Clinical effect of a constant rate infusion of alfaxalone in isoflurane-anesthetized goats undergoing an experimental procedure: a pilot study

Klinisch effect van een infusie van alfaxalone bij geiten tijdens experimenteel onderzoek onder anesthesie met isofluraan: een pilootstudie

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ABSTRACT

Alfaxalone is a synthetic neurosteroid anesthetic agent widely used in veterinary medicine, with a wide margin of safety and good quality of anesthesia. Also, alfaxalone has rapid biotransformation and a low tendency to accumulate in the tissues after repeated doses, which favors its intravenous use as a constant rate infusion. The aim of the study was to assess the isoflurane-sparing property and the clinical effects on the cardiorespiratory system of alfaxalone used as constant rate infusion in goats. Three healthy female goats were included in the study. Each goat was anesthetized twice (interval fifteen days) and received the following treatments in a random order during maintenance of anesthesia: 1. alfaxalone administered as a constant rate infusion at 0.05 mg/kg/min (treatment A); 2. NaCl 0.9% solution at an identical infusion rate (treatment B). Isoflurane vaporizer settings were adjusted according to a flow-chart. The SpO_2 was significantly lower during treatment B than during treatment A. Although no significant differences were demonstrated for the other variables (heart rate, etc.), a clinical effect was noticed, including a modest decrease in the expired isoflurane concentration with treatment A. In conclusion, the co-administration of alfaxalone in isoflurane-anesthetized goats seems to result in only minimal side effects on cardiorespiratory parameters and may reduce the isoflurane requirements, but further studies are needed to confirm these results.

SAMENVATTING

Alfaxalone is een synthetisch neurosteroïde anestheticum dat veel wordt gebruikt in de diergeneeskunde en een goede kwaliteit van anesthesie biedt. Het heeft een snelle biotransformatie en accumuleert weinig in de weefsels na herhaalde dosering, waardoor het geschikt is als continu infuus voor het onderhoud van de anesthesie. Het doel van de studie was om de isofluraansparende eigenschap van alfaxalone toegediend als 'constante snelheid infusie' voor het onderhoud van de anesthesie bij geiten en de klinische effecten ervan op het cardiorespiratoire systeem te beoordelen. Drie gezonde, vrouwelijke geiten werden opgenomen in de studie en tweemaal onder anesthesie gebracht (met een interval van vijftien dagen). Bij elke anesthesie werd een van de volgende behandelingen toegepast (willekeurige volgorde): 1. alfaxalone-infuus aan 0,05 mg/kg/min (behandeling A); 2. 0,9% NaCl-oplossing aan eenzelfde infusiesnelheid (behandeling B). De instellingen van de isofluraanverdamper werden aangepast volgens een stroomdiagram. De SpO_2 was significant lager tijdens behandeling B dan tijdens behandeling A. Hoewel er geen significante verschillen werden aangetoond in de andere variabelen, i. e. hartfrequentie, etc., werd een klinisch effect opgemerkt, waaronder een matige daling in de vereiste dosis isofluraan. De toediening van alfaxalone bleek bij geiten onder isofluraananesthesie minimale cardiorespiratoire veranderingen te veroorzaken en mogelijk een klinisch isofluraansparend effect te hebben. Verder onderzoek is echter nodig om deze resultaten te bevestigen

INTRODUCTION

The term balanced anesthesia is mostly used for a combination of anesthetics, analgesics and adjuvants to achieve analgesia, hypnosis, muscle relaxation, amnesia, maintenance of normal homeostasis and reduction or elimination of autonomic reflexes (Tonner 2005). By combining different agents and techniques, a synergistic effect may be achieved, which helps to reduce the likelihood of side effects during the maintenance of the anesthesia.

Alfaxalone is a synthetic neuroactive steroid anesthetic agent with a wide margin of safety and provides good quality of muscle relaxation, calmness during induction and an uneventful recovery, with minor clinically significant adverse cardiorespiratory effects in sheep and goats (Andaluz et al., 2012; Dzikiti et al., 2014). Previous studies in dogs (Ferré et al., 2006) and cats (Whittem et al., 2008) have demonstrated a dose-dependent systemic exposure with a rapid clearance, short terminal elimination half-life and lack of accumulation after maintenance with repeated boluses (2 and 10 mg/kg for dogs; 5 mg/kg followed by four doses of 2 mg/kg in cats). In a recent study by Dehuisser et al. (2019a and 2019b), cardiovascular function and pharmacokinetic parameters were shown to be stable during continuous administration of alfaxalone in dogs (Dehuisser et al., 2019a, 2019b). Therefore, all the aforementioned properties favor the use of alfaxalone as a constant rate infusion (CRI).

