AN ABSTRACT OF THE THESIS OF

Ana M. del Álamo for the degree of Master of Science in Veterinary Science presented on September 20, 2013

Title: Anesthetic Evaluation of Administration of Intravenous Alfaxalone in Comparison with Propofol and Ketamine/Diazepam Inductions in Alpacas

Abstract approved:

Ron E. Mandsager

Abstract:

Alfaxalone (3a-hydroxy-5a-pregnane-11,20-dione) is a neuroactive steroid that can induce anesthesia. It has recently been reformulated and solubilized in 2-hydroxypropyl-β-cyclodextrin (HCPD). Propofol and the combination of ketamine and diazepam are commonly used induction drugs in all species. The objective of this prospective randomized and crossover design study was to evaluate the safety and efficacy of induction with this new formulation of intravenous alfaxalone in alpacas and to compare these effects to those of propofol and the combination of ketamine/diazepam.

Five healthy alpacas (96.7± 19.9 kg, 9.6± 3.1 years old) were anesthetized on three different occasions with propofol, ketamine/diazepam or alfaxalone by titrated intravenous injection. Quality of induction and intubation was assessed using a simple descriptive scale and quality of recovery was scored: 1 (very poor)- 5 (excellent). The auricular artery was catheterized to measure systolic (SAP), mean (MAP), and diastolic (DAP) arterial pressures and for

collection of arterial blood to obtain blood gases and electrolytes. Electrocardiography, pulse oximetry (SpO_2), respiratory rate, and end-tidal carbon dioxide partial pressure ($P_E'CO_2$) were also monitored. Repeated measures ANOVA was used to assess effects of drug and time. Statistical significance was set at $p < 0.05$.

We observed that the mean dose of alfaxalone sufficient to allow intubation in our alpacas was 2.1 mg kg^{-1}. Induction was excellent with all protocols. Heart rate (HR), SAP and MAP were significantly higher following alfaxalone compared to ketamine/diazepam. Lactate after standing following alfaxalone was higher compared to minutes 1 and 6 after alfaxalone administration and to propofol (standing) ($p < 0.05$). All alpacas required oxygen supplementation due to $P_E'CO_2 > 60$ mmHg and/or $SpO_2 < 90\%$ at minute 1. Time from induction to standing was longer with alfaxalone (34.08± 3.16 minutes) than propofol (19± 4.3 minutes) or ketamine/diazepam (24.9± 1.67 minutes). Recovery quality median scores were clinically and statistically different: 2 (alfaxalone), 4 (ketamine/diazepam), and 5 (propofol). Tremors, paddling, rolling over, seizure-like activity and thrashing characterized recovery from alfaxalone.

In conclusion, all protocols were adequate for induction but recovery quality was worse with alfaxalone. HR, SAP, MAP were increased at minute 1 in all protocols. Transient hypercapnia and hypoxia was observed with all protocols. Based on this study, alfaxalone used alone in un-premedicated alpacas is not recommended due to poor recoveries.

Anesthetic Evaluation of Administration of Intravenous Alfaxalone in Comparison with Propofol and Ketamine/Diazepam Inductions in Alpacas

by

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APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Ana M. del Álamo

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ABBREVIATIONS

HCPD	2-hydroxypropyl-b-cyclodextrin
SAP	Systolic arterial pressure
MAP	Mean arterial pressure
DAP	Diastolic arterial pressure
HR	Heart rate
f_R	Respiratory rate
SpO_2	Hemoglobin saturation of oxygen
$P_E'CO_2$	End-tidal pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
$PaCO_2$	Arterial partial pressure of carbon dioxide
mmHg	Millimeters of mercury
pKa	kilopascal
IV	Intravenous
IM	Intramuscular
BE	Base excess

DEDICATION

I would like to dedicate this work to Javier Benito de la Víbora, who gave me the keys to success.

Anesthetic evaluation of administration of intravenous alfaxalone in comparison with propofol and ketamine/diazepam inductions in alpacas

Chapter 1. Introduction

Alfaxalone (3a-hydroxy-5a-pregnane-11,20-dione) is a synthetic neuroactive steroid that binds to gamma aminobutyric acid type A ($GABA_A$) cell surface receptors enhancing the effect of the endogenous ligand and acting as a ligand itself. It was previously taken off the market when commercialized as Saffan® and Althesin® (veterinary and human product, respectively), a mixture of alfaxalone and alphadolone in Cremophor®-EL due to adverse reactions related to histamine release and anaphylactic reactions. It has been established that these side effects were related to the carrier, Cremophor®-EL, and not to the neuroactive steroids. In recent years, alfaxalone has been solubilized in 2-hydroxypropyl-β-cyclodextrin (HCPD) and has been marketed in several countries as Alfaxan. Cyclodextrins are useful formulation vehicles that increase the amount of drug that can be solubilized in aqueous vehicles. This molecule is has been tolerated when tested in rats, mice and dogs and has shown limited toxicity. It is not associated with histamine release or anaphylactic reactions (Gould&Scott 2005).

The use of the new formulation of alfaxalone has been reported in dogs (Muir et al. 2008; Maddern et al. 2010), cats (Muir et al. 2009; Mathis et al. 2012), horses (Goodwin et al. 2011; Goodwin et al. 2012; Keates et al. 2012), rabbits (Marsh et al. 2009), pigs (Keates 2003; Santos González et al. 2013h), sheep (Walsh et al. 2012; Andaluz et al. 2011), iguanas (Bertelsen & Sauer 2011), amphibians (McMillan & Leece 2011), turtles (Kischinovsky et al. 2013), crocodiles (Olsson et al. 2013), primates, and fish (Kischinovsky et al. 2013). To our knowledge, there are no publications concerning the administration of

alfaxalone in camelids. Alfaxalone undergoes hepatic metabolism: phase I is cytochrome P450 enzyme dependent and phase II is conjugation dependent. It is excreted in the bile and urine (Ferré et al. 2006; Whittem et al. 2008; Lau et al. 2013).

Ketamine, an NMDA (N-methyl-D-aspartate) receptor antagonist, is widely used in all species for induction of anesthesia and, in some instances, for its antihyperalgesic properties. Its use has often been reported in both Old World and New World camelids. Ketamine does not provide good muscle relaxation and pharyngeal reflexes are generally maintained after induction. This minimizes accidental aspiration of rumen contents but hinders intubation (García Pereira et al. 2006). Combinations of ketamine with muscle relaxant drugs, such as diazepam, midazolam, guaifenesin or alpha-2 adrenergic agonists, are recommended in camelids (Abrahamsen 2009). Ketamine stimulates the sympathetic system resulting in elevated heart rate, blood pressure, intracranial pressure and intraocular pressure. Consequently, ketamine is not indicated for cases in which these effects are not recommended.

Propofol (2,6-diisopropylphenol) is an alkyl phenol that is also used as an induction drug. Similarly to alfaxalone, it acts as on $GABA_A$ receptors, enhancing its inhibitory activity and providing hypnosis. The formulation most commonly used is a lipid macroemulsion, which can only be administered intravenously. Although not commonly used in camelids, it provides smooth and reliable induction in llamas (Duke et al. 1997) and camels (Fahmy et al. 1995) with fast and uneventful recoveries. Propofol,

either as an intravenous (IV) bolus or as a constant rate infusion, has been used more extensively in small ruminants ruminants, such as sheep and goats. Smooth inductions along with fast and good quality recoveries have been observed in these species after administration of propofol (Lin et al. 1997; Prassinos et al. 2005). Despite this, its relatively high price has made clinicians rely on cheaper options for induction of general anesthesia in camelids, such as ketamine.

