adequate muscle relaxation (Santos et al. 2016). Thus, it is often combined with other sedatives and analgesics to induce and maintain general anesthesia (Flecknell 2016). Several protocols in rabbits have been reported in the literature, involving ketamine in combination with an α-2 agonist (Borkowski et al. 1990; Hellebrekers et al. 1997; Hedenqvist et al. 2001b; Gil et al. 2002; Difilippo et al. 2004; Henke et al. 2005; Orr et al. 2005; Grint & Murison 2008; Murphy et al. 2010), benzodiazepine (Dupras et al. 2001; Gil et al.

2002; Hedenqvist et al. 2002; Grint & Murison 2008; Oguntoye et al. 2018) or any combination of aforementioned drug classes, with or without an opioid.

One study compared induction of anesthesia using four dose combinations of ketamine and medetomidine in five New Zealand white rabbits (Hedenqvist et al. 2001b). The following doses of ketamine/medetomidine were administered IM: 25/0.25, 15/0.5, 15/0.25 and 10/0.5 mg kg^{-1} on four successive occasions. Induction was smooth and blind orotracheal intubation was achieved with all doses. Duration of surgical anesthesia (determined by loss of righting reflex) was longer for dose groups 25/0.25 and 15/0.15 compared to 15/0.25 and only three rabbits achieved surgical anesthesia in the 10/0.5 groups. Heart rate (HR) and respiratory rate (f_R) decreased from baseline in all groups and was not significantly different between groups. Hypoxemia and hypercapnia occurred with all doses (Hedenqvist et al. 2001b).

Another study compared induction of anesthesia using IM combinations of ketamine (15 mg kg^{-1}) and medetomidine (0.25 mg kg^{-1}) or ketamine (15 mg kg^{-1}) and midazolam (3 mg kg^{-1}) in 50 un-premedicated rabbits of various breeds (Grint & Murison 2008). Time to loss of righting reflex was significantly shorter in the medetomidine group compared to the midazolam group. The number of intubation failures, number of intubation attempts and quality of intubation was not different between groups. The HR and hemoglobin oxygen saturation were lower in the medetomidine group but remained within normal reference ranges (Grint & Murison 2008).

Henke et al. 2005 compared three IM anesthetic induction techniques in 19 chinchilla mixed-breed rabbits (Henke et al. 2005). Rabbits were administered either medetomidine (0.25 mg kg^{-1}) and ketamine (35 mg kg^{-1}) or xylazine (4 mg kg^{-1}) and ketamine (50 mg kg^{-1}) or medetomidine (0.20 mg kg^{-1}), fentanyl (0.02 mg kg^{-1}) and midazolam (1 mg kg^{-1}). All protocols resulted in loss of righting reflex, but the xylazine-ketamine group had a faster time to loss of

reflex. A surgical plane of anesthesia was achieved more commonly in rabbits in the medetomidine-ketamine groups and medetomidine-fentanyl-midazolam groups. Few animals in the xylazine-ketamine group achieved a surgical plane of anesthesia and duration was significantly shorter than the other groups. A similar degree of intubation failures occurred among groups. Rabbits in medetomidine-fentanyl-midazolam group retained the endotracheal tube longer, without chewing or swallowing reflexes, than the other groups. Heart rates were significantly higher in the xylazine-ketamine group and the greatest decrease in blood pressure was also observed in this group. The f_R was significantly lower in the medetomidine-fentanyl-midazolam group with some rabbits experiencing apnea. Hypercapnia was also greatest in this group.

Intramuscular administration may be associated with discomfort and rabbits may react to injection (Orr et al. 2005; Williams & Wyatt 2007; Grint & Murison 2008). Intramuscular administration of ketamine has been documented to cause tissue irritation and muscle necrosis (Aeschbacher 1995). Thus, induction via subcutaneous (SQ) route may be preferred (Wenger 2012). Combinations of ketamine (10-15 mg kg^{-1} SQ) and medetomidine (0.15-0.25 mg kg^{-1} SQ) are sufficient to induce a surgical plane of anesthesia (Hedenqvist et al. 2002; Williams & Wyatt 2007; Grint & Murison 2008) and onset of induction is similar to IM administration of ketamine and medetomidine (Hedenqvist et al. 2002; Williams & Wyatt 2007). In contrast, Orr et al. 2005 reported a significantly slower onset of induction when similar doses of ketamine-medetomidine was given SQ compared to IM (Orr et al. 2005).

Telazol is a commercially formulated combination product containing a dissociative anesthetic (tiletamine) and a benzodiazepine (zolazepam). Studies have reported use of Telazol in rabbits (Brammer et al. 1991; Popilskis et al. 1991; Dupras et al. 2001). One study in New Zealand white rabbits reported that high doses (32 or 64 mg kg^{-1} IM) are required to produce immobility

(Brammer et al. 1991). In addition, these doses did not blunt the response to noxious stimuli and were associated with a delayed recovery (Brammer et al. 1991). Furthermore, these doses were reported to produce dose-dependent renal tubular necrosis within 7 days following injection (Brammer et al. 1991). Lower doses (7.5 mg kg^{-1}) caused mild renal tubular necrosis (Doerning et al. 1992). A subsequent study revealed that tiletamine was responsible for the nephrotoxicity of Telazol in rabbits (Doerning et al. 1992).

2.1.2. Propofol

Propofol is a diisopropyphylphenol hypnotic agent used for IV induction in many veterinary species (Aono et al. 2001). Initial studies evaluating use of propofol in rabbits determined that 6 mg kg^{-1} caused rapid and smooth induction and recovery in non-premedicated rabbits (Aeschbacher & Webb 1993). However, rabbits appear to be highly sensitive to the respiratory depressive effects of injectable anesthetics and these drugs should be administered slowly (Aeschbacher 1995). Apnea may occur even if the drug is administered slowly over 60 seconds (Wenger 2012). Severe respiratory depression appears to occur at the same dose required for induction, which may interfere with the ability to intubate the rabbit (Aeschbacher & Webb 1993). Dose-dependent hypotension and reflex tachycardia may also occur (Aono et al. 2001). Propofol is a commonly used IV induction agent in rabbits (Wenger 2012). Relatively high doses (10-20 mg kg^{-1}) may be needed to induce an anesthetic plane suitable for orotracheal intubation (Flecknell 2016).

One study evaluated induction of anesthesia using either midazolam or propofol in rabbits premedicated with fentanyl and fluanisone (Martinez et al. 2009). In this study, both midazolam and propofol resulted in successful intubation in all but one rabbit. Post-induction apnea was reported in a significantly higher number of rabbits in the midazolam group compared to the

propofol group and mean f_R was higher in the propofol group. Other hemodynamic variables did not differ between groups. Time to recover and quality of recovery were significantly better in the propofol group (Martinez et al. 2009).

A ketamine-propofol (ketofol) combination has been investigated for anesthetic induction of un-premedicated New Zealand white rabbits (Santos et al. 2016). In that study, three doses of ketamine and propofol were evaluated. The drugs were mixed together in a 1:1 mg kg^{-1} ratio so that each treatment group received either 1, 3 or 5 mg kg^{-1} each of ketamine and propofol IV. Time to loss of righting reflex was shortest and the duration of action was longest in the 5 mg kg^{-1} group. Loss of righting reflex was not achieved in the 1 mg kg^{-1} group, nor was intubation possible. Quality of induction was smooth in the 5 mg kg^{-1} group and ranged from fair to smooth in the other groups. Intubation was possible in both 3 and 5 mg kg^{-1}, however intubation quality was variable. Hypoxemia and dose-dependent respiratory depression was observed in all groups (Santos et al. 2016). Respiratory depression was also observed following IV induction with S (+)-ketamine (1 mg kg^{-1}) and propofol (2 mg kg^{-1}) in New Zealand white rabbits premedicated IM with acepromazine (0.1 mg kg^{-1}) and buprenorphine (0.02 mg kg^{-1}) and maintained on propofol alone or in combination with ketamine constant rate infusion (Cruz et al. 2010).

2.1.3. Alfaxalone

Alfaxalone is a synthetic neuroactive steroid that binds to $GABA_A$ receptors and facilitates the inhibitory action of gamma-aminobutyric acid (GABA) resulting in central nervous system depression (Lambert et al. 2003). The initial formulation of alfaxalone contained a castor oil surfactant as a solubilizing agent; however, this component resulted in severe anaphylactic reactions and was later withdrawn from the market (Watt 1975). Years later, alfaxalone was reformulated with 2-hydroxypropyl-β-cyclodextrin (alfaxalone-HPCD) and did not exhibit

histamine-releasing properties associated with earlier formulations (West 2017). However, this new formulation does not include a preservative and according to the USA drug label, once the vial has been broached, the contents should be drawn into a single syringe and used for a single patient with any unused product discarded within six hours (Drug insert, Alfaxan®, Jurox Inc.). In 2018, three preservatives were added to alfaxalone-HPCD formulation, resulting in a new product known as alfaxalone multidose which has an extended shelf life of 28 days following vial broaching (Drug insert, Alfaxan® Multidose, Jurox Inc.). Currently, alfaxalone-HPCD is licenced for IV use in cats and dogs in the United States and several other countries (West 2017). Alfaxalone is also approved for IV use in rabbits in the United Kingdom and Australia and is used off-label in the United states for induction of anesthesia of rabbits.

Alfaxalone produces induction of anesthesia in a dose-dependent manner when injected intravenously in rabbits. In one study, administration of alfaxalone to 20 rabbits of various breeds at doses of 2 or 3 mg kg^{-1} IV following premedication with buprenorphine at 0.03 mg kg^{-1} IM resulted in a smooth and rapid induction of anesthesia with all rabbits achieving an anesthetic plane sufficient enough to allow blind orotracheal intubation (Grint et al. 2008). However, in another study performed in New Zealand white rabbits premedicated with IM morphine (1 or 2 mg kg^{-1}) and medetomidine (0.2 mg kg^{-1}), alfaxalone doses less than 10 mg kg^{-1} IV provided an insufficient anesthetic plane to allow orotracheal intubation using an otoscope and guidewire technique (Navarrete-Calvo et al. 2013).

Alfaxalone produces dose-dependent sedation when injected intramuscularly, with progression to anesthesia occurring at higher doses (Marsh et al. 2009; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019). Preliminary studies investigating IM administration of alfaxalone was first performed in wild rabbits (Marsh et al. 2009). In that study, rabbits were premedicated

with medetomidine (0.25 mg kg^{-1} SQ), induced with alfaxalone (5 mg kg^{-1} IM) and maintained with isoflurane in oxygen (1.5-3%) using a facemask. This protocol resulted in lateral recumbency in all rabbits and a sufficient anesthetic plane to perform surgical preparation. The authors also reported smooth induction and uneventful recovery in all rabbits (Marsh et al. 2009).

Administration of IM alfaxalone at doses of 1, 2.5 or 5 mg kg^{-1} IM resulted in lateral recumbency and loss of righting reflex in Japanese white rabbits (Ishikawa et al. 2019). Administration of IM alfaxalone at doses of 4, 6 or 8 mg kg^{-1} to New Zealand white rabbits resulted in smooth and rapid onset of sedation and loss of righting reflex, however limb withdrawal in response to toe pinch was maintained (Huynh et al. 2014). Similarly, administration of IM alfaxalone 6 mg kg^{-1} to New Zealand white rabbits resulted in good quality of sedation with smooth recovery (Bradley et al. 2019). Furthermore, when the same alfaxalone dose was combined with butorphanol (0.3 mg kg^{-1} IM) and dexmedetomidine (0.2 mg kg^{-1} IM), no response to noxious stimuli occurred, indicating a good anesthetic plane had been achieved. With this drug combination, the onset of effects was more rapid, and duration was longer compared to alfaxalone alone (Bradley et al. 2019).

Respiratory depression occurs in a dose-dependent manner following either IM or IV administration of alfaxalone. Apnea may occur following IV alfaxalone administration, even when administered slowly over 60 seconds (Grint et al. 2008; Tutunaru et al. 2013; Ishikawa et al. 2019). Following administration of 3 mg kg^{-1} IV alfaxalone to un-premedicated New Zealand white rabbits, RR significantly decreased compared to the control group, however values remained within normal physiologic limits (Gil et al. 2012).

Dose-dependent respiratory depression and apnea may also occur following IM administration and is more profound when alfaxalone is combined with other sedative agents

(Navarrete-Calvo et al. 2013; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019). Evidence of significant respiratory depression is difficult to determine in these studies because often only f_R was measured and more sensitive indices of respiratory function (e.g., hemoglobin oxygen saturation, capnography, blood gas analysis) were not monitored.

In one study, administration of IM alfaxalone at doses of 4, 6 or 8 mg kg^{-1} to New Zealand white rabbits, in absence of other sedatives, caused a dose-dependent decrease in f_R (Huynh et al. 2014). In that study, all rabbits experienced a decrease in f_R below the normal physiologic value (defined as f_R < 30 breaths $minute^{-1}$ by the authors). This decrease in f_R was significantly greater and lasted longer in rabbits administered 6 and 8 mg kg^{-1} compared to those administered 4 mg kg^{-1}. In addition, the highest dose (8 mg kg^{-1}) resulted in apnea followed by cardiac arrest in one rabbit (Huynh et al. 2014).

Similar to the study by Huynh et al. 2014, Bradley et al. 2019 also reported a decrease in f_R following administration of 6 mg kg^{-1} IM alfaxalone to New Zealand white rabbits (Bradley et al. 2019). In that study, mean f_R decreased from 196 ± 7 breaths $minute^{-1}$ (baseline) to 35 ± 2 breaths $minute^{-1}$ (10 minutes after injection), representing a decrease in magnitude of greater than 100 breaths $minute^{-1}$. However, f_R remained within the normal acceptable limits (Bradley et al. 2019). In addition, when the same dose of alfaxalone was combined with butorphanol (0.3 mg kg^{-1}) and dexmedetomidine (0.2 mg kg^{-1}), a greater decrease in mean f_R occurred (22 ± 3 breaths $minute^{-1}$) and this was significantly lower than that of alfaxalone alone (Bradley et al. 2019). Although hemoglobin oxygen saturation was monitored in this study, there was difficulties obtaining consistent readings and data were not statistically analyzed.