In goats, a minimum infusion rate of 0.16 mg/kg/min is needed to maintain total intravenous anesthesia, with minimal cardiorespiratory effects and a smooth recovery (Ndawana et al., 2014). Similarly, Moll et al., (2013) showed that the same rate infusion of alfaxalone in unpremedicated sheep maintained clinically acceptable hemodynamic stability, with mild respiratory depression. The use in combination with co-adjuvants midazolam or fentanyl has shown an eight-fold reduction for the alfaxalone constant rate infusion in goats (Dzikiti et al., 2015; Dzikiti et al., 2016). Furthermore, in desflurane-anesthetized sheep, a low alfaxalone CRI rate (0.07 mg/kg/min) reduces the desflurane requirements to maintain a suitable anesthetic depth (Granados et al., 2012).

The aim of the present study was to assess the clinical effects of a CRI of alfaxalone on the cardiorespiratory system and the isoflurane-sparing effects in goats.

MATERIAL AND METHODS

Study design

The study was approved (EC 2013/76) by the ethical committee of the Faculties of Veterinary Medicine and Bioscience Engineering of Ghent University. A prospective, crossover, randomized, blinded pilot study was designed.

Three female, adult goats with a weight of 50 ± 13 kg (mean ± standard deviation) (range 36 – 62), that had already been included in a trial on subcutaneous implantation of a wireless glucometer, were included in the study. The goats were considered healthy on the basis of a physical examination. The goats were housed indoors and fed with a standard commercial diet. The animals were fasted for 12 to 18 hours and deprived of water for 8 to12 hours prior to the experiment, in order to reduce the likelihood of complications associated with recumbency and anesthesia. On the morning of the trials, the physical status of the goats was re-evaluated.

An intravenous (IV) 14 gauge over-the-needle catheter (Optiva 2, Smiths Medical International, UK) was placed aseptically into the left jugular vein. Premedication was performed with IV administration of midazolam (0.3 mg/kg Dormicum, Roche, Belgium) in combination with morphine (0.1 mg/kg Morphine HCl; Oterop, Belgium). Following sedation, the goats were preoxygenated for three minutes by direct flow of 4 L/min of 100% oxygen using a mask. Anesthesia was then induced slowly with IV alfaxalone until endotracheal intubation was possible. After intubation, the goats were placed in lateral recumbency on a smooth, flat and padded surface. The head was positioned so that the salivary secretions and gastric contents, if regurgitated only, could drain from the mouth and not wick between the animal's head and the pad, so that contact with the eyes was avoided. A circulating warm-water heating blanket was used to prevent hypothermia. Anesthesia was maintained with isoflurane (Isoflo, Abbott Laboratories, UK) in a mixture of oxygen and air (70%) delivered through a rebreathing circle system. The animals were allowed to breathe spontaneously. Ringer's lactate solution was administered IV at a rate of 5 mLkg/h.

All goats were anesthetized twice (for SC implantation of the glucometer and the subsequent removal of the device), with a minimum resting period of fifteen days between the two sessions. During anesthesia, one of two treatments was administered. The treatments were allocated in a randomized order, which was determined by lottery (extracting a code from a sealed envelope). Treatment A consisted of the administration of alfaxalone (Alfaxan 10 mg/mL, Vetoquinol, France) as a constant rate infusion (CRI) at 0.05 mg/kg/min; treatment B included the administration of a NaCl 0.9 %-solution (Baxter, Belgium) at an identical infusion rate.

Monitoring included electrocardiography (ECG) and heart rate (HR), pulse oximetry (SpO_2), blood pressure (BP) measurement (invasive method by a 20-gauge over-the-needle catheter placed in the major auricular artery; oscillometric method using a cuff placed on the thoracic limb), capnography ($ETCO_2$), respiratory rate (f_R) and inspiratory and expiratory isoflurane concentrations FI´ISO and FE′ISO, respectively (Datex-Ohmeda Monitor, Datex-Ohmeda, Finland). The anesthetic depth (isoflurane setting) was

adjusted according to a flow-chart (Figure 1). Recovery was performed on a padded surface in a recovery room and the animals were not extubated till the laryngeal reflex returned. Possible adverse events during the recovery were recorded.

All the data were collected at predetermined times until the end of the surgery by a single observer, who was experienced in the clinical methods and scoring systems used in this trial, but who was blinded to the assigned treatments.

Statistical analysis

Data analysis was performed using commercially available computer software (SPSS Statistics 22.0, Chicago, IL). The Shapiro Wilk test was used to assess the normal distribution of data. Normally distributed data were expressed as mean ± standard deviation, and non-parametric data were expressed as median (range). The overall values for HR, SpO_2, BP, $ETCO_2$, f_R, FI´ISO and FE′ISO were compared between treatments by a Mann Whitney U-test. When this test revealed significant differences, the Wilcoxon signed rank test was used to analyze for predetermined timepoints. A value of $p < 0.05$ was considered significant for all statistical tests.