The objective of this study was to evaluate anesthetic induction, maintenance and recovery quality, as well as the cardiovascular, respiratory, and acid-base parameters after administration of alfaxalone titrated intravenously in non-premedicated, healthy alpacas. The results were compared to the administration of intravenous propofol or the combination of ketamine/diazepam in the same group of alpacas. In addition to this, our aim was to determine the dose of intravenous alfaxalone needed to induce and intubate non-premedicated healthy alpacas.

Chapter 2. Materials and Methods

2.1 Ethical approval

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Oregon State University on April, 2011 (ACUP #4124).

2.2 Study design

Five alpacas belonging to the Oregon State Veterinary College Teaching Herd were included in this study. Their weights ranged from 79.7 kg to 101.3 kg (mean 96.7 ± 19.9 kg) and they were between 8 and 10 years old (mean 9.6 ± 3.1 years old). Within this group there was one intact male, one gelding and three females.

Each animal was admitted to the Large Animal Hospital and housed in the same stall as one other alpaca from the same herd to reduce their stress level. Feed was withheld for 12 hours but they were allowed free access to water until the time of induction. A physical exam was performed and weights were obtained every time they were brought into the hospital for the study. No abnormalities were identified on history and physical exam.

This study was designed to have a prospective randomized crossover structure. Each alpaca received, on different occasions, one of each of the three protocols: alfaxalone (2 mg kg^{-1}; Alfaxán®; Vétoquinol, Spain) intravenously (IV) (Group A), propofol (3 mg kg^{-1}; Propoflo®; Abbott Laboratories, IL, USA) IV (Group P), or ketamine (4 mg kg^{-1}; Fort Dodge, KS, USA) combined in one syringe with diazepam (0.2 mg kg^{-1}; Hospira, IL, USA) IV (Group KD). All drugs

were drawn up with an extra 25% of the calculated dose. Each protocol was administered as follows: one fifth (20%) of the volume in the syringe was injected every 15 seconds while a designated assistant assessed the relaxation and sedation of the alpaca. When jaw tone and swallowing reflex were absent, administration of drugs was ceased and intubation was performed. The mean and standard deviation of the amount of drug necessary to achieve absence of jaw tone and swallowing reflex, was later calculated. We established a washout period of at least one week between anesthesia periods for each alpaca. No drugs, other than the aforementioned ones, were administered to these alpacas during the duration of the study. All alpacas were kept in sternal recumbency during the length of the anesthetic procedure and recovery.

2.3 Catheterization

Before each trial, the alpacas were placed in a camelid restraining chute and had two catheters placed, without use of any sedatives. A 16 gauge x 14 cm Abbocath catheter was inserted in the right jugular vein and sutured and bandaged in place. A 22 gauge x 2.5 cm winged catheter was placed in one of the caudal auricular arteries to provide a means for measuring invasive blood pressure and obtaining arterial blood samples. Prior to catheterization of this artery, the base of the ear was blocked with 0.5-1 mL of subcutaneous 2% lidocaine (Vedco, MO, USA) to keep the unsedated alpacas from shaking their head and to minimize discomfort during catheter placement.

After the catheters were in place, all animals were returned to their stalls for 1 hour prior to induction.

2.4 Endotracheal intubation

The same experienced anesthetist assessed the quality of induction and quality of intubation in all animals. Assessing quality of induction was based on muscle relaxation, absence of tremors or twitches and depth of anesthesia of the alpacas. Assessing quality of intubation was based on jaw muscles relaxation and ease to open the mouth and visualize the anatomical features of the larynx. Both quality of induction and intubation were described as excellent, good, fair, poor or very poor.

Intubation was performed when the alpacas were in sternal recumbency, had relaxed jaw tone, and when swallowing reflex was absent. Loss of palpebral reflex was not considered to be a good indicator of adequate anesthetic depth because most of the alpacas retained a palpebral reflex even when intubation conditions were optimal. Intubation was performed with the aid of a 30 cm long laryngoscope blade and blunt-end 60 cm plastic stylet. The endotracheal tube cuff was immediately inflated after placement to a volume that was considered subjectively adequate judging by the relative size of the endotracheal tube in comparison to the tracheal diameter.

2.5 Instrumentation

Instrumentation consisted of: an electrocardiogram (ECG) read on lead II, pulse oximetry probe placed on the tongue, invasive arterial blood pressure and side-stream capnography. All of this was displayed on a multiparameter monitor (Spectrum, Datascope Corp, NJ, USA). The blood pressure transducer

(Argon Critical Care Systems, Singapore) was calibrated with room atmospheric pressure and clipped to the fiber at the level of the right heart. Parameters recorded included heart rate (HR) in beats minute^{-1}, respiratory rate (f_R) in breaths minute^{-1}, pulse oximetry (SpO_2), end tidal carbon dioxide partial pressure (P_E' CO_2) in mmHg, systolic, mean and diastolic arterial blood pressure (SAP, MAP, and DAP, respectively) in mmHg.

The alpacas were allowed to breathe room air if their SpO_2 was more than 90% and their P_E' CO_2 was less than 60 mmHg. If either one of these parameters were outside of the specified values, or if their f_R was below 1-2 breaths minute^{-1} they were connected to 100% O_2 delivered by a semi-closed circle anesthesia machine (Modulus, Ohio Medical Products, WI, USA). Manual breaths were delivered at a rate of 3 breaths minute^{-1}. When spontaneous ventilation returned, and SpO_2 was over 90%, and P_E' CO_2 was below 60 mmHg they were disconnected from the anesthesia machine and allowed to resume breathing room air.

2.6 Recovery

The alpacas were kept in sternal position propped against a padded wall with their heads elevated and were allowed to quietly recover from the induction drugs without applying any stimulation. An assistant was present to prevent them from injury in case of falls or sudden head movements.

Once alpacas were holding their own head up and were swallowing, chewing and actively trying to expel the endotracheal tube they were extubated and allowed to stand on their own.

The same person scored recovery on every occasion. Recovery time was considered to be the time between extubation and standing without assistance. Alpacas received a recovery score from 1 to 5 according to a non-validated recovery scoring system developed by the authors (Table 1).

2.7 Timeline

Cardiopulmonary parameters were recorded 5-10 minutes before induction, 1 minute after intubation, and then at 5-minute intervals until extubation. Arterial blood was collected into 1 mL heparinized syringes from the caudal auricular artery catheter at the same time points. One more arterial blood sample was obtained approximately 60 minutes after extubation, once the alpacas were standing and back in their stalls exhibiting normal behavior. The blood was stored in ice-water slurry for less than an hour before analysis for pH, arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), arterial hemoglobin oxygen saturation (SaO_2), electrolytes (Na^+, K^+, Cl^-, and Ca^{++}), bicarbonate (HCO_3^-), base excess (BE), lactate and glucose concentrations (Siemens 1265 Rapidlab Analyzer; Siemens Healthcare Diagnostics.Inc, NY, USA), and were corrected for body temperature.

2.8 Statistical analysis

Data were analyzed with SAS Version 9.2 (SAS Institute, Cary, NC). Analysis of variance procedures were used to determine differences in drug and time. A repeated measures ANOVA with an autoregressive (period 1) covariance

structure was used to model the within subject variation. Simple effects of drug given time and time given drug were assessed with planned contrasts. Means and standard errors were reported, and significance was set at $p < 0.05$. Recovery scores were analyzed using a nonparametric ANOVA and the results are given as medians and ranges.

Table 1. Recovery scoring system used by a single observer to determine quality of recovery after extubation in all groups.

Recovery Score		
1	Obstructed airway, vocal, dysphoric, prolonged	Very poor
2	Dysphoric, vocal, uncoordinated, prolonged	Poor
3	Mildly uncoordinated, mild dysphoria	Fair
4	Quiet, coordinated	Good
5	Quiet, well coordinated, stood up smooth and fast	Excellent

Chapter 3. Results

There were no significant differences when comparing the baseline cardiopulmonary measurements (HR, f_R, SAP, DAP, and MAP) between groups A, P and KD ($p > 0.05$).