In another study, decrease in respiratory rate was observed in Japanese white rabbits following IM administration of alfaxalone at doses of 2.5 mg kg^{-1} and 5 mg kg^{-1} but not 1 mg kg^{-}

[1] (Ishikawa et al. 2019). In this study, the decrease in f_R occurred earlier and lasted longer in rabbits administered 5 mg kg^{-1} compared to 2.5 mg kg^{-1} and median minimum f_R values were lower for the 5 mg kg^{-1} group compared to the 2.5 mg kg^{-1} group, suggesting a dose-dependent response. However, at no point did any value decrease below normal physiologic reference range. In addition, abnormally low (< 95%) hemoglobin oxygen saturation occurred in one rabbit in the 2.5 mg kg^{-1} group and three rabbits in the 5 mg kg^{-1} group. For these rabbits, the decrease in hemoglobin oxygen saturation corresponded with the decrease in f_R, suggesting hypoxemia may have been present (Ishikawa et al. 2019).

Clinically significant hypoxemia (defined as PaO_2 < 88 mmHg) has been documented in New Zealand white rabbits following IM administration of alfaxalone (4 mg kg^{-1}) in combination with dexmedetomidine (0.1 mg kg^{-1}) and midazolam (0.2 mg kg^{-1}) (Rousseau-Blass & Pang 2020). In that study 100% oxygen supplementation via facemask resolved the hypoxemia. However, the increase in PaO_2 was associated with a decrease in f_R and subsequent hypercapnia (defined as $PaCO_2$ > 45 mmHg). The authors speculated that drug-induced hypoventilation resulted in hypoxemia, leading to hypoxic ventilatory drive and increased minute ventilation. Upon provision of 100% oxygen supplementation, the hypoxic drive was attenuated resulting in a decrease in f_R and hypercapnia associated with drug administration (Rousseau-Blass & Pang 2020).

Alfaxalone exhibits minimal cardiovascular depression in both dogs and cats at clinically relevant doses administered IV (Muir et al. 2008; Muir et al. 2009). Similar findings have also been reported in rabbits. In one study, HR significantly decreased following administration of 3 mg kg^{-1} IV alfaxalone to un-premedicated New Zealand white rabbits compared to the control group, however values remained within normal physiologic limits (Gil et al. 2012).

In New Zealand white rabbits, no significant change in HR was observed in rabbits administered IM alfaxalone at doses of 4, 6 or 8 mg kg^{-1} (Huynh et al. 2014). Similarly, Ishikawa et al. 2019 did not observe a significant change in HR or oscillometric blood pressure following IM alfaxalone administration at doses of 1, 2.5 or 5 mg kg^{-1} in Japanese white rabbits. However, HR tended to increase transiently following treatments (Ishikawa et al. 2019). Bradley et al. 2019 also reported no change in HR following administration of 6 mg kg^{-1} IM alfaxalone to New Zealand white rabbits (Bradley et al. 2019). However, when the same dose of alfaxalone was combined with dexmedetomidine (0.2 mg kg^{-1}), the mean HR was lower (146 ± 8 beats $minute^{-1}$) than the HR of alfaxalone alone (285 ± 10 beats $minute^{-1}$) but higher than the HR of dexmedetomidine alone (133 ± 3 beats $minute^{-1}$) ten minutes following treatment administration (Bradley et al. 2019). The authors speculated that the increase in HR observed in the alfaxalone-dexmedetomidine group compared to the dexmedetomidine group could have been a result of arterial hypotension causing a reflex increase in HR, however blood pressure was not monitored in this study (Bradley et al. 2019).

2.1.4. Inhalant induction

Inhalant induction through use of a facemask may be considered when IV catheterization is unsuccessful, IM injectable agents are unavailable, or in situations when the associated stress of handling is detrimental to the animal. Induction of general anesthesia using volatile anesthetics is commonly performed using isoflurane or sevoflurane (Eatwell & Mancinelli 2013). The inhalant is delivered via a tight-fitting face mask or sealed induction chamber and is delivered at a high concentration of isoflurane (5%) or sevoflurane (8%) in oxygen (Eatwell & Mancinelli 2013). Induction of anesthesia using inhalants is relatively long compared to rapid-acting intravenous induction agents (Flecknell et al. 1999). Rabbits may become sufficiently stressed and struggle

violently during the induction process, potentially leading to spinal and limb injuries (Flecknell et al. 1999; Eatwell & Mancinelli 2013; Foot 2020). The pungent odour associated with isoflurane may cause rabbits to breath-hold, resulting in prolonged periods of apnea, leading to hypercapnia, hypoxemia and respiratory acidosis (Flecknell et al. 1999; Hedenqvist et al. 2001a; Bateman et al. 2005; Longley 2009). However, use of inhalants with a less pungent odour, such as sevoflurane or desflurane, did not prevent the breath-holding response in rabbits (Flecknell et al. 1999; Hedenqvist et al. 2001a). Slowly increasing the percentage of anesthetic agent every 30 seconds does not reduce induction time or the occurrence of breath-holding, anxiety or struggling (Hedenqvist et al. 2001a). In addition, induction of anesthesia using volatile inhalants is associated with marked bradycardia (Flecknell et al. 1999; Hedenqvist et al. 2001a).

2.2. Intubation techniques

Orotracheal intubation involves placement of a tube from the oral cavity into the trachea (Foote 2020). It serves many purposes including effective delivery of inhalational anesthetic gas, and oxygen supplementation (Nordin 1977). In addition, it provides a patent airway and allows intermittent positive pressure ventilation when indicated. Furthermore, appropriate airway management prevents pulmonary aspiration of foreign material and limits personnel exposure to waste anesthetic gasses.

Both cuffed and uncuffed endotracheal tubes can be used in rabbits. However, uncuffed tubes may be preferred to prevent damage to tracheal mucosa induced by cuff pressure when inflated (Nordin 1997). Use of uncuffed tube also allows passage of the largest diameter tube possible, whereas presence of a cuff increases the outer diameter which may make it too large to pass through the larynx (Johnson 2010; Flecknell 2016). In addition, cuffed endotracheal tubes may not be suitable for use in smaller breeds or young rabbits due to lack of availability of smaller

sized tubes (Johnson 2010). Use of cuffed endotracheal tubes may increase the success rate of intubation during blind orotracheal intubation of larger breed rabbits (Smith et al. 2004; Lee et al. 2019). Lee et al. 2019 documented an increased risk of failure during intubation of New Zealand white rabbits when uncuffed tubes were used in comparison to cuffed tubes. The uncuffed endotracheal tubes used in this study were made of a softer material than the cuffed tubes, and often bent during blind insertion into the oral cavity preventing further passage of the tube into the trachea (Lee et al. 2019).

Prior to intubation, an adequate depth of anesthesia should be present, and the arytenoid cartilages should be desensitized with lidocaine to prevent laryngospasm (Eatwell & Mancinelli 2013; Varga 2014). Endotracheal tube length should be premeasured from the incisors to the thoracic inlet, to ensure correct placement in the trachea and to avoid endobronchial intubation (Eatwell & Mancinelli 2013; Thompson 2017; Varga 2017). In addition, airway devices should be lubricated before use to decrease the risk of trauma to respiratory tissues (Eatwell & Mancinelli 2013). Correct placement of the endotracheal tube in the trachea can be confirmed via, capnography, respiration monitors, airflow detection devices (e.g., Beck airway airflow monitor), visualization of condensation on the inside of the endotracheal tube, or by listening for respiratory sounds at the distal end of the tube (Eatwell & Mancinelli 2013; Thompson 2017; Varga 2017). Often, elicitation of a cough reflex occurs and indicates passage of the endotracheal tube into the trachea (Weinstein et al. 2000).

Contrary to canine and feline patients, orotracheal intubation is often difficult to perform in rabbits as a result of their complex oropharyngeal anatomy (Aeschbacher 1995; Brodbelt 2009; Stephens De Valle 2009; Crotaz 2010; Flecknell 2016; Varga 2017). The rabbit oral cavity consists of a small oral commissure and with limited ability to extend the temporomandibular joint. The

oropharynx is long and narrow, with large incisors and a large fleshy tongue base that impedes visualization of the glottis (Eatwell & Mancinelli 2013; Varga 2017). The glottis is small and ventrally situated. Rabbits are obligate nasal breathers, and the epiglottis is normally positioned dorsally over the soft palate, so the soft palate requires disengagement from the epiglottis in order to visualize the larynx (Eatwell & Mancinelli 2013; Varga 2017). These anatomical features greatly hinder direct visualization of the larynx, thus orotracheal intubation can be a challenge in this species, especially in smaller breeds (Varga 2014). As a result, several different intubation techniques have been described in the literature in an attempt to provide a technically simplistic, timely and easily performed method of obtaining a patent airway in the rabbit.

2.2.1. Laryngoscope-Assisted Orotracheal Intubation

In contrast to canine and feline patients, laryngoscope-assisted orotracheal intubation is comparatively difficult to perform in rabbits, especially in small breed or young rabbits (Varga 2014). Due to the anatomical challenges associated with rabbit intubation, the size of the standard laryngoscope further impedes visualization of the glottis and hinders placement of an endotracheal tube (Davis & Malinin 1974). Smaller laryngoscope blades such as Flecknell or Miller (size 00-1) blades, are appropriate for this technique in rabbits (Gografe et al. 2003; Eatwell & Mancinelli 2013; Thompson 2017; Varga 2017; Lee et al. 2019). Proper positioning is important for successful intubation often requiring hyperextension of the head and neck causing the larynx to align with the oropharynx, to allow for direct visualization of the glottis (Inglis and Strunk 2009; Johnson 2010).

The rabbit is positioned in sternal recumbency with the tongue extended to one side of the oropharynx and grasped with forceps (Eatwell & Mancinelli 2013). The head and neck are then hyperextended, and the laryngoscope is introduced through the opposite side of the oropharynx.

The laryngoscope is positioned to elevate the soft palate dorsally, exposing the glottis and the endotracheal tube is then passed along the laryngoscope blade and advanced into the trachea (Eatwell & Mancinelli 2013; Varga 2017). Alternatively, the rabbit can be positioned in dorsal recumbency to facilitate visualization of the glottis (Eatwell & Mancinelli 2013; Flecknell 2016). Davies et al. 1996 reported improved visualization of the larynx when attempting intubation from behind the rabbit positioned in sternal recumbency with the head held upright at a 90° angle to the surface at which the rabbit was positioned upon (Davies et al. 1996).

A variation to the classic laryngoscope technique involving use of a polypropylene guide catheter (Seldinger technique) has been described in New Zealand white rabbits (Thompson 2017). For this technique, the rabbit is placed in sternal recumbency with the head extended dorsally and the neck extended cranially. The endoscope blade is placed behind the incisors in the left oropharynx and advanced caudally until the soft palate is visualized. The blade is tipped forward to compress the base of the tongue and displace the epiglottis and expose the glottis. The guide catheter is then advanced through the larynx and into the trachea and the endotracheal tube is advanced over the catheter into the trachea. The laryngoscope and guide catheter are removed and the endotracheal tube secured in place (Thompson 2017). Similar techniques in New Zealand white rabbits have also been described in the literature (Macrae & Guerreiro 1989; Gografe et al. 2003). This technique was found to be simplistic, time efficient and did not require use of specialized equipment or an assistant (Thompson 2017). However, only large breed rabbits were used in the aforementioned studies and use of these techniques in young or small breed rabbits may be challenging as a result of an anatomically smaller oral cavity.

A standard otoscope and ear speculum can be used as an alternative to the laryngoscope (Eatwell & Mancinelli 2013; Varga 2017) and this may allow better visualization in young or small

breed rabbits (Weinstein et al. 2000). The otoscope can be used to visualize the glottis as described for the laryngoscope, or it can be used to position a stylet in the trachea which allows an endotracheal tube to be advanced over the stylet and into the trachea (Weinstein et al. 2000; Eatwell & Mancinelli 2013; Varga 2017).

Laryngoscope-assisted intubation is advantageous because equipment is widely available and inexpensive, and the technique is simplistic and easy to learn. In addition, there is minimal risk of damage to upper respiratory tissues and successful intubation can be visually confirmed (Varga 2017). However, aggressive insertion of a laryngoscope may result in injury to the oral cavity, tongue or teeth (Thompson 2017). Aggressive stylet placement or use of rigid guidewires may result in damage to the larynx and peri-laryngeal structures (Gografe et al. 2003). Furthermore, the laryngoscope-assisted technique, with or without use of a guidewire, may not be suitable for young or small rabbits due to the difficulty in placement of the laryngoscope and visualization of laryngeal structures (Varga 2014; Thompson 2017). Thus, use of standard otoscope and ear speculum may be preferred (Weinstein et al. 2000).

2.2.2. Blind Orotracheal Intubation

Blind orotracheal intubation involves placement of an endotracheal tube through the oral cavity without direct visualization of the glottis (Varga 2017). The classic technique involves placing the rabbit in sternal recumbency and positioning the head and neck craniodorsally (Fick & Schalm 1987; Flecknell 2016). The endotracheal tube is inserted blindly into the oropharynx through the diastema and advanced towards the larynx until respiratory sounds are heard at the opening of the glottis or condensation is visualized on the inside of the tube, indicating that the endotracheal tube is correctly positioned at the opening of the glottis. The endotracheal tube is

advanced into the trachea as the rabbit inhales (Fick & Schalm 1987; Morgan & Glowaski 2007; Flecknell 2016).