The mean dose of alfaxalone required to achieve an adequate plane of anesthesia to allow tracheal intubation was 2.0± 0.0 mg kg^{-1} IV. To obtain the same effect, alpacas required administration of a mean dose of 3.3± 0.4 mg kg^{-1} IV of propofol or a mean dose of 4.4± 0.6 mg kg^{-1} IV ketamine in combination with 0.2 ± 0.0 mg kg^{-1} IV diazepam.

Induction quality for all protocols was scored as good to excellent. There was one case of mild excitement and rigidity in one alpaca after administration of the first 25% dose of propofol. This resolved after administration of a second 25% of propofol and intubation was easily performed. There was no rigidity, twitching, or tremors observed during induction with alfaxalone. The time taken from the beginning of drug administration until intubation was not found to be significantly different between groups ($p = 0.83$). These times were 2.1 ± 1.1 minutes, 2.4 ± 1 minutes, and 2.1 ± 0.5 minutes for Groups A, P and KD, respectively.

Duration of anesthesia was defined as the time from the beginning of drug administration (induction) until extubation. Mean duration of anesthesia for groups A, P, and KD was 12 ± 1.9 minutes, 10.9 ± 2.5 minutes, and 10 ± 1.5 minutes, respectively. Although duration was slightly longer in the alfaxalone group, this difference was not statistically significant ($p = 0.146$).

The mean time from induction until standing in the alpacas that received alfaxalone was significantly longer than when they received propofol or ketamine/diazepam (p = 0.017). The mean times were: 34 ± 7 minutes, 19 ± 8.6 minutes, and 24.8 ± 3.7 minutes, respectively. The difference between groups P and KD was not statistically significant.

At minute 1, all alpacas in all groups needed respiratory support due to apnea and hypoventilation except for 1 alpaca from group A and 1 alpaca from group P.

However, at minute 1, the mean PaO_2 for groups A, P, and KD was 51.9 ± 17.2 mmHg (6.9 ± 2.3 kPa) , 50.5 ± 20.4 mmHg (6.7 ± 2.7 kPa), and 50.5 ± 20.4 mmHg (6.7 ± 2.7 kPa), respectively (Table 2). These differences were not significant between groups. Mean PaO_2 at the same time point was significantly different from baseline for all groups (p = 0.01, p = 0.04, and p = 0.04, respectively). All alpacas in all groups had returned to acceptable ventilation level, according to our criteria and to the subsequent arterial blood gas analysis, with or without 100% O_2 and respiratory support, by minute 6.

Similar changes in $PaCO_2$ and SaO_2 were observed at minute 1 in all groups in comparison to baseline (Table 2). $PaCO_2$ was significantly higher than baseline and SaO_2 was significantly lower. At minute 6, however, there were no differences compared to baseline $PaCO_2$ and SaO_2 levels.

Administration of alfaxalone resulted in increased HR at minute 1 compared to the baseline value (p = 0.0001). When comparing the HR of Group A to the rest of the groups, HR at minute 1 was significantly higher than Group KD (p =

0.02) and at minute 6 it was significantly higher than Groups P and KD (p = 0.002). (Table 4) (Figure 1).

SAP and MAP were elevated at minute 1 (Figure 2) after administration of alfaxalone in comparison to the baseline value (p = 0.011, and p = 0.045, respectively). Also, at minute 1, SAP and MAP were elevated in group A in comparison to group P (p = 0.003) and KD (p = 0.03).

Median recovery scores for groups A, P and KD were as follows: 2 ± 3, 4 ± 1, 5 ± 2, respectively (Table 1). Group A had a significantly lower recovery score that the other groups (Table 3). Clinical observation agreed with the statistical results of this analysis. No alpacas had respiratory complications after extubation, such as obstructed airway, or during recovery from any of the three protocols.

Lactate mean values (minute 60) were 2.93 ± 1.2 mmol L^{-1}, 2.25 ± 1.4 mmol L^{-1}, and 1.29 ± 0.8 mmol L^{-1}, for groups A, KD, and P, respectively. Mean lactate values at minute 60 did not show significant differences between groups A and KD. However, mean lactate values for both of these groups at minute 60 were both significantly higher than the values of group P (p = 0.003).

Mean lactate values obtained from Group A was significantly higher than baseline and minutes 1 and 6 (p = 0.0001) (Table 2) (Figure 3). There were only two lactate measurements at minute 11 in Group A because only three alpacas were still intubated at that time point and we were unable to obtain lactate values in one of them due loss of patency of the arterial and venous catheters during sudden seizure-like activity. The values (minute 11) obtained from Group A were 0.7 and 5.66 mmol/L. The former increased to 2.66 mmol L^{-1} and the latter

decreased to 4.27 mmol L^{-1} by minute 60. Both of these alpacas had poor recoveries according to our scoring system. Brief, intense paddling and seizure-like activity followed by 16 minutes of lateral recumbency characterized the former. The alpaca with the higher lactate values had a prolonged period of paddling, twitching and uncontrolled rolling before standing which could be one of the causes of the hyperlactatemia.

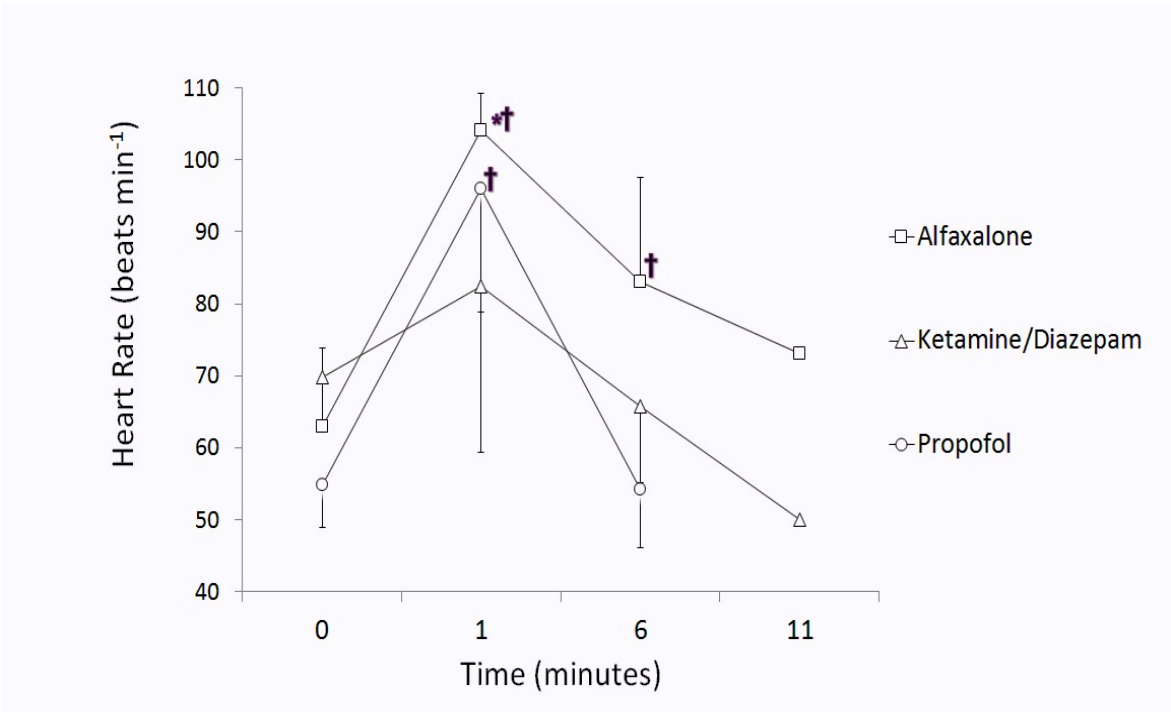

Figure 1. Heart rate (beats minute^{-1}) over time (minutes) in five alpacas anesthetized with titrated intravenous alfaxalone, propofol or a combination of ketamine/diazepam. Data are represented as mean ± SD. Minute 0 (baseline): is considered to be intubation in all groups. *Significant difference ($p < 0.05$) from baseline of the same group. †Significant difference ($p < 0.05$) between groups at the same time.

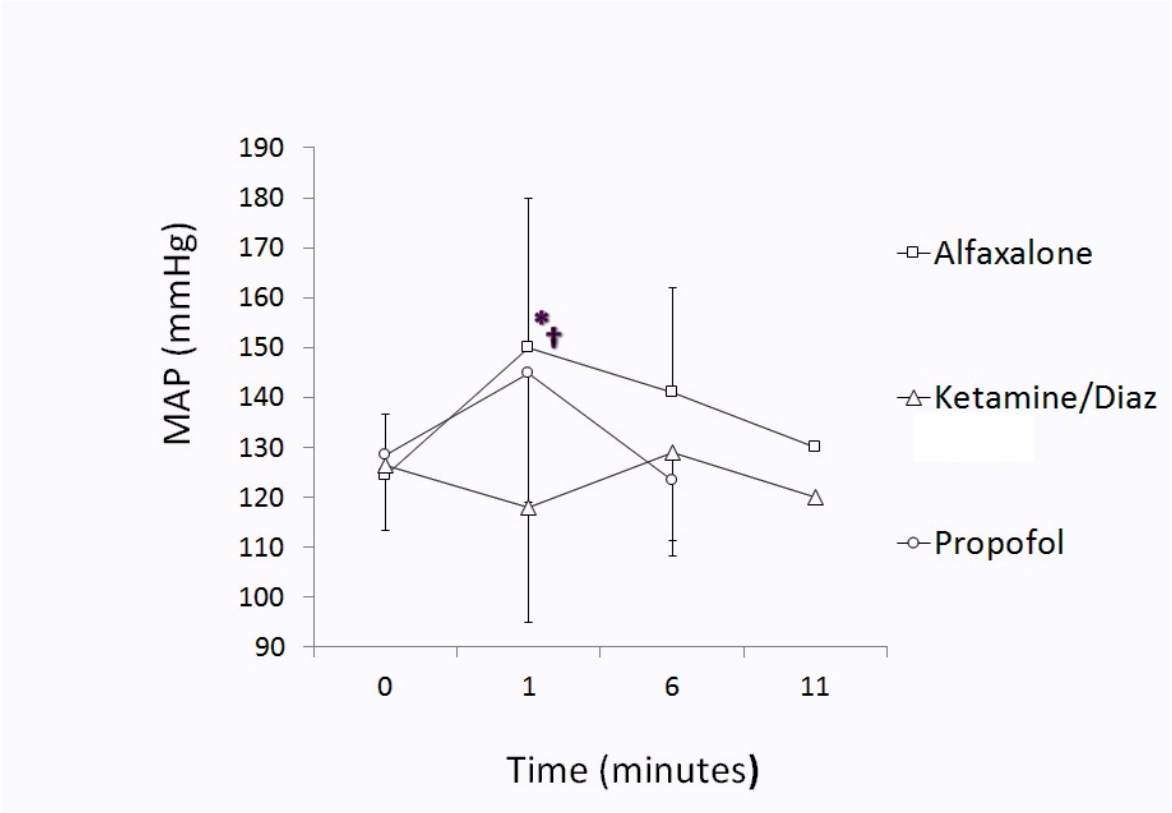

Figure 2. Mean arterial pressure (mmHg) over time (minutes) in five alpacas anesthetized with titrated intravenous alfaxalone, propofol or a combination of ketamine/diazepam. Data are represented as mean ± SD. Minute 0 (baseline): is considered to be intubation in all groups. *Significant difference ($p < 0.05$) from baseline of the same group. †Significant difference ($p < 0.05$) between groups at the same time.

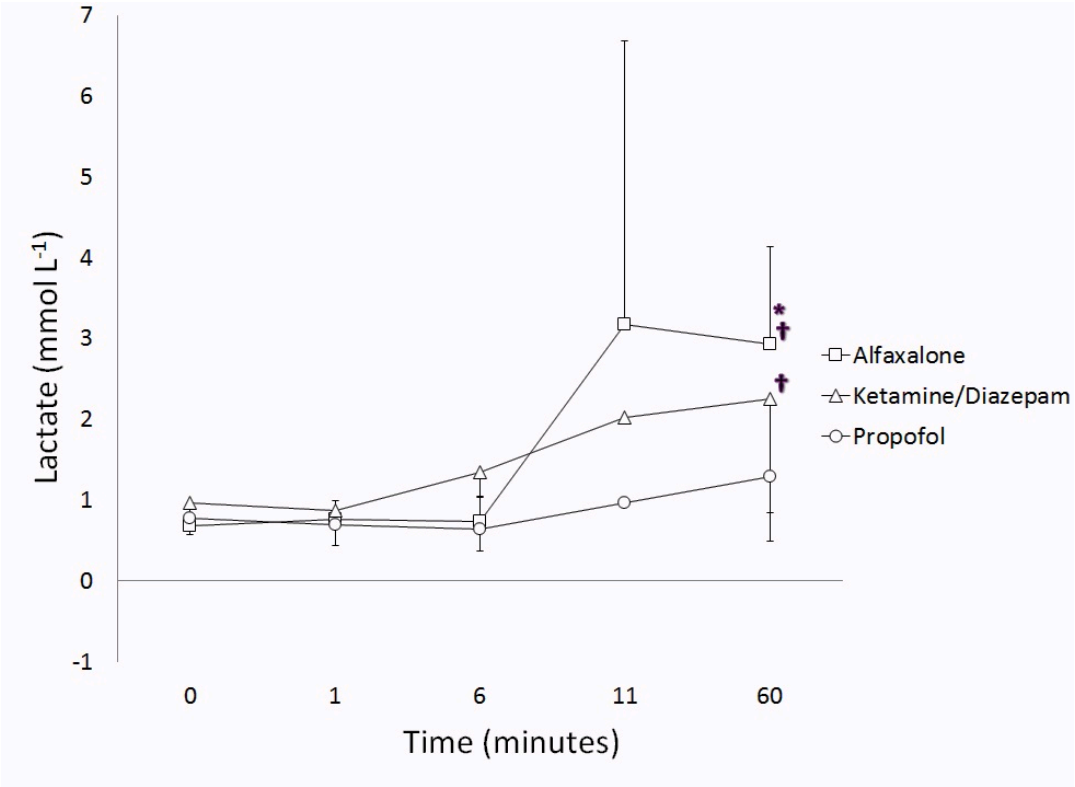

Figure 3. Lactate values (mmol L^{-1}) measured from arterial blood sample over time (minutes) in five alpacas anesthetized with titrated intravenous alfaxalone, propofol or a combination of ketamine/diazepam. Data are represented as mean ± SD. Minute 0 (baseline): is considered to be intubation in all groups. *Significant difference ($p < 0.05$) from baseline of the same group. †Significant difference ($p < 0.05$) between groups at the same time.

Table 2. Blood gas and end tidal carbon dioxide values at baseline (= 0) and at 1, 6, 11 after intubation and 60 minutes after extubation in alpacas receiving alfaxalone (Group A), propofol (Group P) or the combination of ketamine/diazepam (Group KD) for induction of anesthesia.