Several variations in the blind technique have been evaluated for efficiency and rapidity of intubation (Varga 2017). In one such technique, the rabbit was placed in lateral recumbency and it was demonstrated to be more time consuming to perform and difficult to master compared to intubation in sternal recumbency (Morgan & Glowaski 2007). Another technique involves temporary insertion of an endotracheal tube into the esophagus to facilitate blind placement of a second tube into the trachea (Falcão et al. 2011). This study reported a 100% success rate of orotracheal intubation in New Zealand rabbits with no reported complications, however the number of intubation attempts and overall time required to successfully intubate was not reported. Yet another technique involves blind insertion of a stylet with a small light source attached to the distal end, placed through the inside of an endotracheal tube (Su et al. 2013). Correct placement of the stylet at the opening of the glottis was confirmed by anterior neck transillumination causing a bright glow, at which point the endotracheal tube could be advanced off the stylet into the trachea, whereas incorrect placement in the esophagus resulted in a dim glow. Intubation was shown to be faster using the lighted-stylet technique (mean ± SD time 20.34 ± 17.15 seconds) when compared to laryngoscope-assisted intubation (mean ± SD time 57.58 ± 64.21 seconds) in New Zealand white rabbits.

One study compared capnography-assisted blind orotracheal intubation to laryngoscope-assisted orotracheal intubation in New Zealand white rabbis and found that the use of capnography allowed for faster intubation with a higher success rate (Lee et al. 2019). In this study, capnography was used during the blind orotracheal technique to determine the correct placement of the endotracheal tube by detecting carbon dioxide in exhaled gas. Use of mainstream capnography

resulted in rabbits being intubated approximately three times faster compared to sidestream capnography and approximately eight times faster compared to rabbits intubated using a laryngoscope. The superiority of the mainstream capnograph in this study was attributed to the availability of real-time measurements to guide correct placement of the endotracheal tube at the glottis. Furthermore, a higher intubation success rate was achieved using the blind technique compared to using a laryngoscope.

The advantage of using the blind technique is that it requires no specialized equipment (Varga 2017). However, blind orotracheal intubation is technically challenging, time consuming and requires experience to become efficient (Krüger et al. 1994; Morgan & Glowaski 2007; Thompson 2017; Varga 2017). It often requires multiple attempts to correctly place the endotracheal tube, which has been speculated to result in laryngeal and/or tracheal trauma, hemorrhage, edema or cause laryngospasm (Grint et al. 2006; Phaneuf et al. 2006; Engbers et al. 2017). Because correct placement of the endotracheal tube is reliant on respiration, the occurrence of hypoventilation or apnea may make it difficult to confirm orotracheal intubation (Thompson 2017). In addition, food material may be inadvertently pushed into the trachea by the endotracheal tube, potentially causing aspiration pneumonia (Varga 2017).

2.2.3. Endoscopic-Guided Orotracheal Intubation

In absence of specialized equipment, direct visualization of the glottis from the oral cavity is difficult (Varga 2017). For endoscopic-guided orotracheal intubation, an endoscope, video laryngoscope or video-optical stylet is used to visualize the larynx and trachea (Johnson 2010). Typical equipment for endoscopic-guided intubation includes an endotracheal tube, an endoscope and a light source and potentially a video camera and video display system (Johnson 2010). The endoscope can be utilized in one of two ways: The side-by-side intubation technique involves

insertion of the endoscope into the oropharynx and advancing it until the glottal opening is visualized at which point the endotracheal tube is advanced parallel to the endoscope into the trachea (Johnson 2010). Alternatively, the over-the-endoscope intubation technique involves passing the endoscope through the centre of the endotracheal tube and once positioned at the opening of the glottis, the endotracheal tube is advanced off the endoscope and into the trachea (Johnson 2010; Eatwell & Mancinelli 2013; Varga 2017).

Many studies have assessed use of endoscopic-guided intubation in rabbits. In one study, New Zealand white rabbits were placed in dorsal recumbency with the head and neck hyperextended and a rigid 30° endoscope connected to a light source was used for intubation using an over-the-endoscope technique (Tran et al. 2001). The authors reported that all 60 rabbits were rapidly intubated within 30-120 seconds with no instances of incorrect placement in the esophagus or failed intubation. No complications were reported in the perianesthetic period or long-term. In addition, no histopathologic evidence of orotracheal injury was present following necropsy at 10 or 30 days (Tran et al. 2001). Another study described use of a semi-rigid 0° fibre-optic endoscope connected to a light source to intubate dorsally recumbent rabbits using a side-by-side technique (Worthley et al. 2000). Six New Zealand white rabbits were used, and each was intubated 6 times followed by euthanasia and necropsy. Mean intubation time was 60.8 ± 8.8 seconds and the majority of rabbits were successfully intubated on the first attempt. Only 3 of 36 intubation attempts resulted in esophageal intubation, with the subsequent attempt resulting in correct placement in the trachea. No complications were reported and there were no gross lesions reported on post-mortem examination of the oropharynx, other than mild abrasions reported in one rabbit (Worthley et al. 2000). Similar techniques of endoscopic-guided orotracheal intubation have been

report with rabbits positioned in sternal recumbency with the head and neck extended and the mouth held open using a mouth gag placed on the upper and lower jaws (Varga 2017).

The main advantage of endoscopy-guided orotracheal intubation is that it allows direct visualization of the glottis, which decreases the risk of trauma to laryngeal and tracheal tissues and allows evaluation of the oropharynx for food or foreign material (Eatwell & Mancinelli 2013; Varga 2017). Correct placement of the endotracheal tube in the trachea can be visually confirmed (Johnson 2010). This method is technically simplistic and requires little skill to become efficient (Worthley et al. 2000; Tran et al. 2001; Johnson 2010). However, specialized equipment is necessary, expensive and may be of limited availability (Varga 2017).

2.2.4. Nasotracheal Intubation

Nasotracheal intubation involves the insertion of an endotracheal tube into the external naris, through the nasopharynx and directly into the trachea (Varga 2017). This technique has not been well described in the literature and only one study, performed by Stephens De Valle 2009, has assessed feasibility of the technique in rabbits compared to the blind orotracheal approach (Stephens De Valle 2009). In this study, New Zealand white rabbits were premedicated with buprenorphine (0.03 mg kg^{-1} SQ), ketamine (35 mg kg^{-1} IM), xylazine (5 mg kg^{-1} IM) and glycopyrrolate (0.01 mg kg^{-1} SQ), followed by induction with isoflurane (3.0% in oxygen) via facemask. The rabbits were then placed in dorsal or sternal recumbency with the head and neck hyperextended. A lubricated 2.0-2.5 mm uncuffed endotracheal tube was inserted into the external naris and directed ventromedially into the trachea. Correct placement was confirmed by observing visualization of condensation inside the tube (Stephens De Valle 2009). The author reported nasotracheal intubation to be technically easier to perform with less attempts to achieve successful intubation when compared to blind orotracheal intubation. However, the methodology used to

perform orotracheal intubation was not described in this study, making it difficult to interpret results or make comparisons between techniques. It was also reported that endotracheal tubes placed nasally were less easily dislodged with patient movement when compared to orally placed tubes. In addition, no complications associated with the nasotracheal technique and no evidence of respiratory clinical signs were observed during the two-month follow up period (Stephens De Valle 2009).

Nasotracheal intubation is advantageous as rabbits are obligate nasal breathers and the epiglottis is naturally engaged with the dorsal soft palate; thus, advancement of the endotracheal tube through the naris should result in direct access to the trachea (Stephens De Valle 2009; Varga 2017). This technique may be beneficial in smaller rabbit breeds where orotracheal intubation is difficult or has failed (Varga 2014; Varga 2017). Although not documented in the study by Stephens De Valle 2009, a potential disadvantage of nasotracheal intubation is an increased risk of introducing pathogens such as *Pasteurella multocida* from the nasal cavity into the lungs (Varga 2014; Varga 2017).

2.2.5. Supraglottic Airway Devices

A supraglottic airway device (SGAD) consists of a tube connected to a species-specific, anatomically shaped cuff (Foote 2020). Insertion of SGADs is easy, rapid and do not require direct visualization of the larynx for successful placement (Varga 2017). Similar to endotracheal tube placement, capnography can be used to confirm correct placement of SGADs (Richardson 2015). When correctly placed, they form a tight seal around the ventral pharynx, larynx and the upper esophagus (Foote 2020). This allows delivery of oxygen and inhalant anesthetics and allows sufficient ventilation without intubation of the trachea (Varga 2017). Because these devices do not engage the larynx or enter the trachea, the risk of tracheal damage and laryngeal spasm is reduced

(Eatwell & Mancinelli 2013; Varga 2017; Foote 2020). However, lack of direct tracheal seal increases the risk of gastric tympany or personnel exposure to waste anesthetic gases if the SGAD becomes dislodged or if the glottis is insufficiently sealed (Varga 2017). Use of SGADs in humans is associated with an increased risk of gastroesophageal reflux, however this potential complication has not been documented in rabbits (Valentine et al. 1994). An additional concern is that the lack of complete air-tight seal hinders use of these devices during positive pressure ventilation (Smith et al. 2005; Bateman et al. 2005; Wenger et al. 2017).

Many human SGADs are commercially available including laryngeal tubes (LT), laryngeal mask airway (LMA) and i-gel. Efficient use relies on the device mimicking and conforming to the specific pharyngeal and peri-laryngeal anatomy of the species it was designed for, thus use of human SGADs may be less reliable in rabbits due to anatomical differences between the species (Eatwell & Mancinelli 2013).

An LT consists of a tube containing two cuffs that direct air flow into the trachea, with the larger proximal cuff forming a seal in the upper pharynx and the smaller distal cuff forming a seal at the esophageal inlet when inflated (Yamamoto et al. 2007). One study described the blind insertion of a size 0 LT in New Zealand white rabbits and evaluated its efficacy during positive pressure ventilation (Yamamoto et al. 2007). The results of this study indicate that the LT was relatively easy to place with four of six rabbits having the device successfully placed on the first attempt. For the other two rabbits, device placement was successful on the second attempt. Efficacy of ventilation was assessed by paralysing the rabbits followed by manual application of positive pressure ventilation and observing adequate chest movement and by presence of normal end-tidal carbon dioxide via capnograph. Efficient ventilation was observed in all rabbits. The

authors report that use of an LT in rabbits was effective for airway management with no complications reported (Yamamoto et al. 2007).

The LMA consists of a tube connected to an inflatable elliptical mask at the distal end that forms a seal around the pharynx when inflated (Bateman et al. 2005; Kazakos et al. 2007). For device placement, the rabbit is positioned in lateral recumbency with the head tilted upward at a 90° angle. The device is turned laterally and placed in the oral cavity with the aperture facing the tongue and convex side against the buccal wall. The device is advanced blindly until resistance is met and then is rotated countrerclockwise so that the cuff covers the glottal opening. The cuff is then inflated to form a seal around the larynx (Smith et al. 2004; Bateman et al. 2005; Kazakos et al. 2007). Inflation of the LMA has been reported to cause lingual cyanosis due to compression of the lingual artery (Kazakos et al. 2007).

One study investigated use of an LMA in 50 New Zealand White rabbits weighing between 2.3 and 4.5 kg and demonstrated that the LMA fits well over the rabbit larynx and provides a tight seal (Kazakos et al. 2007). In addition, device placement was rapid and successful on the first attempt in all cases. The authors determined that the size 1.0 LMA was more suitable for rabbits weighing less that 4 kg, whereas the size 1.5 LMA was more suitable for rabbits weighing more than 4 kg (Kazakos et al. 2007). Four of the rabbits in this study developed lingual cyanosis following insertion of the LMA. This resolved without complication following deflation of the cuff in two rabbits, repositioning of the device in one rabbit and using a smaller-sized LMA in the last rabbit (Kazakos et al. 2007).

Cruz et al. 2000 compared use of a size 1.0 LMA to blind orotracheal intubation in Norfolk rabbits (2.7 ± 0.4 kg) and evaluated use of the LMA during isoflurane anesthesia and spontaneous ventilation (Cruz et al. 2000). The results indicated that the LMA was easier and faster to place

compared to blind orotracheal intubation. A significantly lower dose of induction agent was needed to place the LMA and the LMA was successfully placed in all eight rabbits on the first attempt, whereas only three of eight rabbits were successfully orotracheally intubated on the first attempt. Hemodynamic variables were similar between groups and no regurgitation or other complications were reported (Cruz et al. 2000).

Another study evaluated use of a size 1.0 LMA in eight New Zealand white rabbits (weighing between 3.3 and 5.0 kg) and the ability of the LMA to limit emission of waste anesthetic gases compared to cuffed and non-cuffed endotracheal tubes (Smith et al. 2004). These authors also reported that the LMA was easier and faster to place compared to blind orotracheal intubation. A surgical plane of anesthesia was maintained and no complications such as gastric tympany or regurgitation were observed with the LMA. Isoflurane concentration was measured at the rabbit's oral commissure and in the operator's breathing zone using an infrared spectrophotometer. Isoflurane was detected only at the oral commissure for all three devices and a significantly higher concentration of isoflurane was detected with the LMA compared to both cuffed and non-cuffed endotracheal tubes (Smith et al. 2004). However, Kazakos et al. 2007 reported no leakage of gas in the oral commissure during spontaneous or mechanical ventilation of rabbits with an LMA.

Bateman et al. 2005 compared use of a size 1.0 LMA to use of a facemask during isoflurane anesthesia in spontaneously breathing and mechanically ventilated Giant Flemish cross Chinchilla rabbits (4.1 ± 0.8 kg). All rabbits anesthetized using a facemask developed signs suggestive of airway obstruction whereas none of the rabbits in in the LMA groups developed signs of airway obstruction, indicating that LMAs maintained better airway patency compared to facemasks. Overall, there was no difference in mean cardiopulmonary variables between spontaneously ventilating rabbits in the facemask and LMA groups; however, mild hypoxemia (defined as PaO_2

= 70 mmHg) occurred in one rabbit and severe hypercapnia (defined as $PaCO_2$ > 100 mmHg) occurred in two rabbits, all in the facemask group. Partial pressures of carbon dioxide were decreased and partial pressures of oxygen were increased in mechanically ventilated rabbits in the LMA group compared to rabbits that were spontaneously breathing, indicating that positive pressure ventilation is effective in rabbits with an LMA. Four of six rabbits in the mechanically ventilated LMA group developed gastric tympany suggesting that the LMA provided an incomplete seal over the larynx, however the cuffs were not inflated during the study for fear of causing inguinal cyanosis. In addition, one mechanically ventilated rabbit in the LMA group regurgitated (Bateman et al. 2005).

More recently, the v-gel, a modified LMA with a non-inflatable cuff, has been designed for use in rabbits (Crotaz 2010). The device consists of a plastic tube with a tapered end that is inserted into the pharynx and lodges over the glottis, forming a seal around the opening of the larynx (Varga 2017). The device is manufactured with silicone material which is unlikely to cause damage to pharyngeal structures and the material does not harden following cleaning or sterilization (Eatwell & Mancinelli 2013). In addition, these devices come in a variety of sizes and conform to the specific anatomy of the rabbit airway, thus proper placement should prevent aspiration of gastric contents and allow intermittent positive pressure ventilation without leakage of waste anesthetic gases (Crotaz 2013; Eatwell & Mancinelli 2013; Wenger et al. 2017). During insertion, the device deflects the soft palate dorsally to disengage the epiglottis (Eatwell & Mancinelli 2013). The rabbit is placed in sternal recumbency and the tongue is extended outside the mouth. An appropriately sized v-gel is blindly inserted into the oral cavity until the baffle plate at the end of the device is placed between the upper and lower incisors and correct placement is confirmed using capnography (Crotaz 2013).

A preliminary feasibility study on use of an i-gel in rabbits was performed using five cadaver rabbits (Crotaz 2010). Modifications of the device allowing it to conform the specific pharyngeal and laryngeal anatomy of the rabbit, lead to the development of a rabbit-specific v-gel. (Crotaz 2010). Since then, investigations on the clinical use of the rabbit v-gel have demonstrated a high success rate. However, the device was again redesigned to allow easier insertion and placement (Crotaz 2013). This redesigned v-gel was clinically tested in 29 rabbits undergoing anesthetic procedures and the v-gel was easily and rapidly placed in 27 of these rabbits with a mean insertion time of 8 seconds. During the anesthetic period, hemodynamic variables remained within clinically acceptable limits. No respiratory complications were noted, however mild lingual cyanosis was reported in one rabbit which resolved with jaw manipulation and repositioning of the device. It was speculated that lingual cyanosis was a result of compression of the tongue and subsequent venous congestion rather than arterial compression. It was also noted that the v-gel became easily dislodged if the patient is repositioned following placement. Based on this initial assessment, the v-gel design was again modified to reduce the incidence of these problems (Crotaz 2013). For these two studies, both failed to mention specific characteristics of the rabbits utilized (e.g., breed, weight, age). Therefore, it is difficult to determine clinical applicability based on these assessments alone.

Following the initial studies performed by Crotaz 2010 and 2013, several other studies have investigated use of the v-gel in rabbits. Many studies have reported that the rabbit v-gel is faster to place with less attempts when compared to blind orotracheal intubation (Richardson 2015; Engbers et al. 2017), laryngoscope-assisted orotracheal intubation (Uzun et al. 2015), endoscopic-guided orotracheal intubation (Comolli et al. 2020) and LMA insertion (Uzun et al. 2015). Lingual

cyanosis is a common complication associated with v-gel use; however, this is typically resolved with repositioning of the device (Richardson 2015; Uzun et al. 2015; Wenger et al. 2017).

2.2.6. Facemasks

Successful intubation may require excess time, multiple intubation attempts, and ultimately may result in failure to intubate (Smith et al. 2004; Grint & Murison 2008; Richardson 2015; Wenger et al. 2017). Thus, as a result of the difficulties associated with orotracheal intubation, alternate methods of administering inhalant anesthetic agents and oxygen supplementation are often used in the rabbit (Foote 2020). Anesthetic maintenance via facemask is the most simplistic method of providing general anesthesia; however, it is associated with a number of disadvantages.

Use of facemasks may not allow for adequate oxygenation or ventilation during anesthesia and does not ensure maintenance of a patent airway which may predispose to airway obstruction (Bateman et al. 2005). In addition, use of a facemask can increase exposure of anesthetic gases to personnel (Bateman et al. 2005; Eatwell & Mancinelli 2013). Trace inhalant concentration exposure in humans may be associated with detrimental effects such as damage to chromosomes and developmental abnormalities in utero during pregnancy and cognitive issues (Hoerauf et al. 1999; Smith et al. 2004; Nilsson et al. 2005).

2.3. Respiratory Tissue Trauma

Damage to laryngeal and tracheal tissues following orotracheal intubation has been documented in rabbits; however, causes for this damage are largely unknown (Grint et al. 2006; Phaneuf et al. 2006; Engbers et al. 2017). Compared to other veterinary species, rabbits may be more prone to laryngeal and tracheal trauma as a result of a highly vascularized mucosa and submucosa (Phaneuf et al. 2006). Contact of the endotracheal tube with the larynx and trachea may result in tracheal irritation leading to vascular congestion, edema and hemorrhage (Phaneuf et al.

2006). Severe lesions may result in tracheal stricture formation with clinical signs becoming evident days later (Grint et al. 2006).

Potential causes of laryngeal and tracheal trauma have been widely speculated and include aggressive intubation or multiple intubation attempts (Phaneuf et al. 2006), movement of the endotracheal tube during patient positioning (Grint et al. 2006; Phaneuf et al. 2006) or during mechanical ventilation (Phaneuf et al. 2006) and chemical burns caused by disinfectant residues following endotracheal tube sterilization (Grint et al. 2006). Improperly secured endotracheal tubes may result in movement or rotation of the tube within the trachea causing trauma to laryngeal and tracheal tissues (Eatwell & Mancinelli 2013).

One case series documented three reports of tracheobronchial stricture formation in rabbits during post-mortem evaluation following a previous anesthetic event in which the rabbits underwent blind orotracheal intubation (Grint et al. 2006). All three rabbits recovered from anesthesia uneventfully; however, all rabbits developed dyspnea and clinical signs of dyspnea 17-24 days later. A definitive cause was never identified following gross and histopathological examination (Grint et al. 2006). It is possible that trauma induced by the endotracheal tube could have resulted in stricture formation as evidenced by experimental studies using a nylon brush to mimic endotracheal tube trauma in rabbits and resulting in tracheal stricture formation within 14 days of the experiment (Nakagishi et al. 2005; Steehler et al. 2011). A second case series evaluated gross and histologic damage to laryngeal and tracheal tissues documented in 15 New Zealand white rabbits following anesthesia where rabbits underwent blind or endoscopic-guided orotracheal intubation (Phaneuf et al. 2006). Six of these rabbits developed respiratory clinical signs in the post-anesthetic period, of which two rabbits acutely died and one was euthanized due to ongoing clinical signs. The remainder of rabbits (including three rabbits that developed mild

respiratory clinical signs) were euthanized for research-related purposes, unrelated to respiratory disease. Post-mortem examination of the 15 rabbits revealed a wide range of gross and histopathological lesions of laryngeal and tracheal tissues, however causative factors were not definitively identified (Phaneuf et al. 2006).

Another study compared blind orotracheal intubation and insertion of a v-gel in 15 New Zealand white rabbits and evaluated damage to respiratory tissues caused by these techniques (Engbers et al. 2017). They concluded that placement of a v-gel causes less histological trauma to tracheal tissues compared to blind orotracheal intubation. In addition, all rabbits were successfully intubated using the blind technique after one or two attempts, indicating that single attempts at intubation can result in sufficient tracheal injury (Engbers et al. 2017). A more recent study compared endoscopic-guided orotracheal intubation and insertion of a v-gel in 14 New Zealand white rabbits and evaluated gross and histological damage to laryngeal and tracheal tissues (Comolli et al. 2020). The results indicated that histological evidence of damage was present following intubation with both techniques, however degree of damage was similar between groups (Comolli et al. 2020).

Ancillary intubation equipment may also result in respiratory tissue damage. In one study, three of six rabbits experienced respiratory clinical signs following laryngoscope-assisted orotracheal intubation and use of a rigged, plastic-coated wire stylet (Gografe et al. 2003). One rabbit developed pulmonary edema, evidenced by marked serosanguinous fluid aspirated from the endotracheal tube, following observed laryngeal trauma during stylet placement. This rabbit became dyspneic and was euthanized. The second rabbit experienced laryngeal trauma during intubation, resulting in pulmonary edema and wheezing pulmonary rales but recovered following supportive therapy. The third rabbit developed dyspnea with inspiratory and expiratory stridor

following extubation with wheezing and crackles present on pulmonary auscultation. This rabbit became opisthotonic and died before emergency measurements could be undertaken. After switching to a flexible guidewire, the remaining 33 animals in the study were intubated without complication (Gografe et al. 2003).

Chapter Three. Assessment of Three Doses of Intramuscular Alfaxalone Combined with Hydromorphone and Dexmedetomidine to Allow Endoscopic-Guided Orotracheal Intubation in Domestic Rabbits (*Oryctolagus Cuniculus*)

3.1. Introduction

Domestic rabbits have a 1.39% risk of anesthetic-related mortality compared to 0.17% and 0.24% in dogs and cats respectively (Brodbelt et al. 2008). Drug-induced cardiorespiratory depression may be one factor contributing to this increased risk. Intubation is inherently difficult in rabbits and the use of injectable sedatives and anesthetics (opioids, benzodiazepines, α-2 adrenergic agonists, dissociative anesthetics) is often necessary to facilitate intubation by providing sedation, immobilization and muscle relaxation (Borkowski & Karas 1999). Specific drug combinations such as medetomidine and ketamine are associated with variable and potentially severe adverse effects warranting the investigation of safer alternatives (Hellebrekers et al. 1997; Grint & Murison 2008).

Alfaxalone is a synthetic neuroactive steroid that binds to $GABA_A$ receptors and facilitates the inhibitory action of gamma-aminobutyric acid (GABA) resulting in central nervous system depression (Lambert et al. 2003). It is formulated with 2-hydroxypropyl-β-cyclodextrin (alfaxalone-HPCD) and licenced for intravenous (IV) use in cats and dogs in the United States and other countries. Alfaxalone is also approved for IV use in rabbits in the United Kingdom and Australia. Intramuscular (IM) administration is approved for cats in Australia however off-label IM use is common in other species.

Several studies have investigated the sedative and anesthetic effects of IM alfaxalone in rabbits. In one study, IM alfaxalone was administered at doses of 4, 6 or 8 mg kg^{-1} to New Zealand white rabbits (Huynh et al. 2014). All doses resulted in smooth and rapid onset of sedation and dose-dependent respiratory depression; however, one rabbit receiving 8 mg kg^{-1} experienced apnea

followed by cardiac arrest. Another study in New Zealand white rabbits, reported good quality of sedation with smooth recovery and minimal cardiorespiratory depression following administration of 6 mg kg^{-1} alfaxalone IM as a sole agent (Bradley et al. 2019). Furthermore, when the same dose was combined with butorphanol (0.3 mg kg^{-1} IM) and dexmedetomidine (0.2 mg kg^{-1} IM), no response to noxious stimuli occurred, indicating a good anesthetic plane had been achieved. With this drug combination, the onset of effects was more rapid, and duration was longer compared to alfaxalone alone. There was also a greater magnitude of cardiovascular and respiratory depression, however values remained within normal limits (Bradley et al. 2019).

Intravenous administration of alfaxalone at 2 or 3 mg kg^{-1} following premedication with buprenorphine at 0.03 mg kg^{-1} IM has been shown to result in a smooth and rapid induction of anesthesia with minimal cardiorespiratory effects and a high success rate of blind orotracheal intubation in rabbits (Grint et al. 2008). However, there is currently no data evaluating the suitability of IM alfaxalone for orotracheal intubation of rabbits. Induction of anesthesia using IM alfaxalone is advantageous due to less handling and associated stress with catheter placement for IV administration, in addition to it being a non-controlled substance with limited abuse potential.

The objective of this study was to determine the dose of IM alfaxalone combined with dexmedetomidine and hydromorphone that would allow endoscopic-guided orotracheal intubation of rabbits without causing a decrease in respiratory rate (f_R) and/or apnea. We hypothesized that when administered IM with hydromorphone (0.1 mg kg^{-1}) and dexmedetomidine (0.005 mg kg^{-1}), increasing the dose of alfaxalone (2, 5 or 7 mg kg^{-1}) would increase the success rate of endoscopic-guided orotracheal intubation but would progressively depress respiratory rate and/or increase the occurrence of apnea.

3.2. Materials and Methods

3.2.1. Animals

This study was approved by the Louisiana State University (LSU) Institutional Animal Care and Use Committee (Protocol No. 17-101). Fifteen intact, Miniature Lop rabbits were used for this study. There were 9 females and 6 males, ranging from 4 to 9 months in age and weighing a mean ± SD of 2.3 ± 0.3 kg. Rabbits were deemed healthy based on results of physical examination, complete blood count and serum biochemical analysis. Upon arrival, rabbits were housed separately in pens grouped by sex and allowed to acclimatize for at least two weeks prior to the experimental period. They had free access to fresh water and Alicia or Alfalfa hay and were fed a commercial pelleted diet (Country Acres Rabbit Pellet 16%, Country Acres Feed Company, MN, USA) twice daily. Food, but not water, was withheld 12 hours prior to the experiment.

At the completion of the study, male rabbits were neutered by the Louisiana State University Division of Laboratory Medicine personnel and all rabbits were made available for adoption.