Values are expressed as mean ± SD. $PaCO_2$, arterial partial pressure of carbon dioxide; $P_E'CO_2$, end-tidal carbon dioxide; SaO_2, arterial hemoglobin saturation with oxygen; HCO_3^-, bicarbonate; BE, base excess; Na^+, Sodium; K^+, potassium; Ca^{++}, ionized calcium; Cl^-, chloride. * Values significantly different from baseline (=0); [a,b,c] Values significantly different between groups at the same time point. $p < 0.05$

	Alfaxalone					Ketamine/diazepam					Propofol				
Time (minutes)	0	1	6	11	60	0	1	6	11	60	0	1	6	11	60
pH	7.46 ± 0.02	7.38 ± 0.04*	7.45 ± 0.05	7.44 ± 0.14	7.44 ± 0.01	7.44 ± 0.05	7.35 ± 0.06*	7.39 ± 0.08	n/a	7.38 ± 0.03	7.45 ± 0.03	7.39 ± 0.04*	7.45 ± 0.02	n/a	7.4 ± 0.04
$PaCO_2$ (mmHg)	39.4 ± 4.1	48.1 ± 3.4*	39 ± 9	40 ± 12	38 ± 2.4	37.3 ± 3	46 ± 1.5*	36.6 ± 6.7	n/a	39.6 ± 7.3	37.5 ± 5.2	45.6 ± 7.1*	38.2 ± 5	n/a	39.3 ± 3.2
$P_E'CO_2$ (mmHg)	n/a	48.3 ± 13	40 ± 13	n/a	n/a	n/a	40 ± 11.2	36.4 ± 7	n/a	n/a	n/a	45.8 ± 12	43.2 ± 10.5	n/a	n/a
PaO_2 (mmHg)	99.6 ± 13.8	51.9 ± 17.2*	294 ± 205.5	78 ± 32.2	82 ± 14	98.7 ± 7	50.5 ± 20.4*	250.8 ± 164.2	n/a	93.4 ± 17	99 ± 15.1	50.5 ± 20.4*	242 ± 168	n/a	96.8 ± 15.5
SaO_2 (%)	99 ± 0.9	88.4 ± 10.5*	99.7 ± 0.3	95 ± 5.6	98.7 ± 1	99.1 ± 0.7	87 ± 17.4*	99.4 ± 0.8	n/a	98.7 ± 1	99 ± 0.8	82.8 ± 10.9*	99.8 ± 0.3	n/a	98.5 ± 1.5
HCO_3^- (mmol L^{-1})	26.96 ± 2.3[a]	27.6 ± 2.2	25.7 ± 3.5	25.65 ± 0.5	24.76 ± 1.2	22.86 ± 5.72[b]	25.12 ± 3.62	23.46 ± 3.74	n/a	22.64 ± 3.7	24.4 ± 3.17[c]	26.72 ± 4.52	28.87 ± 4.27	n/a	23.92 ± 0.71
Na^+ (mmol L^{-1})	151 ± 2.1	151 ± 1.4	150 ± 1.6	151 ± 4.2	151.2 ± 2.2	150.7 ± 1.7	150.3 ± 1	150.7± 1.4	n/a	152.2 ± 4.9	149.9 ± 0.9	149.1 ± 1.7	149.7 ± 1.6	n/a	149.6 ± 2.5
K^+ (mmol L^{-1})	3.6 ± 0.4	3.4 ± 0.3	3.2 ± 0.4	3.2	3.5 ± 0.5	3.6 ± 0.5	3.5 ± 0.5	3.4 ± 0.3	n/a	3.6 ± 0.8	3.7 ± 0.5	3.6 ± 0.2	3.6 ± 4.	n/a	3.8 ± 0.8
Ca^{++} (mg dL^{-1})	4.3 ± 0.28	4.14 ± 0.3	4.02 ± 0.09[a]	3.76 ± 0.5	4.12 ± 0.35	4.1 ± 0.3	4.1 ± 0.4	4.1 ± 0.26[a]	n/a	4.06 ± 0.6	4.32 ± 0.37	4.26 ± 0.35	4.42 ± 0.25[b]	n/a	4.35 ± 0.27
Glucose (mmol L^{-1})	136 ± 13	133 ± 11	138 ± 19	146 ± 21	148 ± 15	143 ± 28	144 ± 29	145 ± 26	n/a	146 ± 25	156 ± 33	155 ± 26	174 ± 28*	n/a	177 ± 25*
Lactate (mmol L^{-1})	0.68 ± 0.28	0.76 ± 0.23	0.74 ± 0.31	3.1 ± 3.5*	2.9 ± 1.2*[a]	0.95 ± 0.34	0.86 ± 0.12	1.35 ± 0.61	n/a	2.25 ± 1.4*[a]	0.77 ± 0.23	0.69 ± 0.25	0.64 ± 0.27	n/a	1.29 ± 0.8[b]

Table 3. Recovery score, time, and observations in the alpacas that received alfaxalone (Group A). Recovery time was considered to be the time elapsed from extubation until alpacas were standing unassisted.

Recovery score: excellent=5; good=4; fair=3; poor= 2; very poor=1.

Alpaca	**Recovery time**	**Score (1 to 5)**	**Observations**	**Ataxia when standing**
1	17 minutes	3	Lateral recumbency, tremors, and rigidity.	Yes
2	30 minutes	2	Dysphoria, rolling several times, tremors, incoordination	Yes - Mild
3	18 minutes	1	Dysphoric, paddling in lateral recumbency, violent thrashing, seizure-like activity	No
4	20 minutes	2	Dysphoric, paddling in lateral recumbency, short seizure –like activity. Laid in lateral recumbency for 16 minutes.	No
5	16 minutes	4	Uncoordinated. Stood up and fell. Stood on 2nd attempt after 10 minutes.	Yes

Table 4. Cardiovascular parameters and respiratory rate (f_R) measured at baseline (= 0) and at 1, 6, 11 minutes after intubation in alpacas receiving alfaxalone (Group A), propofol (Group P) or the combination of ketamine/diazepam (Group KD) for induction of anesthesia.

Values are expressed in means ± SD. HR, heart rate; f_R, respiratory rate; SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure; MAP, mean arterial blood pressure. * Values significantly different from baseline (=0); [a,b,c] Values significantly different between groups at the same time point. $p < 0.05$

	Alfaxalone			**Ketamine/diazepam**			**Propofol**		
Time (minutes)	0	1	6	0	1	6	0	1	6
HR (beats minute^{-1})	63 ± 11	104 ± 5*[a]	83 ± 15*[a]	70 ± 6	82 ± 23[b]	66 ± 11	55 ± 6	96 ± 17	54 ± 8
f_R (breaths minute^{-1})	23 ± 13	11 ± 8	11 ± 4	26 ± 8	21 ± 6	21 ± 6	24 ± 6	12 ± 6	15 ± 5
SAP (mmHg)	161 ± 9	211 ± 52*[a]	190 ± 27	156 ± 18	143 ± 27[b]	157 ± 25	160 ± 2	176 ± 39	169 ± 32
DAP (mmHg)	108 ± 18	121 ± 42	113 ± 25	102 ± 13	97 ± 28	109 ± 17	102 ± 8	114 ± 21	105 ± 9
MAP (mmHg)	124 ± 12	150 ± 30*	141 ± 21	126 ± 13	118 ± 23[b]	129 ± 21	128 ± 3	145 ± 25	123 ± 12

Chapter 4. Discussion

4.1 Alfaxalone dose

In this study, we found that the mean dose of alfaxalone necessary to induce general anesthesia and allow intubation in non-sedated alpacas was 2.1 mg Kg^{-1} intravenously (IV). Alfaxalone can also be administered intramuscularly (IM) and, although we did not evaluate this route of administration, it is reasonable to postulate that the dose needed to achieve the same effect would be slightly higher than 2 mg Kg^{-1}.

The only ruminants in which alfaxalone has been investigated to date are sheep. Andaluz et al. (2012), Moll et al. (2013), and Torres et al. (2012) reported that 2 mg Kg^{-1} IV administered over 120 seconds achieved rapid loss of consciousness in sheep. Walsh et al. (2012) used 2 mg Kg^{-1} IV of alfaxalone in combination with medetomidine at 2 ug Kg^{-1} IV to induce anesthesia and all sheep needed to be given additional drugs to achieve intubation.

The dose used in this study is not only consistent with the dose of alfaxalone used in reports in ruminant species but with other non-ruminant species such as dogs (1.9-2.6 mg Kg^{-1}) (Muir et al. 2008; Pasloske et al. 2005; Jiménez et al. 2012; Maney et al. 2013) and rabbits (2-3 mg Kg^{-1}) (Grint et al. 208). In horses and ponies, all previous investigations have included drugs for premedication, such as butorphanol and xylazine or romifidine and guaifenesin or diazepam for induction of anesthesia in combination with alfaxalone (Leece et al. 2009; Goodwin et al. 2011 and 2012; Klöppel & Leece 2011; Keates et al. 2012). In all these studies, the dose of alfaxalone used was 1 mg Kg^{-1} IV. Clinically, it is rare to induce general

anesthesia in a horse with just one drug, which is probably why the dose of alfaxalone administered alone to un-premedicated horses is yet to be determined.

4.2 Ketamine/diazepam and propofol dose

In this study, mean induction dose for ketamine and diazepam used was 4.4 mg Kg^{-1} IV and 0.2 mg Kg^{-1} IV, respectively. Most of the publications in South American camelids suggest using ketamine for induction in combination with a muscle relaxant, such as diazepam or guaifenesin, or with an alpha-2 adrenergic agonist, such as xylazine (Riebold et al. 1989; Mama 2000; García-Pereira et al. 2006). A mixture of equal volumes of ketamine (100 mg ml^{-1}) and diazepam (5 mg ml^{-1}) can be administered intravenously at a dose of 3.5 mg Kg^{-1} and 0.17 mg Kg^{-1}, respectively, to produce a graceful transition to sternal recumbency approximately 1 minute after administration (Abrahamsen 2009). As was expected, the doses used in this study were very similar.

Mean induction dose for propofol in our alpacas was 3.33 ± 0.41 mg Kg^{-1} IV. To our knowledge, there is only one other research publication in New World camelids using propofol in which the authors investigated the cardiopulmonary effects of two different rates of propofol infusion over 1 hour in healthy llamas (Duke et al. 1997). In this study, un-premedicated llamas were induced with a dose of 2 mg Kg^{-1} but they were not intubated, which eliminates the stimulatory effect of an endotracheal tube passing through the laryngeal area. It has been suggested (personal communication) that alpacas usually require 10-20% higher doses of sedative drugs in comparison to llamas, possibly due to the nature of their temperament and larger total body surface area. Furthermore, Fowler in 1998 published that it has been suggested that alpacas require 10-15% higher dose of

xylazine than llamas. This, and the fact that laryngeal manipulation during intubation is very stimulatory, may explain how Duke et al. (1997) were able to induce their llamas with such a low dose of propofol.

Also, in this same report, they considered the quality of anesthesia to be smooth and reliable and they state that 2 mg Kg^{-1} might have been the minimal induction dose of propofol needed for this species because 2 out of 5 llamas in their study momentarily paddled their limbs and the rest appeared "lightly" anesthetized.

The only other published data on propofol in camelids is in camels (Fahmy et al. 1995). They observed a smooth induction, maintenance and good recovery from anesthesia after administration of xylazine, diazepam and 2 mg Kg^{-1} of propofol (IV).

We recommend that at least 3.3 mg Kg^{-1} IV of propofol needs to be used to induce general anesthesia and intubate unsedated and unpremedicated alpacas.

4.3 Quality of induction

Alfaxalone is known to produce smooth inductions, but can sometimes be associated with muscle tremors, paddling and twitching, even in animals premedicated with other sedatives or muscle relaxants (Walsh et al. 2012). Most of the studies conducted in horses (Leece et al. 2009; Goodwin et al. 2011) and dogs (Muir et al. 2008) found good to excellent inductions with alfaxalone (Leece et al. 2009; Goodwin et al. 2011) characterized by sinking smoothly to the ground (Klöppel&Leece 2011). There was no rigidity, twitching, or tremors observed in any of the inductions in which alfaxalone was administered in our alpacas.

Alfaxalone did have a higher incidence of muscle tremors in horses in one study after administration of intravenous xylazine at 0.5 mg Kg^{-1}, guaifenesin at 35 mg

Kg^{-1}, and alfaxalone at 1 mg Kg^{-1} (Keates et al. 2012) and in another study in sheep (Walsh et al. 2012).

Quality of induction in our study was scored to be good/excellent in all groups. We had only one case of mild excitement or rigidity in one alpaca after administration of the first 25% (0.75 mg Kg^{-1}) of the dose of propofol. As mentioned before, induction of llamas with propofol has shown some excitatory effects, such as paddling with their limbs (Duke et al. 1997), but these effects were not seen in premedicated camels (Fahmy et al. 1995). Since the mild excitement and rigidity in our study resolved after immediate administration of a second 25% of propofol, we postulate that, if the rate of administration of propofol would have been faster, none of the alpacas in the study would have had any undesired side effects from the induction drugs.

There have been previous reports of the lipid macroemulsion formulation of propofol used in this study causing muscle twitching, paddling, opisthotonus, panting and pain on intravenous injection in dogs. In the literature, ketamine and alfaxalone have not been associated with these excitatory phenomena. In one recent study, it was found that the administration of the lipid macroemulsion of propofol caused moderate to severe pain at injection in dogs but alfaxalone did not (Michou et al. 2012). It is possible that our alpacas felt slight to moderate pain after the first injection of propofol and reacted to it, before further injections of propofol could induce hypnosis.

4.4 Quality of intubation

Quality of conditions for intubation in our study, similarly to quality of induction, was considered to be excellent in all groups. The same researcher, with experience

in intubating camelids, intubated all alpacas. In the study in sheep by Walsh et al (2012), they assessed quality of intubation between veterinary students and experienced anesthetists. Intubation scores between the group intubated by veterinary students and the group intubated by experienced anesthetists were not significantly different. This implies that successful intubation is consistent and not difficult for inexperienced anesthetists.

Both sheep and alpacas have a relatively elongated and narrow intermandibular space, limited ability to open their mouth, larynxes located far from the lips (Byers et al. 2009), and are prone to regurgitation and excessive salivation during induction and intubation. Due to this, it is possible to compare the quality of intubation between these two different species. We believe that the person assisting by extending the head and holding the mouth open during intubation is crucial for an easy and correct placement of the endotracheal tube in any species. In our study, the person that was holding the head was the same for all alpacas and was also an experienced anesthetist. This could have contributed to the excellent quality of intubation observed in all of our groups, however, we also believe that these different drugs may have equal ability to induce hypnosis and laryngeal relaxation for intubation.

4.5 Respiratory effects

Respiratory depression has been reported after the administration of alfaxalone in other species (Grint et al 2008; Muir et al. 2009; Maney et al. 2013).