3.2.2. Experimental Design

For each rabbit, age, sex, weight, and f_R were recorded at the beginning of the experimental period (T = 0). Rabbits were randomly assigned using a random number generator (www.randomnumbergenerator.com) to one of three treatment groups (n = 5 per group). All rabbits were administered 0.1 mg kg^{-1} hydromorphone (Hydromorphone Hydrochloride; Hospira Inc., IL, USA) and 0.005 mg kg^{-1} dexmedetomidine (Dexdormitor; Zoetis Inc., MI, USA) combined with one of three doses of alfaxalone (Alfaxan multidose; Jurox Inc., MO, USA): low dose alfaxalone (A2; 2 mg kg^{-1}), mid dose alfaxalone (A5; 5 mg kg^{-1}) and high dose alfaxalone (A7; 7 mg kg^{-1}). Drug selection and doses used were based on clinical use at the LSU veterinary

teaching hospital and by conducting a pilot study using two rabbits. The drugs were mixed together immediately prior to injection and administered intramuscularly into the semimembranosus or semitendinosus muscles of the right or left hindlimb of each rabbit by the same investigator (PQW).

Rabbits were placed in a small animal carrier, under observation, for 10 minutes to allow for maximal sedative effects. Subsequently, f_R was recorded (T = 10) and quality of anesthesia was subjectively evaluated using a semi-quantitative rating scale modified from Raekallio et al. 2002 (Appendix A.1). The rating scale consisted of 5 categories and their scores were summed for a total anesthesia score ranging from 0 to 12: spontaneous posture (0-4), response to toe pinch (0-2), palpebral reflex (0-2), eye rotation (0-1) and jaw tone (0-3). The investigator assessing the anesthesia quality score (SNR) was blinded to the treatment group. Rabbits were then positioned in sternal recumbency with the head and neck extended and 2 mg of Lidocaine (Lidocaine Injectable, 2%; Vetone, ID, USA) was blindly splashed over the arytenoids. Thirty seconds later, orotracheal intubation was attempted by the same investigator (PQW) using an uncuffed, 2.0 mm inner diameter, murphy-eyed endotracheal tube (Mallinckrodt Pharmaceuticals, MO, USA) placed over a semi-rigid endoscope (FM-1.9X6 Micro-Endoscope; MDS-VET, Canada). Once the endoscope was inserted into the trachea, the endotracheal tube was advanced off the endoscope and into the trachea. Only one intubation attempt was performed per animal. An intubation attempt was defined as the advancement of the endotracheal tube from the mouth, at the level of the incisors, to the opening of the larynx. End-tidal partial pressure of carbon dioxide was measured using a multiparameter monitor (CARESCAPE monitor B850; GE Healthcare, Finland). Intubation was considered successful when up to 6 consecutive end-tidal carbon dioxide readings were present on the capnograph and considered unsuccessful if no readings were present. For

rabbits that were intubated, quality of intubation was subjectively evaluated by one investigator (PQW) using a semi-quantitative rating scale ranging from 0 to 3 (Appendix A.2).

Immediately following the end of the experiment, male rabbits were placed under general anesthesia with isoflurane in 100% oxygen for orchiectomy. Female rabbits were immediately extubated. All rabbits recovered under supervision and were placed back in their pen once they regained the ability to ambulate.

3.2.3. Statistical Analysis

Statistical analysis was performed with commercially available software (JMP Pro 15.0.0, SAS Institute Inc., NC, USA). One-way ANOVA was used to analyze the continuous response variable (weight) with the treatment group as the fixed effect. Respiratory rates at time 0 and 10 minutes were compared with a paired-t test. Assumptions of these models (linearity, normality of residuals and homoscedasticity of residuals) and influential data points were assessed by examining standardized residual and quantile plots and the normality of residual was confirmed with Shapiro-Wilk test. Ordinal scores (intubation and anesthesia quality scores) were analyzed by Kruskal-Wallis test followed by post hoc pairwise comparisons. Sex against treatment groups was tested with Fisher's exact test. Values of $P < 0.05$ were considered significant. Non-normally distributed data are reported in median (range).

3.3. Results

All rabbits completed the study without any adverse events. There were no significant differences in sex or weight between rabbits of the three groups. Respiratory rates were significantly lower at T = 10 compared to T = 0 ($P < 0.001$) for all treatment groups (Table 1). No apnea occurred in any rabbit in any treatment group.

Table 1. Respiratory rate data (breaths per minute; median (range)) at baseline and 10 minutes after administration of dexmedetomidine (0.005 mg kg^{-1}), hydromorphone (0.1 mg kg^{-1}), and either alfaxalone 2 mg kg^{-1} (group A2), 5 mg/kg (group A5), or 7 mg kg^{-1} (group A7).

	Group A2	**Group A5**	**Group A7**
Baseline (T = 0)	160 (100-240)	180 (160-210)	170 (120-200)
10 min after injection (T = 10)	72 (28-168)	36 (28-68)	40 (32-48)

The median total anesthesia quality scores for the A2, A5 and A7 treatment groups were 3 (2-5), 6 (5-6) and 6 (4-9), respectively (Table 2). Rabbits in treatment groups A7 ($P = 0.04$) and A5 ($P = 0.02$) had significantly higher total anesthesia quality scores compared to rabbits in group A2.

Table 2. Median (range) anesthesia quality scores in rabbits following administration of dexmedetomidine (0.005 mg kg^{-1}), hydromorphone (0.1 mg kg^{-1}) and either alfaxalone 2 mg kg^{-1} (group A2), 5 mg kg^{-1} (group A5), or 7 mg kg^{-1} (group A7). The maximum possible anesthesia quality score is 12. * Indicates significant difference ($P < 0.05$) from group A2.

	Group A2	**Group A5**	**Group A7**
Spontaneous posture	1 (1-2)	2 (2-2)	3* (2-4)
Response to toe pinch	0	0	0
Palpebral reflex	1 (0-1)	1 (1-1)	1 (1-2)
Eye rotation	0	0	0
Jaw tone	1 (1-2)	3* (2-3)	2*(2-3)
Total anesthesia score	3 (2-5)	6* (5-6)	6* (4-9)

The median values for each category of the anesthesia quality score for each treatment group are presented in Table 2. Group A7 had a significantly higher spontaneous posture score compared to A2 ($P = 0.03$). Jaw tone score was significantly higher in group A7 and A5 when compared to A2 ($P = 0.02$ and $P = 0.02$, respectively). Scores for response to toe pinch, palpebral response, and eye rotation were not significantly different between groups.

Successful intubation occurred in 0/5 (0%), 3/5 (60%), and 4/5 (80%) of rabbits with median intubation quality scores of 0 (0-0), 2 (0-3) and 2 (0-3) in the A2, A5 and A7 treatment

groups, respectively. Treatment group A7 had significantly higher intubation quality scores compared to group A2 ($P = 0.02$).

3.4. Discussion

This study demonstrates that IM alfaxalone in combination with hydromorphone and dexmedetomidine causes dose-dependent anesthetic induction of rabbits, facilitating endoscopic-guided orotracheal intubation with minimal changes in respiratory rate.

The total anesthesia quality scores for rabbits in treatment groups A5 or A7 were similar, and higher than rabbits in treatment group A2. This indicates a dose-dependent effect progressing from mild sedation to anesthesia. For each dose, the onset of effects following IM alfaxalone injection was smooth and rapid, with maximal sedative effects occurring within 10 minutes of injection. These findings are similar to those reported in other studies (Huynh et al. 2014; Ishikawa et al. 2019).

Lateral recumbency (as indicated by spontaneous posture score) was achieved only in treatment group A7, whereas rabbits in groups A2 or A5 remained in sternal recumbency. This is in contrast to data reported by Huynh et al. 2014, who reported rapid loss of consciousness and loss of righting reflex in rabbits administered a single dose of either 4, 6, or 8 mg kg^{-1} alfaxalone IM. Ishikawa et al. 2019 also reported lateral recumbency and loss of righting reflex at lower doses (1, 2.5 or 5 mg kg^{-1} IM) of alfaxalone than the doses used in the current study. Furthermore, it was expected that addition of hydromorphone and dexmedetomidine in the current study had an additive or synergistic effect on level of sedation compared to alfaxalone alone. One reason for the observed differences seen is based on methods used to evaluate spontaneous posture. Both aforementioned studies evaluated loss of righting reflex by positioning the rabbits in lateral or dorsal recumbency and documenting the resistance of the animals trying to obtain an upright

position. In the current study, spontaneous posture was assessed without interference by human handling or positioning of the subjects. It is possible that if rabbits in treatment groups A2 or A5 were placed in lateral recumbency, loss of righting reflex may have been observed.

Jaw tone scores for treatment groups A5 and A7 were similar and higher than that of treatment group A2. Decreased jaw tone at these higher doses provided sufficient muscle relaxation to facilitate opening the mouth and this was likely a contributing factor to successful intubation in these rabbits. Ishikawa et al. 2019 reported poor or slight jaw relaxation in rabbits administered 1 or 2.5 mg kg^{-1} IM alfaxalone (poor) or 5 mg kg^{-1} IM alfaxalone (slight); whereas the current study reports minimal or no jaw tone at 5 or 7 mg kg^{-1} IM alfaxalone with hydromorphone and dexmedetomidine. This suggests that alfaxalone alone may not provide sufficient jaw relaxation to allow intubation and higher doses and the addition of muscle relaxants such as α-2 agonists or opioids may be necessary to increase the success rate of intubation. Even though jaw tone scores were similar for treatment groups A5 and A7, group A7 had a higher success rate of intubation (80% vs. 60%). As previously mentioned, group A7 rabbits exhibited a higher spontaneous posture score indicating a deeper plane of anesthesia, which likely contributed to the greater success rate of intubation in group A7.

All rabbits responded to the toe pinch with a complete withdrawal of the forelimb, indicating that response to noxious stimulation was still present at all alfaxalone doses tested. These findings are in accordance with other studies assessing a withdrawal response following a single IM injection of alfaxalone at similar doses in rabbits (Bradley et al. 2019; Huynh et al. 2014). However, in the study by Bradley et al. 2019, when alfaxalone (6 mg kg^{-1}) was combined with butorphanol (0.3 mg kg^{-1}) and dexmedetomidine (0.2 mg kg^{-1}) and administered IM, the withdrawal response was consistently absent. This suggests that sufficient muscle relaxation and

analgesia was present in the drug protocol to suppress noxious stimulation associated with the toe pinch. This lack of response could possibly be a result of the relatively high dose of dexmedetomidine used, which provides dose-dependent analgesia in addition to muscle relaxation. In the current study, the dose of dexmedetomidine was comparatively lower than that used in the previous study by Bradley et al. 2019 and may have not been sufficient to abolish the toe pinch withdraw.

Variable doses of IV alfaxalone allowing for orotracheal intubation have been reported, ranging from 2 mg kg^{-1} to 10 mg kg^{-1} (Grint et al., 2008; Navarrete-Calvo et al. 2013). These differences may reflect age-related and/or breed-specific characteristics and type or dose of premedication used. Rabbits are frequently difficult to intubate, and this is largely a result of their unique oropharyngeal anatomy (Varga 2017). They have a long, narrow oral cavity with a large, fleshy tongue and large incisors, making visualization of the larynx difficult. As such, an adequate plane of anesthesia to allow proper extension of the head and opening of the mouth, is required to visualize the larynx with a scope and to facilitate intubation. In this study, none of the rabbits in treatment group A2 could be intubated. However, 60% of rabbits in treatment group A5 and 80% of rabbits in A7 could be successfully intubated, representing a 20% increase in intubation success rate. Based on this dose-related progression, we speculate that doses greater than 7 mg kg^{-1} may further increase the success rate of intubation; however, the risk of respiratory complications such as hypoventilation and/or apnea may also increase in a dose-dependent fashion, and further studies are needed to ascertain these risks. In addition, increasing doses of alfaxalone will increase the total injectate volume. With the only currently available concentration (10 mg ml^{-1}) of alfaxalone, larger doses may become impractical to inject or result in pain or discomfort, as is the case in dogs and cats (Grubb et al. 2013; Tamura et al. 2016).

Even though rabbits in the treatment groups A5 and A7 exhibited similar total anesthesia quality scores, a higher percentage of rabbits were successfully intubated in the A7 treatment groups versus the A5 treatment group (80% vs. 60%). Rabbits within the A5 treatment group had lower individual total anesthesia score values (maximum score = 6/12) and overall a narrow range of values (5-6), whereas rabbits within the A7 treatment group had higher individual total anesthesia score values (maximum score = 9/12) and a wider range of values (4-9), resulting in similar median total anesthesia scores.

Intravenous administration of alfaxalone can cause apnea in dogs and cats, especially following rapid IV administration (Muir et al. 2009; Amengual et al. 2013; Bigby et al. 2017). In rabbits, apnea was also shown to occur, even when IV alfaxalone was administered slowly over a period of 60 seconds (Grint et al. 2008; Tutunaru et al. 2013; Ishikawa et al. 2019). In contrast, IM alfaxalone is less likely to cause apnea, presumably due to slower drug uptake and absorption; however clinically significant, dose-dependent respiratory depression may occur, especially when other sedative agents are used in combination (Navarrete-Calvo et al. 2013; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019). In the current study, no apnea occurred. However, respiratory rates were lower following administration of all three drug protocols but remained within normal physiologic values (30-60 breaths $minute^{-1}$) for rabbits (Meredith & Crossley 2002). These results are in agreement with other studies evaluating similar doses of alfaxalone IM (Marsh et al. 2009; Ishikawa et al. 2019). The reasons for the decreased respiratory rates reported in some studies and the lack of respiratory adverse effects observed in others, including this study can only be speculated, and may be a result of several factors such as: differences in breed-specific or strain-specific drugs sensitiveness, age- or sex-related factors, environmental factors (e.g., stress, study design) and presence of underlying or sub-clinical disease.

Several limitations were identified in the current study. Bias could have been introduced into the study design as the investigator performing intubation (PQW) was aware of the treatment group. In addition, only young and healthy Mini-Lop rabbits were used in this study, indicating that results can only be applied to this subset of animals. Potential differences in drug-related effects may arise in older rabbits, different breeds of rabbits, or rabbits with clinical illness or underlying disease. The suitability of drug protocols tested in the current study were only evaluated for endoscopic-guided orotracheal intubation, when in fact there are several intubation techniques employed in rabbits. The intubation success rates with different techniques could vary with the drug protocols used in this study. Finally, the evaluation of respiratory depression in this study was only based on respiratory rate and presence or not of apnea, while other methods of respiratory function monitoring, such as capnography and blood gas, would provide better measurements of the depressant effects on ventilation and oxygenation.