All animals in our study received 100% O_2 through a semi-closed circle anesthesia machine for respiratory support, except for 2 alpacas: one in the alfaxalone group and one in the propofol group (2/15 =13.33%). A $P_E'CO_2$ of 60 mmHg was chosen

as the cutoff to support ventilation. This threshold, which is significantly higher than normal ($P_E'CO_2$ between 35-45 mmHg) (Bedenice 2009), was chosen because we expected some degree of respiratory depression and we considered our alpacas to be sufficiently healthy to withstand a brief period of moderate hypercapnia. Since one of our objectives was to evaluate the respiratory effects of the three treatments we tried to assist their ventilation as little as possible. Alpacas in our study showed apnea, shallow breathing or low respiratory rate in response to the 3 induction protocols. Hypoventilation, caused by low respiratory rate after intubation, was the most common finding in all induction protocols.

Arterial blood samples were not analyzed immediately after extraction. They were iced and analyzed approximately an hour after extraction. Because of this, we did not notice that we had misjudged the need for respiratory support in some of the alpacas.

$PaCO_2$ at min 1 in all three groups was very similar and mildly above the high end of normal parameters (35-45 mmHg), ranging from 45.5 ± 7.1 mmHg to 48.1 ± 3.4 mmHg. In a similar manner, $P_E'CO_2$, at the same time point, was also very similar between groups and on the upper end of the normal range. Arterial carbon dioxide partial pressure is usually considered a good indicator of adequacy of ventilation in all species. In this case, even though our clinical judgment led us to support ventilation in most of the alpacas in this study, both P_aCO_2 and $P_E'CO_2$ indicated that this may not have been necessary.

At minute 1 after intubation, mean PaO_2 for groups A, P, and KD was 51.88 ± 17.2 mmHg, 50.46 ± 20.4 mmHg, and 55.06 ± 22.4 mmHg, which was considered to be hypoxemia (Tranquilli et al. 2009) and was significantly lower than baseline. However, cyanosis wasn't noted after any of the induction protocols. Cyanosis is

the blue/purple appearance of the mucous membranes or non-pigmented skin that develops when there is at least 5 g/dl of deoxyhemoglobin in the blood. Since alpacas have higher affinity for oxygen than other non high-altitude mammals, this can explain why cyanosis wasn't observed at such low PaO_2.

There are five causes for hypoxemia: low F_iO_2, hypoventilation, diffusion impairment, V/Q mismatch and vascular shunts. Diffusion impairment of gases across the alveolar capillary membrane can be a cause of decreased PaO_2 and normal or close to normal $PaCO_2$ (Bedenice 2009) but this cause was unlikely in our healthy alpacas. Studies in sheep have shown that hypoxemia occurs after administration of alpha-2 agonists because of activation of pulmonary intravascular macrophages (PMI's) promoting lung vascular and tissue injury with subsequent edema formation, congestion, extravasation of red blood cells, and increase in pulmonary arterial pressure (Aharonson-Raz & Singh 2010). To our knowledge, there are no reports of alfaxalone, propofol or ketamine/diazepam promoting this same effect. Furthermore, there are no reports on the role of PMI's in llamas or alpacas. However, we did not obtain lung biopsies to definitively rule out this cause. In healthy animals, V/Q mismatch or shunting can be due to body positioning or increased intraabdominal pressure due to tympany. Our alpacas were kept in sternal position and had no signs of abdominal distension.

Apnea and decreased respiratory rate has been described after administration of propofol in many species (Schumacher et al. 1997; Sams et al. 2008; Martínez Taboada & Murison 2012). In sheep anesthetized with 2 mg Kg^{-1} of alfaxalone minimal respiratory depression and no apnea was noted (Andaluz et al. 2012). Prassinos et al. (2005) reported that PaO_2 was minimal at the time of connection to 100% O_2 but cyanosis was not noted in goats anesthetized with either propofol (3

mg Kg^{-1}) or intravenous ketamine (10 mg Kg^{-1}). At this time, both groups exhibited PaO_2 of 43.3 mmHg and 54.3 mmHg, respectively, which is similar to our study. In another study where sheep received either propofol (3 mg Kg^{-1}) or xylazine-ketamine (0.11 mg Kg^{-1} and 2.2 mg Kg^{-1}, respectively) as induction agents, it was seen that propofol significantly reduced respiratory rate and oxygen tensions for a longer period of time (up to 45 min) compared to xylazine-ketamine (Lin et al. 1997).

Due to the lack of consistency in the F_iO_2 used throughout the study, we were not able to draw conclusions from the PaO_2 measured after minute 1. At recovery, 60 minutes after induction, all animals had normal PaO_2 and $PaCO_2$. This indicates that whatever the cause of the initial hypoxemia, it had resolved within one hour.

4.6 Cardiovascular effects

In this study, heart rate was elevated after induction (minute 1) in all groups (Figure 1).

This change is somewhat expected in the ketamine/diazepam group, in which despite an increase in HR at minute 1, arterial blood pressure decreased and subsequently increased slightly (Figure 2). Induction of anesthesia with ketamine generally produces cardiovascular stimulation, with an increase in HR, cardiac output and arterial blood pressure, with a greater depression in myocardial contractility compared to propofol or etomidate (Kawakubo et al. 1999). Mild myocardial depression and not a high enough compensatory heart rate could be related to the decrease in SAP and MAP observed in the alpacas in our study. However, a larger number of animals in our study could have yielded different results and further research in this area is warranted.

As for alfaxalone, in dogs (Muir et al. 2008) 2 mg Kg^{-1} IV has been noted to produce a transient increase in HR and a decrease in systemic vascular resistance, which resulted in minimally affected arterial blood pressure and cardiac output. In cats (Muir et al. 2009), at a dose of 5 mg Kg^{-1} IV of alfaxalone, the cardiovascular changes were even less pronounced.

However, sheep anesthetized with alfaxalone at 2 mg Kg^{-1} IV had a significant increase in HR, as seen in our study, but this was attributed to a change in position from sternal to lateral recumbency (Andaluz et al. 2012) which did not happen in the alpacas in our study. In rats, a bolus of alfaxalone was correlated to slight elevations of serum noradrenaline (Horishita et al. 2002). Noradrenaline was not measured in our study, therefore, we cannot conclude that the cardiovascular changes we reported were caused by this.

Laryngoscopy and tracheal intubation can lead to adrenergic stimulation and an increase in circulatory catecholamines, which act by increasing heart rate and arterial blood pressure, amongst other functions (Hong et al. 2011). Although induction conditions had been excellent and intubation was performed with ease in all of our alpacas, this minimal laryngeal manipulation could have transiently stimulated the sympathetic system of our un-premedicated alpacas. In both the alfaxalone and propofol groups, HR, SAP, and MAP had a tendency to return to lower levels during the subsequent minutes after intubation, possibly reflecting a decline in catecholamine release.

4.7 Duration of anesthesia

Since the starting point of loss of consciousness is sometimes difficult to assess, to be consistent we chose the duration of anesthesia to be defined as the time from the

beginning of drug administration until extubation. Other studies consider duration of anesthesia the time from intubation to extubation. Intubation of alpacas is not always easy due anatomical characteristics, which is why we expected the intubation process to be longer in some of our animals. If this had been the case, the animals would have been unconscious and likely anesthetized during the time it had taken to place an endotracheal tube, which should have been considered part of the duration of anesthesia. In our study, however, we found that there were no significant differences for the time to achieve intubation between the three treatment groups.

Both, duration of anesthesia (induction until extubation) and time from intubation to extubation, showed no statistically significant differences between the three groups. Despite this, the duration of alfaxalone anesthesia (12 ± 1.9 minutes) was slightly longer than the durations of groups P and KD.

In previous dog studies, it has been reported that a similar dose of alfaxalone without premedication, such as in our case, yielded 9.8 ± 2.4 min (Muir et al. 2008), 6.4 ± 2.9 minutes (Ferre et al. 2006) and 11 ± 7 minutes (Maney et al. 2013) from intubation until extubation. In sheep (Andaluz et al. 2012), duration of anesthesia after 2 mg Kg^{-1} of alfaxalone was 6.4 ± 3.6 minutes. Rabbits anesthetized with 3.3 mg $Kg^{-1}$of alfaxalone regained a pedal withdrawal reflex after 8.9 minutes (Gil et al. 2012).

The somewhat longer duration in our alfaxalone group could be attributed to two factors: (a) we included the time it took to induce and intubate in our statistical analysis and (b) dogs and alpacas exhibit different signs for extubation. Dogs and cats are usually extubated when they are swallowing whereas camelids and sheep should be extubated when they are chewing, coughing (Riebold et al. 1994), and

actively trying to expel the endotracheal tube to decrease the chance of post-extubation airway obstruction.

Muir et al. (2009) did report a time of 26 ± 10.7 minutes from intubation to extubation in cats after 5 mg Kg^{-1} IV of alfaxalone. Thus, duration of action of alfaxalone in cats without noxious stimulation seems to be much longer than most of the other species, as was corroborated by Whittem et al. (2008) in their pharmacokinetic study in cats in which they saw that the time to head lift after 5 mg Kg^{-1} IV of alfaxalone was 45 minutes.

4.8 Recovery from anesthesia

Time from the beginning of induction until standing unassisted was longer for group A compared to groups P and KD (34 ± 7 min, 19 ± 8.6 min, and 24.8 ± 3.7 min, respectively). Recovery after administration of either propofol or ketamine/diazepam was rapid and uneventful, as has been previously reported in camelids, with or without premedication, probably due to their temperament (Riebold et al. 1989; Abrahamsen 2009). All the alpacas in this study remained in sternal position for a few minutes and, after holding their own head up with slight swinging from side to side, they smoothly stood up. While being lead back to their stalls, they were slightly ataxic but fully capable of walking at a normal speed. Recovery in camelids is also uneventful when mask induction with inhalants, such as isoflurane, is performed (Mama et al. 1999). Mean recovery time in llamas after an infusion of 0.2 mg Kg^{-1} min^{-1} of propofol was 11 minutes (Duke et al. 1997). Camels, after administration of xylazine (0.25 mg kg^{-1}), diazepam (0.25 mg Kg^{-1}), and propofol (2 mg Kg^{-1}), were standing by 15.1 ± 1.93 minutes (Fahmy et al. 1995). Both reports had remarkably good recoveries: smooth and free of ataxia.

Recovery after administration of alfaxalone in our alpacas was statistically and clinically significantly worse compared to groups P and KD. Median recovery score assigned to group A was 2 ± 1 out of 5 points, which was considered to be poor (Table 1). All of our alpacas exhibited some sort of abnormal behavior around the time of extubation such as tremors, hind limb rigidity, twitching, paddling wildly in lateral recumbency, rolling over, seizure-like activity, prolonged recovery (compared to groups P and KD), and ataxia once standing (Table 3). We did not measure temperature during anesthesia in our alpacas, so we cannot exclude hypothermia as a cause for the tremors observed, although we believe it is an unlikely contributor.

Although most reports in other species state that alfaxalone produces smooth recoveries, when compared to other induction agents, such as propofol, quality of recovery is worse. Walsh et al (2012) reported some excitatory effects, mainly muscle twitching after recovery from medetomidine and alfaxalone induction in sheep. Mathis et al. (2012) investigated the quality of recovery in ninety-three cats after premedication with meloxicam, acepromazine and buprenorphine, and induction with either propofol or alfaxalone to effect. These cats were maintained on isoflurane for a short surgical procedure. The mean duration of anesthesia for the alfaxalone group was 20 minutes. According to previous studies (Muir et al. 2009), 20 minutes is less than the duration of action of alfaxalone in cats. In this study one cat had a seizure and they saw more frequent paddling and tremors in the alfaxalone group. However, they concluded that alfaxalone was safe enough to use for short procedures in cats. Also, Jiménez et al. (2012) reported worse recoveries in dogs premedicated with methadone and induced with alfaxalone in comparison to propofol but, nonetheless, overall recovery quality was considered to be good.

In our alpacas, we considered that the recoveries observed were clinically undesirable. Two out of five alpacas exhibited seizure-like activity and thrashed wildly enough that they could not be restrained without exposing personnel to harm. None of the alpacas or personnel sustained any injuries from their uncontrolled recoveries partly because the study was conducted in a padded large animal recovery stall. It is possible that premedication and maintenance with an inhalant agent could attenuate this effect and perhaps future studies should be aimed at investigating that theory.

4.9 Lactate measurements

Lactate is the end product of anaerobic metabolism and is a good indicator of tissue perfusion. Hyperlactatemia can result from hypoxemia, anemia, cytokine inhibition of pyruvate dehydrogenase, hypermetabolic states, relative thiamine deficiency, decreased clearance of lactate by the liver or increased production of lactate (Bedenice 2009). Lactate measurements in our study were positively correlated to worse recovery quality but not to hypoxemia. At minute 1, although PaO_2 was decreased in all three groups, lactate values were still below 1.4 mmol L^{-1} (minutes 1 and 6). Based on this information, we postulate that the increase in lactate values was not related to the short period of hypoxemia experienced by all alpacas immediately after induction.

The highest lactate values were noted in those alpacas with the worst observed recoveries, possibly due to the sudden increase in muscle activity and tissue O_2 demand. We were unable to obtain a blood gas sample from alpaca number 3 (Table 3) due to loss of the venous and the arterial catheters during the thrashing

but we assume that the lactate value would have been significantly elevated as well.

4.10 Limitations and thoughts for future studies

We recognize that the biggest limitation in this study is the small sample size of alpacas, which is why we suggest interpreting our results with care. Due to the number of alpacas available at the time of the data collection and the fact that one alpaca was excluded because we were unable to place a jugular catheter on 3 different occasions without sedation, we could only use five animals.

Although our goal was to evaluate the effects of alfaxalone in non-premedicated and non-stimulated alpacas and compare these effects to propofol and ketamine/diazepam, it would have been interesting to include noxious stimulation to simulate most clinical situations. Thus, our results are more relevant for short diagnostic procedures in which these animals would need to be under general anesthesia, such as for ECG-gated computed tomography scans (CT).

As to the cardiovascular parameters measured in this study, we cannot draw conclusions as to how alfaxalone affected cardiac output, systemic vascular resistance or pulmonary pressures. Our results are based on parameters that are routinely obtained in a normal clinical setting, such as heart rate and invasive arterial blood pressure.

Further investigation as to the cause of the hyperlactatemia observed in our study is warranted in this species and could include the determination of tissue perfusion and oxygen delivery to tissues using cardiac output measurement methods, such as lithium dilution techniques.

Assessment of the degree of regurgitation and salivation was not one of the objectives of this study but we can say that, clinically, there appeared to be no differences regarding regurgitation or salivation between the three groups. All alpacas seemed to salivate profusely and have a small amount of ruminal contents present on the outer surface of their endotracheal tubes upon extubation.

Chapter 5. Conclusions

In conclusion, induction of anesthesia was smooth and intubation conditions were good after administration of titrated alfaxalone, propofol or ketamine/diazepam in healthy un-premedicated alpacas. Mild respiratory depression and a mild increase in heart rate were observed after induction with any of the three protocols. Duration of anesthesia was slightly longer after alfaxalone in comparison to propofol or ketamine/diazepam. Recovery from alfaxalone was statistically and clinically significantly worse than recovery from propofol or ketamine/diazepam.

Based on the results of this study, at this time alfaxalone used alone without premedication cannot be recommended for induction of anesthesia in healthy alpacas.

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