In conclusion, when administered with 0.1 mg kg^{-1} hydromorphone and 0.005 mg kg^{-1} dexmedetomidine, increasing doses (2, 5, 7 mg kg^{-1}) of IM alfaxalone progressively increased the success rate of endoscopic-guided orotracheal intubation. Furthermore, increasing the dose of alfaxalone had no significant effect on respiratory rate. Higher doses of alfaxalone and/or dexmedetomidine may be necessary to increase the success rate of endoscopic-guided orotracheal intubation.

Chapter Four. Comparison of Endoscopic-Guided and Blind Orotracheal Intubation Techniques and their Correlation with Anesthesia Quality and Respiratory Tissue Damage in Domestic Rabbits (*Oryctolagus Cuniculus*)

4.1. Introduction

Domestic rabbits are amongst the most commonly anesthetized companion animal species (Brodbelt et al. 2008). However, the reported 1.39% overall risk of anesthetic-related mortality is much higher than that reported in dogs (0.17%) and cats (0.24%) (Brodbelt et al. 2008). Several factors may contribute to the increased risk including drug-induced cardiorespiratory depression (Grint & Murison 2008; Navarrete-Calvo et al. 2013; Bradley et al. 2019), difficulty of intubation (Grint & Murison 2008; Wenger et al. 2017), damage to respiratory tissues during intubation (Grint et al. 2006; Phaneuf et al. 2006; Engbers et al. 2017) and underlying, subclinical respiratory disease (Johnson-Delaney & Orosz 2011).

Rabbit intubation can be challenging and one possible reason may be a result of their complex oropharyngeal anatomy. The oral cavity of the rabbit is small consisting of a large, fleshy tongue and large incisors. In addition, the oropharynx is long and narrow with a ventrally situated glottis (Eatwell 2014). These anatomical features hinder visualization of the larynx and make orotracheal intubation technically challenging.

Amongst the many intubation techniques described in rabbits, blind orotracheal intubation is one of the most commonly utilized in clinical practice as it requires no specialized equipment (Varga 2017). However, because direct visualization of the larynx is not achievable, repeated attempts may be necessary for successful intubation. Multiple intubation attempts may result in trauma to the delicate tissues of the larynx and trachea (Phaneuf et al. 2006). Endoscopic-guided intubation may facilitate visualization of the larynx and minimize the number of intubation attempts potentially resulting in less risk of respiratory tissue damage.

Trauma to the respiratory tissues following orotracheal intubation has not been extensively documented in rabbits. In one study, tracheobronchial stricture formation was observed post-mortem following anesthesia and blind orotracheal intubation, using an uncuffed, 2.5 mm inner diameter endotracheal tube, of three clinically healthy rabbits (Grint et al. 2006). The authors speculated that lesions could have been the result of trauma caused by movement of the endotracheal tube during anesthesia or by chemical burns caused by disinfectant residues following endotracheal tube sterilization. Another study observed gross and histopathologic evidence of tracheal and laryngeal damage following blind or endoscopic-guided orotracheal intubation of 15 rabbits, using either uncuffed or cuffed (2.5-3.5 mm inner diameter) endotracheal tubes (Phaneuf et al. 2006). The authors suggested that tracheal vascular anatomy, tube movement during mechanical ventilation, body position during anesthesia and repeated intubation attempts may have been associated with tracheal mucosal injury in rabbits. A third study reported significantly more tracheal damage in rabbits intubated using a blind orotracheal technique (2.0-3.5 internal diameter, cuffed endotracheal tubes) compared to rabbits where a supraglottic airway device was placed (Engbers et al. 2017). Finally, a more recent study demonstrated a similar degree of histopathological damage to laryngeal and tracheal tissues in 14 rabbits following endoscopic-guided orotracheal intubation (using 3.5 internal diameter, cuffed endotracheal tubes) or placement of a supraglottic airway device (Comolli et al. 2020). The authors of this study speculated that tracheal lesions could have been pre-existing or possibly caused by inadvertent insertion of the v-gel tip into the trachea during device placement.

Relatively few studies have investigated the occurrence of respiratory tissue damage following orotracheal intubation and to the authors' knowledge, none have quantitatively correlated the number of attempts at tracheal intubation to the degree of tissue damage. Thus, the

main objective of this study was to compare damage to respiratory tissues caused by blind and endoscopic-guided orotracheal intubation of rabbits. A secondary objective was to correlate the number of intubation attempts with degree of respiratory tissue damage. We hypothesized that blind orotracheal intubation would cause a greater degree of damage to respiratory tissues and would be associated with a higher number of intubation attempts compared to endoscopic-guided orotracheal intubation. Additionally, we hypothesized that the number of intubation attempts would be positively correlated to the degree of damage to respiratory tissues.

4.2. Materials and Methods

4.2.1. Animals

This study was approved by the Louisiana State University Institutional Animal Care and Use Committee (Protocol No. 17-101). Twenty-four female, intact, New Zealand white rabbits with a mean ± standard deviation (SD) age and weight of 2.10 ± 0.03 months and 2.2 ± 0.2 kg, were used. The rabbits were considered healthy based on results of physical examination, complete blood count and serum biochemical analysis. Upon arrival, rabbits were housed as a single group in a large pen and allowed to acclimatize for at least 2 weeks prior to the experimental period. They had free access to fresh water and Alicia or Alfalfa hay and were fed a commercial pelleted diet (Country Acres Rabbit Pellet 16%, Country Acres Feed Company, MN, USA) twice daily. Food, but not water, was withheld 12 hours prior to the experiment.

4.2.2. Experimental Design

Age, weight, heart rate (HR) and respiratory rate (f_R) were recorded at the beginning of the experimental period (T = 0) for each subject. Rabbits were assigned to undergo either blind orotracheal intubation (group B; n = 12) or endoscopic-guided orotracheal intubation (group E; n = 12) using a random number generator (www.randomnumbergenerator.com). Each rabbit was

administered 0.1 mg kg^{-1} hydromorphone (Hydromorphone Hydrochloride; Hospira Inc., IL, USA), 0.005 mg kg^{-1} dexmedetomidine (Dexdormitor; Zoetis Inc., MI, USA) and 7 mg kg^{-1} alfaxalone (Alfaxan multidose; Jurox Inc., MO, USA). The drugs were mixed together immediately prior to injection and administered intramuscularly (IM) into the semimembranosus or semitendinous muscles of the right or left hindlimb of each rabbit by a single investigator (PQW).

Rabbits were placed in a small animal carrier, under observation for 10 minutes to allow for onset of sedative effects. Subsequently, HR and f_R were recorded (T = 10) and quality of anesthesia was subjectively evaluated by the same investigator (SNR), using a semi-quantitative rating scale modified from Raekallio et al. 2002 (Appendix A.1). The rating scale consisted of five categories and their scores were summed for a total anesthesia score ranging from 0 to 12: spontaneous posture (0-4), response to toe pinch (0-2), palpebral reflex (0-2), eye rotation (0-1) and jaw tone (0-3).

Rabbits were positioned in sternal recumbency with the head and neck well extended. Lidocaine was not used to desensitize the arytenoids to avoid confounding the results, as lidocaine may exhibit an anti-inflammatory effect (Caracas et al. 2009). Orotracheal intubation was performed by a single investigator (PQW) using an uncuffed, 2.0 mm inner diameter, murphy-eyed endotracheal tube (Mallinckrodt Pharmaceuticals, MO, USA). For blind intubation, the endotracheal tube was inserted into the oral cavity and advanced into the trachea once condensation from respiration was visualized in the tube. For endoscopic-guided intubation, the endotracheal tube was placed over a semi-rigid endoscope (FM-1.9X6 Micro-Endoscope; MDS-VET, Canada) and was advanced off the endoscope and into the trachea, once positioned in the cranial trachea. An intubation attempt was defined as the advancement of the endotracheal tube from the opening

of the mouth, at the level of the incisors, to the entrance of the larynx, which was identified via palpation of the ventral neck region. Following an intubation attempt, end-tidal partial pressure of carbon dioxide concentration ($P_{E'}CO_2$) was measured using a multiparameter monitor (CARESCAPE monitor B850; GE Healthcare, Finland). Intubation was considered successful when up to six consecutive $P_{E'}CO_2$ readings were present on the capnograph. If no capnograph readings were present, intubation was considered unsuccessful and the process was repeated until intubation was successful. Additional alfaxalone (3 mg kg^{-1}) and dexmedetomidine (0.0025 mg kg^{-1}) were administered IM if quality of anesthesia was inadequate to allow for proper body positioning or if excessive jaw tone, swallowing or tongue retraction were present to prevent insertion of the endotracheal tube. If anesthesia and/or intubation quality were still insufficient, additional alfaxalone (2 mg kg^{-1}) was administered IM. Once successful intubation was achieved, the number of intubation attempts was recorded and the quality of intubation was subjectively evaluated by an investigator (PQW) using a semi-quantitative rating scale ranging from 0 to 3 (Appendix A.2).

Rabbits were connected to a Bain system and administered isoflurane (IsoFlo; Abbott Laboratories, IL, USA) in 100% oxygen at 0.2 L kg^{-1} $minute^{-1}$ for 2 hours. Rabbits were instrumented for monitoring of HR and cardiac rhythm via electrocardiography, $P_{E'}CO_2$ and f_R via capnography, capillary hemoglobin oxygen saturation (SpO_2) via pulse oximetry, systolic (SBP), diastolic (DBP) and mean (MBP) arterial pressures via direct technique and rectal temperature via the use of a thermistor (CARESCAPE monitor B850; GE Healthcare, Finland). A 22-gauge, 2.5 cm catheter (Sur-Vet Surflo ETFE; Terumo Medical Corp., NJ, USA) was aseptically placed into the marginal vein of the left or right ear for administration of crystalloid fluids (Lactated Ringer's; Hospira, IL, USA) at a rate of 10 mL kg^{-1} $hour^{-1}$. A 22-gauge, 2.5 cm

catheter (Sur-Vet Surflo ETFE; Terumo Medical Corp., NJ, USA) was aseptically placed into the auricular artery, in either the same or opposite ear containing the intravenous (IV) catheter, for measurement of direct arterial pressure using a disposable pressure transducer system (Truewave 3.6M/12FT; Edwards Lifesciences, Germany) connected to a multiparameter monitor (CARESCAPE monitor B850; GE Healthcare, Finland). The pressure transducer was aligned with the glenohumeral joint (approximating the level of the heart) and zeroed prior to performing measurements. Rabbits were allowed to spontaneously ventilate when $P_{E'}CO_2$ was between 35 and 45 mmHg. If hypoventilation ($P_{E'}CO_2$ > 45 mmHg) was observed, then positive-pressure ventilation was applied using the reservoir bag of the Bain circuit. If hypotension (MBP < 60 mmHg) occurred, then rabbits were administered dopamine (Dopamine Hydrochloride; Hospira Inc., IL, USA; 40-60 µg kg^{-1} $minute^{-1}$) as a constant rate infusion (CRI). If hypotension persisted, then dobutamine (Hospira Inc., IL, USA; 40-60 µg kg^{-1} $minute^{-1}$) CRI was administered in addition to dopamine. A combination of dobutamine and norepinephrine (Norepinephrine Bitartrate; Baxter, IL, USA; 0.5-1.0 µg kg^{-1} $minute^{-1}$) CRI was administrated for treatment of persistent hypotension. Rectal temperature was maintained between 37-38°C using a forced-air warming blanket (Bair Hugger; Arizant Inc., MN, US) placed underneath the rabbit. Cardiopulmonary data was recorded every 5 minutes during the two-hour anesthetic period.

At the end of the anesthetic period, rabbits were euthanized with IV pentobarbital (Fatal-Plus; Vortech Pharmaceuticals, MI, USA) at a dose of 195 mg kg^{-1}. Necropsy was immediately performed for collection of laryngeal, tracheal and pulmonary tissues. Tissues were fixed in 10% neutral buffered formalin and submitted to the Louisiana Animal Disease Diagnostic Laboratory for gross and histopathologic examination. Tissues were processed and stained with hematoxylin and eosin following standard procedures. The degree of histologic tissue damage was subjectively

evaluated using a semi-quantitative scoring system modified from Phaneuf et al. 2006 (Appendix A.3). Histologic tissue damage from samples of the cranial larynx (at the level of the vocal cords), caudal larynx, cranial trachea (approximately 1 cm distal to the larynx) and caudal trachea (at the level of the tracheobronchial bifurcation) of each rabbit were individually scored. Subsequently, a total cumulative score was obtained by adding each independent score from each of the four sections examined, ranging from zero to 16 for each rabbit. Scoring was performed by a veterinary pathologist in training (MC) and by a board-certified veterinary pathologist (IML) who were blinded to the treatment groups.

4.2.3. Statistical Analysis

Statistical analysis was performed using commercially available software (JMP Pro 15.0.0; SAS Institute Inc., NC, USA). Weight and age were assessed by a student *t*-test and respiratory and heart rates were evaluated with a mixed ANOVA with treatment, time and their interaction as the fixed effects and each rabbit as the random effect. Anesthesia quality, intubation quality and histopathology scores were tested against treatments via Mann-Whitney test. Additional drug needed (Y/N) was evaluated using chi-squared test. The number of intubation attempts was fitted with a Poisson regression against the treatment effect. Assumptions of the parametric models (linearity, normality of residuals and homoscedasticity of residuals) and influential data points were assessed by examining standardized residual and quantile plots and the normality of the residual was confirmed with Anderson-Darling tests. The correlation of respiratory tissue damage and the number of intubation attempts in the study was also evaluated using Spearman's correlation coefficient. A post hoc power analysis was performed with G*Power 3.1 (Faul et al. 2009) using the data of the number of intubation attempts and additional drug needed (Y/N) and demonstrated that the group size was adequate to detect a significant difference between the two

treatments, with 90% and 98% power, respectively. For all analyses, a value of $P < 0.05$ was considered significant.

4.3. Results

Twenty-one rabbits completed the study without any adverse events occurring during the anesthetic period. One rabbit in group E developed a ventricular arrhythmia which progressed to severe bradycardia and hypotension despite treatment with dobutamine and norepinephrine. Resuscitation was not performed, and this rabbit was euthanized at 1 hour and 36 minutes into the anesthetic period. Two rabbits, both in group B, developed a light plane of anesthesia and began moving causing extubation. The first rabbit extubated at 1 hour and 50 minutes into the anesthetic period and was euthanized immediately as only 10 minutes remained until the end of the study period. The second rabbit extubated at 1 hour and 15 minutes and was delivered isoflurane via a tight-fitting facemask for the remainder of the anesthetic period. All 24 rabbits experienced hypotension and were treated accordingly.

There were no differences in age or weight between rabbits in each treatment group. Heart rates were higher at T = 10 compared to T = 0 ($P = 0.04$) with no difference between treatment groups (Table 3). Respiratory rates were lower at T = 10 compared to T = 0 ($P < 0.001$) with no difference between treatment groups (Table 3).

Table 3. Mean (± standard deviation) respiratory rate (fR; breaths minute^{-1}) and heart rate (HR; beats minute^{-1}) data at baseline (T = 0) and 10 minutes (T = 10) following administration of dexmedetomidine (0.005 mg kg^{-1}), hydromorphone (0.1 mg kg^{-1}) and alfaxalone (7 mg kg^{-1}) of rabbits undergoing blind (group B) or endoscopic-guided (group E) orotracheal intubation.

	f_R		**HR**	
	T = 0	T = 10	T = 0	T = 10
Group B	108 (± 36)	44 (± 15)	158 (± 20)	176 (± 25)
Group E	119 (± 32)	50 (± 20)	154 (± 29)	172 (± 47)

The median (range) total anesthesia quality scores for groups B and E were 7.5 (6-8) and 7.0 (5-8) respectively (Table 4). Total anesthesia quality scores and individual values for each category were not different between treatment groups. A higher number of rabbits in group E (11/12 rabbits) required additional alfaxalone and dexmedetomidine to successfully intubate the trachea compared to rabbits in group B (2/12 rabbits; $P < 0.001$).

Table 4. Median (range) anesthesia quality scores following administration of dexmedetomidine (0.005 mg kg^{-1}), hydromorphone (0.1 mg kg^{-1}) and alfaxalone (7 mg kg^{-1}) of rabbits undergoing blind (group B) or endoscopic-guided (group E) orotracheal intubation. The maximum possible score is 12.

	Group B	**Group E**
Spontaneous posture	4 (3-4)	4 (3-4)
Response to toe pinch	0	0
Palpebral reflex	1 (0-1)	1 (0-1)
Eye rotation	0	0
Jaw tone	2 (2-3)	2 (1-3)
Total anesthesia score	7.5 (6-8)	7 (5-8)

The median (range) intubation quality scores were 2.5 (2-3) for group B and 3 (2-3) for group E, and no difference was detected between treatment groups. However, a higher number of intubation attempts was required for group B compared to group E, with median (range) intubation attempts of 2 (1-8) and 1 (1-3) for groups B and E, respectively ($P = 0.01$).

Median cumulative histopathology scores and pooled scores for the cranial and caudal larynx and trachea are presented in Figure 1. No inter-observer variability occurred between scores. The median (range) cumulative histopathology scores for treatment groups B and E were 6 (3-10) and 6 (2-9) respectively. Location-specific histopathology scores, cumulative histopathology scores and pooled scores were not different between treatment groups and respiratory tissue damage was not correlated to the number of intubation attempts.

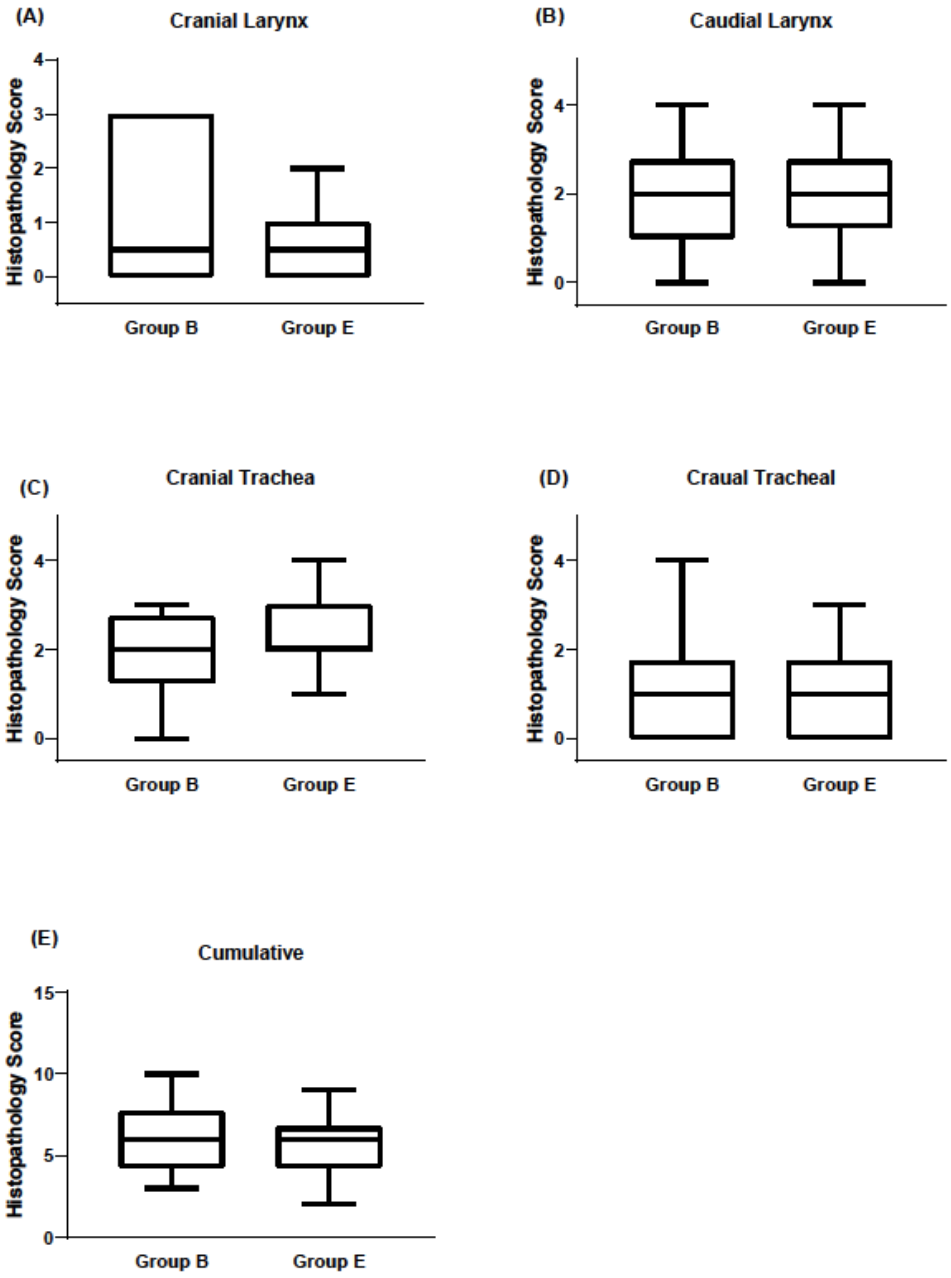

Figure 1. Individual and cumulative histopathology scores following administration of dexmedetomidine (0.005 mg kg^{-1}), hydromorphone (0.1 mg mg kg^{-1}) and alfaxalone (7 mg kg^{-1}) of rabbits undergoing blind (group B) or endoscopic-guided (group E) orotracheal intubation. A) cranial larynx, B) caudal larynx, C) cranial trachea, D) caudal trachea, E) cumulative histopathology score.

4.4. Discussion

The main finding of this study is that both blind and endoscopic-guided orotracheal intubation produced a similar degree of respiratory tissue damage despite the higher number of intubation attempts needed with the blind technique.

In both groups, rabbits exhibited similar total anesthesia quality scores; however, 91.6% of rabbits in group E required additional alfaxalone and dexmedetomidine to allow intubation compared to only 16.7% of rabbits in group B. Rabbits requiring additional drugs initially exhibited low intubation quality scores. However, following administration of additional alfaxalone and dexmedetomidine, intubation quality scores increased and were similar between groups. An adequate plane of anesthesia is necessary to attenuate laryngeal reflexes, provide muscle relaxation, extrude the tongue out of the mouth and allow for proper positioning to facilitate intubation. Also, the rabbits could only be intubated once the swallowing and coughing reflexes had been abolished, emphasizing the need for an adequate anesthetic plane. In addition, these results suggest that positioning associated with the intubation technique influenced overall success rate of intubation. Endoscopic-guided intubation requires hyperextension of the head to allow direct visualization of the larynx (Johnson 2010). Rabbits in group E appeared to exhibit insufficient muscle relaxation and became sufficiently stimulated during hyperextension of the head and neck, therefore necessitating administration of additional drugs. In comparison, rabbits in group B required a lesser degree of head extension and did not become stimulated during intubation despite requiring more attempts to successfully intubate.

Blind orotracheal intubation is frequently associated with repeated intubation attempts in rabbits (Richardson 2015; Wenger et al. 2017). Rabbits in group B required a greater number of intubation attempts compared to group E and had a wider range of values, with one rabbit requiring

eight attempts. The blind technique often requires multiple attempts as correct placement of the endotracheal tube is based on occurrence of breath sounds or condensation on the tube and not on visualization of the glottis. In contrast, endoscopic techniques allow for direct visualization of the glottis (Varga 2017), potentially increasing the success rate with fewer intubation attempts. However, because endoscopic-guided intubation requires hyperextension of the head and neck causing a higher degree of stimulation, a deeper anesthetic plane is required. Thus, higher drug doses are required which could potentially increase risk of cardiorespiratory adverse effects.

Repeated intubation attempts have been implicated as a cause of laryngeal and tracheal tissue damage in rabbits (Phaneuf et al. 2006; Engbers et al. 2017). However, none have investigated a correlation between the number of intubation attempts and the degree of damage. In the present study, total cumulative histopathology scores and range of values were similar between groups despite group B having a higher number of intubation attempts. The highest cumulative histopathology score was 10/16, occurring in a rabbit from group B who was successfully intubated after only one attempt. In contrast, the highest number of intubation attempts was eight, occurring in a rabbit from group B who obtained a low cumulative histopathology score of 2/16. It is not known why such a difference existed; however, the results suggest that the number of intubation attempts is not correlated with the degree of respiratory tissue damage.

All rabbits exhibited some degree of tissue damage, with similar scores reported between the different tissue types. Histological damage to laryngeal and tracheal tissues were also documented in the studies by Phaneuf et al. (2006), Engbers et al. (2017) and Comolli et al. (2020). According to these authors, rabbits may be more prone to development of laryngeal and tracheal lesions as a result of the highly vascularized mucosa (Phaneuf et al. 2006). Mucosal irritation due to contact with the endotracheal tube may result in vascular congestion, edema and hemorrhage

and varying degrees of these lesions were observed histologically in this study. An alternative explanation for the presence of the observed tissue damage is that these lesions existed prior to initiation of the study; however, a control group of non-intubated rabbits was not present in the current study to allow comparison.

Intubation-induced lesions may progress over time, potentially resulting in stricture formation (Nakagishi et al. 2005; Grint et al. 2006). In the present study, rabbits were euthanized and the airway tissues were histologically evaluated two hours following intubation, which allows sufficient time for the potential airway damage induced by intubation to develop histological changes associated with acute inflammation (Bochsler & Slauson 2002). Following a traumatic insult to a tissue, the initial neutrophilic infiltration generally occurs within 2 hours, followed by peak infiltration in 4-6 hours, after which mononuclear infiltrates predominate and peak in 18-24 hours (Bochsler & Slauson 2002). The rabbits were euthanized two hours following intubation, thus only the initial stages of the inflammatory response were evaluated in this study. In a clinically applicable scenario, the extent of respiratory tissue damage may be more severe if an inflammatory response has sufficient time to fully develop. Therefore, based on the results of this study, the long-term consequences of damage caused to laryngeal and tracheal tissues are not known and cannot be predicted.

Alfaxalone can cause respiratory depression when administered IV or IM in rabbits, especially when used in combination with other sedatives (Navarrete-Calvo et al. 2013; Huynh et al. 2014; Bradley et al. 2019; Ishikawa et al. 2019). Thus, it is not surprising that a decrease in respiratory rate was observed following administration of alfaxalone in this study, with some rabbits exhibiting respiratory rates lower than the normal physiologic range of 30-60 breaths $minute^{-1}$ (Meredith & Campbell-Ward 2010). It is unclear if a resultant decrease in minute

ventilation occurred causing hypoventilation and/or hypoxemia, as rabbits were not yet instrumented for monitoring of SpO_2 or $P_{E'}CO_2$. Previous studies have reported the occurrence of hypoxemia in rabbits following administration of IM alfaxalone (Rousseau-Blass & Pang 2020; Ishikawa et al. 2019). In the study by Rousseau-Blass and Pang (2020), 100% oxygen supplementation via facemask resolved the hypoxemia but was associated with hypercapnia. Thus, rabbits should be carefully monitored following alfaxalone administration and intubation is recommended in anesthetized rabbits to provide oxygen supplementation and positive pressure ventilation.

Alfaxalone has been shown to maintain cardiovascular stability and increase heart rate in dogs but not cats at clinical doses (Muir et al. 2008; Muir et al. 2009; Okushima et al. 2015; Lagos-Carvajal et al. 2017; Lagos-Carvajal et al. 2020). In dogs, the positive chronotropic effect is thought to be the result of a baroreceptor-induced increase in heart rate in response to a decrease in arterial blood pressure (Okushima et al. 2015). However, whether a similar mechanism occurs in rabbits has yet to be elucidated. In the current study, heart rates were higher following administration of alfaxalone but remained within the normal range (180-300 beats $minute^{-1}$) reported for rabbits (Meredith & Campbell-Ward 2010). This is in contrast to studies reporting a decrease in heart rate following alfaxalone administration in rabbits (Gil et al. 2012; Tutunaru et al. 2013; Bradley et al. 2019). One explanation for this difference is that a higher dose of alfaxalone was used in the current study, potentially causing a larger decrease in blood pressure and initiating a reflex baroreceptor response. Hypotension commonly occurred; however, direct arterial blood pressure monitoring was only initiated after rabbits were introduced to isoflurane anesthesia. Thus, it is unclear if hypotension occurred as a result of the vasodilatory properties of the inhalant anesthesia alone or if it was pre-existing to initiation of inhalant anesthesia as a result of alfaxalone

administration. Nonetheless, the fact that all rabbits became hypotensive is an important finding as this could contribute to the higher mortality rate reported for rabbits if not treated appropriately.

The main limitation of this study was that a control group containing non-intubated rabbits was not present, so it was impossible to know if there was any pre-existing respiratory disease. However, it is the authors' opinion that including a control group in this study did not fit within the reduction principle of the humane use of animals in scientific research because it would have required the euthanasia of rabbits for the sole purpose of examining the respirator tract. Instead, we elected to reduce animal use by only comparing variables between the two intubation techniques. The use of control animals could be replaced by examination of rabbits at slaughterhouses or retrospective analysis of post-mortem reports. An additional study limitation is that only young, apparently healthy New Zealand white rabbits were used, so the results cannot be extrapolated to other breeds, older rabbits or rabbits with clinical illness.

In conclusion, a similar degree of respiratory tissue damage was observed following orotracheal intubation using both blind and endoscopic-guided techniques. Blind intubation required a higher number of attempts but was not correlated with a higher degree of tissue damage. Endoscopic-guided intubation required the rabbits to be more sedate than for blind intubation. In a clinical setting, this information provides novel evidence that the number of intubation attempts may not contribute to the increase in anesthesia-related mortality seen in domestic rabbits as suggested in the past by other authors. The level of anesthesia must be considered appropriate for each intubation technique, perhaps not because of possible respiratory tissue damage but simply for ease of intubation. Further investigation is warranted to determine the long-term consequences of respiratory tissue damage caused by orotracheal intubation in rabbits.

Chapter Five. Conclusions

Rabbits have one of the highest perianesthetic mortality rates reported in veterinary species (Brodbelt et al. 2008). Several factors may contribute to this increased risk in rabbits including drug-induced cardiovascular depression, lack of appropriate airway management due to unsuccessful intubation, stress associated with handling, and underlying, subclinical respiratory disease (Phaneuf et al. 2006; Grint & Murison 2008; Navarrete-Calvo et al. 2013; Engbers et al. 2017). In an attempt to reduce anesthetic-related mortality, there is a myriad of research investigating improved intubation techniques that are simple and easy to perform, as well as induction protocols using specific drug combinations in order to reduce the adverse effects associated with using large volumes of a single drug.

Alfaxalone is a synthetic neuroactive steroid than binds $GABA_A$ receptors, resulting in central nervous system depression (Lambert et al. 2003). Use as an induction agent in dogs and cats is widespread and well-reported in the literature (Muir et al. 2008; Muir et al. 2009); however, data on pharmacokinetics, safety and clinical efficacy is largely lacking in some species such as rabbits. Intramuscular administration is advantageous, especially in easily stressed species such as rabbits, because it allows dose-dependent sedation and anesthesia without handling the rabbit to place an IV catheter. However, dose-dependent cardiorespiratory depression may also be seen with IM administration, especially at high doses (Huynh et al. 2014). Currently, no study has investigated the suitability of IM alfaxalone for orotracheal intubation of rabbits.

Orotracheal intubation in rabbits is technically challenging, often resulting in multiple, unsuccessful attempts. It has been speculated that multiple intubation attempts can result in trauma to the delicate laryngeal and tracheal tissues that comprise the rabbit upper respiratory tract; however, there is a lack of scientific evidence in the literature to support this claim. Few studies

have investigated the occurrence of respiratory tissue damage following orotracheal intubation in rabbits, and none have attempted to correlate the number of intubation attempts to degree of tissue damage.

Based on the lack of studies evaluating IM alfaxalone use for induction of anesthesia in rabbits, one of the objectives of this thesis research was to determine the optimal dose of IM alfaxalone in combination with dexmedetomidine and hydromorphone that would allow endoscopic-guided orotracheal intubation in rabbits without causing excessive respiratory depression and/or apnea. Similarly, due to lack of data surrounding potential damage to respiratory tissues caused by intubation, another major objective of this research was to compare damage to laryngeal and tracheal tissued caused by blind or endoscopic-guided orotracheal intubation. This study also aimed to correlate the number of intubation attempts with the degree of respiratory tissue damage.

In order to achieve the objectives of this thesis, two experimental studies were designed. In the first study, 15 Mini-Lop rabbits received either 2, 5, or 7 mg kg^{-1} of IM alfaxalone in combination with dexmedetomidine and hydrophone. Quality of anesthesia and intubation were scored, and intubation was attempted using an endoscopic-guided technique. Quality of anesthesia, quality of intubation, and intubation success rate all increased in a dose dependent manner, with the highest alfaxalone dose producing the highest success rate of intubation (80% of rabbits). Furthermore, no respiratory adverse effects were noted in any of the rabbits.

Higher doses of alfaxalone and/or dexmedetomidine may be necessary to increase the success rate of intubation; however, higher doses of these drugs may result in clinically significant cardiorespiratory depression. Previous studies have reported occurrence of respiratory depression following IM administration of alfaxalone at similar doses used in the current study (Grint et al.

2008; Navarrete-Calvo et al. 2013; Huynh et al. 2014; Ishikawa et al. 2019). In this study, evaluation of respiratory function was based only on respiratory rate and the presence, or absence, of apnea. Future studies are warranted to investigate the respiratory depressant effects of alfaxalone in rabbits, using more sensitive methods of respiratory function monitoring (e.g., hemoglobin oxygen saturation, capnography, blood gas analysis).

In the second study, 25 New Zealand white rabbits were administered 7 mg kg^{-1} of IM alfaxalone in combination with dexmedetomidine and hydrophone. Quality of anesthesia and intubation were scored, and intubation was performed using either an endoscopic-guided technique or a blind technique. Additional alfaxalone and dexmedetomidine were administered if intubation was unable to be performed, and following successful intubation, the number of intubation attempts was recorded. Rabbits were euthanized following two hours of general anesthesia, and laryngeal, tracheal and pulmonary tissues were submitted for histopathologic evaluation. The degree of tissue damage was scored for each tissue type in each rabbit.

A higher number of intubation attempts were required in rabbits undergoing blind intubation compared to endoscopy-guided intubation. This result is not surprising as blind orotracheal intubation is commonly reported to be technically challenging with a high rate of failure (Grint & Murison 2008; Wenger et al. 2017). In contrast, endoscopy-guided intubation allows direct visualization of the larynx, facilitating proper placement of the endotracheal tube and allowing for a reduced number of intubation attempts. In addition, approximately 92% of rabbits intubated using the endoscopy-guided technique required additional alfaxalone and dexmedetomidine compared to only 17% of rabbits blindly intubated. These results suggest that endoscopy-guided intubation was more stimulating to rabbits due to insufficient muscle relaxation.

For example, endoscopy-guided intubation required a greater degree of head extension to properly visualize the larynx, causing the rabbits to move warranting administration of additional sedatives.

Histopathological evaluation revealed no difference in the histopathology scores between rabbits intubated using the blind or endoscopy-guided techniques, even though blind orotracheal intubation required a higher number of intubation attempts. These results suggest that repeated intubation attempts may not be associated with respiratory tissue damage and a subsequent increase in the perianesthetic mortality rate of rabbits. In this study, rabbits were only anesthetized for two hours, meaning that a fully developed inflammatory response may not have had sufficient time to occur. Thus, the long-term effects on the respiratory tissues could not be evaluated. Further studies are needed to assess the long-term damage to respiratory tissues and the potential clinical consequences associated with repeated intubation attempts in rabbits.

In conclusion, this thesis research provides valuable information on the potential adverse effects associated with repeated intubation attempts in rabbits. The results of this research suggest that an increased number of intubation attempts may not be associated with respiratory tissue damage and thus, it may not be a contributing factor to the increased perianesthetic mortality rate seen in domestic rabbits. Furthermore, this research demonstrated that an anesthetic plane deep enough to allow orotracheal intubation is possible following IM administration of alfaxalone in combination with other sedatives such as α-2 agonists and opioids, without risk of clinically significant bradypnea or apnea. In this study, the success rate of intubation increased in a dose-dependent manner, with the 7 mg mg^{-1} resulting in a success rate of 80%. Further studies are needed to evaluate this dose of alfaxalone in a clinical setting, both in healthy and sick rabbits.

The decision to perform intubation is based on various factors including type and duration of procedure, available equipment, experience, size/breed of rabbit and overall health status. Based

on the results of this thesis research, several recommendations can be made with respect to increasing the success rate of intubation and minimizing the risk of mortality in rabbits. First, anesthetic events should be carefully planned beforehand, having all necessary equipment within reach and additional anesthetic drug available if needed. In addition, a secondary plan of action should be arranged should intubation ultimately fail and the clinician should designate a specific time period in which intubation is attempted before implemented the secondary plan. Third, every attempt possible should be made to minimize the stress of the rabbit in the preoperative period, including minimizing handling using IM sedatives/anxiolytics and allowing the animal to achieve maximal sedative effects in a quiet location. Drug selection should ideally involve agents that provide adequate sedation/immobilization with minimal cardiovascular and respiratory depressive effects, such as the protocol devised from this research. Forth, selection of intubation technique should be based on clinician preference and experience. Intubation should be performed only by experienced personnel taking care to avoid aggressive or forceful movements that could cause trauma to the larynx and trachea. In addition, the rabbit should be preoxygenated via facemask using 100% oxygenation prior to intubation and the clinician should ensure an adequate anesthetic depth has been achieved to facilitated positioning and placement of the endotracheal tube. Lidocaine should be administered to desensitize the arytenoid cartilages to prevent laryngospasm. Finally, the rabbit should be appropriately monitored in the post anesthetic period for signs of respiratory distress.

Appendix. Semi-Quantitative Scoring System for Quality of Anesthesia, Intubation and Respiratory Tissue Damage

Appendix A.1. Semi-quantitative scoring system adapted from a previously described scoring system (Raekallio et al. 2002) to quantify the degree of anesthesia quality in rabbits. Scores for each category were summed to determine the total anesthesia quality score for each treatment group (maximum possible score of 12).

Category	Score	Description
Spontaneous posture	0	Normal
	1	Sternal recumbency, head up
	2	Sternal recumbency, head down
	3	Lateral recumbency, response to stimuli
	4	Lateral recumbency, no response to stimuli
Response to toe pinch	0	Complete withdrawal of limb
	1	Partial withdrawal of limb
	2	No withdrawal of limb
Palpebral reflex	0	Strong
	1	Weak
	2	Absent
Eye rotation	0	Central
	1	Ventromedial
Jaw tone	0	Unable to open mouth
	1	Able to open mouth with strong resistance, unable to extrude tongue
	2	Able to open mouth with minimal resistance, +/- tongue extrusion, chewing/tongue retraction
	3	Able to open mouth and extrude tongue with no resistance

Appendix A.2. Semi-quantitative scoring system to quantify the degree of intubation quality in rabbits.

Score	Description
0	Swallowing and/or coughing not allowing intubation
1	Minimal swallowing and/or coughing not allowing intubation
2	Minimal swallowing and/or coughing allowing intubation
3	No swallowing and/or coughing allowing intubation

Appendix A.3. Semi-quantitative scoring system adapted from a previously described scoring system (Phaneuf et al. 2002) to quantify the degree of respiratory tissue damage in rabbits. Four samples (cranial and caudal larynx, cranial and caudal trachea) from each rabbit were individually scored and subsequently, a total cumulative score was obtained by adding each independent score from each of the four sections examined (maximum possible cumulative score of 16).

Score	Description
0	Within normal limits
1	Focal attenuation of epithelium Heterophil exocytosis and/or few heterophils in lamina propria
2	Focal attenuation and/or erosion/ulceration affecting ≤ 5% of epithelial lining Few heterophils within lamina propria and epithelium (exocytosis) Mild or moderate edema of lamina propria
3	Focal/multifocal/extensive erosion/ulceration affecting 5-30% of epithelial lining Heterophilic infiltration in lamina propria with cell debris/fibrin exudation Focal/multifocal hemorrhages within lamina propria Congestion/edema of submucosa or submucosal infiltration with heterophils and hemorrhage
4	Focal/multifocal/extensive erosion/ulceration affecting >30% of epithelial lining Heterophilic infiltration in lamina propria with cell debris/fibrin exudation focal/multifocal hemorrhages within lamina propria Congestion/edema of the submucosa or submucosal infiltration with heterophils and hemorrhage

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Vita

Stephanie Nicole Reabel was born in Brockville, Ontario, Canada in January 1984. She successfully completed the Doctor of Veterinary Medicine program at the University of Guelph, Ontario Veterinary College in May 2017. In July 2017, she started a one-year small animal surgery and medicine rotating internship at VCA Hollywood Animal Hospital in Hollywood, Florida. Following her internship, Dr. Reabel was accepted into a three-year residency program in veterinary anesthesia and analgesia at the Louisiana State University School of Veterinary Medicine in Baton Rouge, Louisiana. At the same time, she was accepted into a Master's degree program in the Department of Veterinary Clinical Sciences, School of Veterinary Medicine. Dr. Reabel plans to receive her Masters this May 2021 and to complete her residency program in July 2021.