RESULTS

The total mean duration of the procedure with either the saline or alfaxalone CRI was 68.3 ± 17.2 minutes (min). Time 0 (t0) was considered 10 minutes before starting the surgery. Although there were no significant differences between the groups, the duration of the procedure was forty minutes in two cases. Therefore, statistical analysis was only performed on data collected during the first forty minutes of every experimental animal.

Moderate differences were found for some variables, but these were not significant for HR, BP, $ETCO_2$, f_R, FI´ISO and FE′ISO (Figure 2). Systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were initially lower with treatment A, but gradually increased towards the end of the trial. The HR values were lower in the goats in group A (98.1 ± 15.7) than in group B (107.5 ± 13.1), but the difference was not significant. The FE′ISO gradually decreased over time with treatment A, and although the overall value during the observational period was lower (0.91 ± 0.26%) than in group B (1.03 ± 0.23%), the difference was not significant.

The only significant difference between the treatments was found for SpO_2, which was lower during treatment B than during treatment A at t0, t10, t20, t30 and t40 ($p = 0.027$, $p = 0.027$, $p = 0.027$, $p = 0.028$ and $p = 0.027$, respectively) (Figure 2).

No adverse events were noticed during the recovery period.

DISCUSSION

In this present pilot study, the use of a CRI of alfaxalone at 0.05 mg/kg/min is demonstrated to result in minimal cardiorespiratory changes, and even to

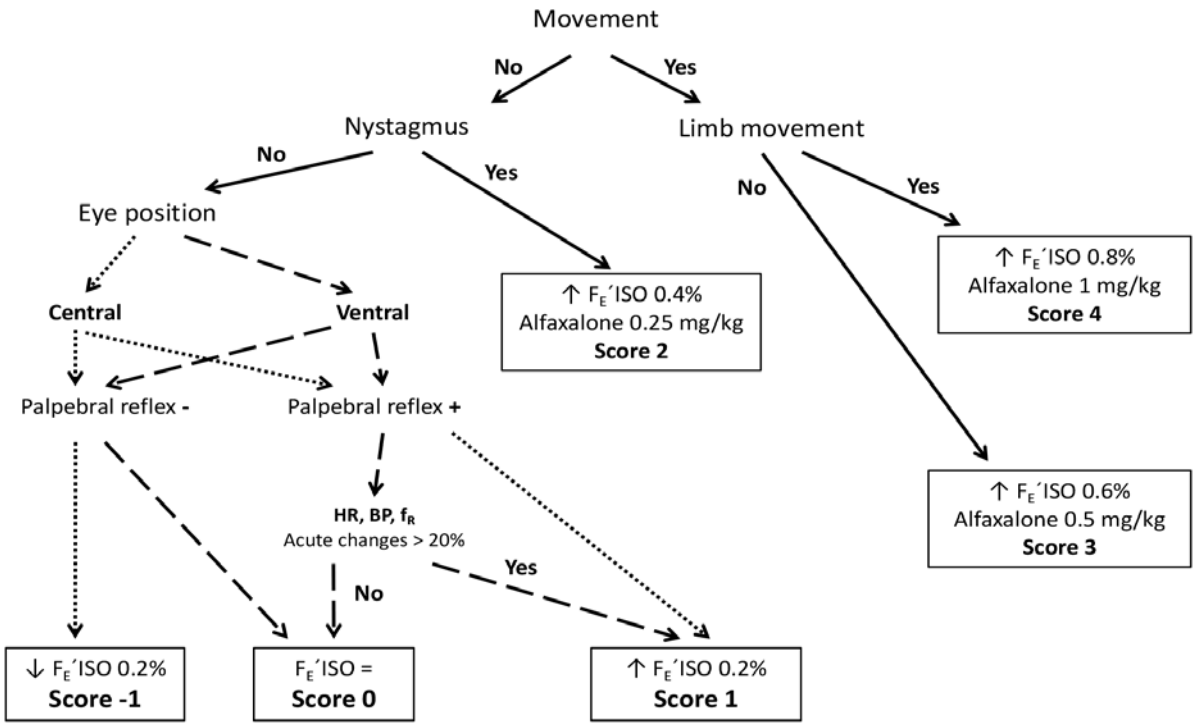

Figure 1. Flow chart used to modify isoflurane settings in isoflurane-anesthetized goats (n =3) combined with an alfaxalone constant rate infusion at 0.05 mg/kg/min.

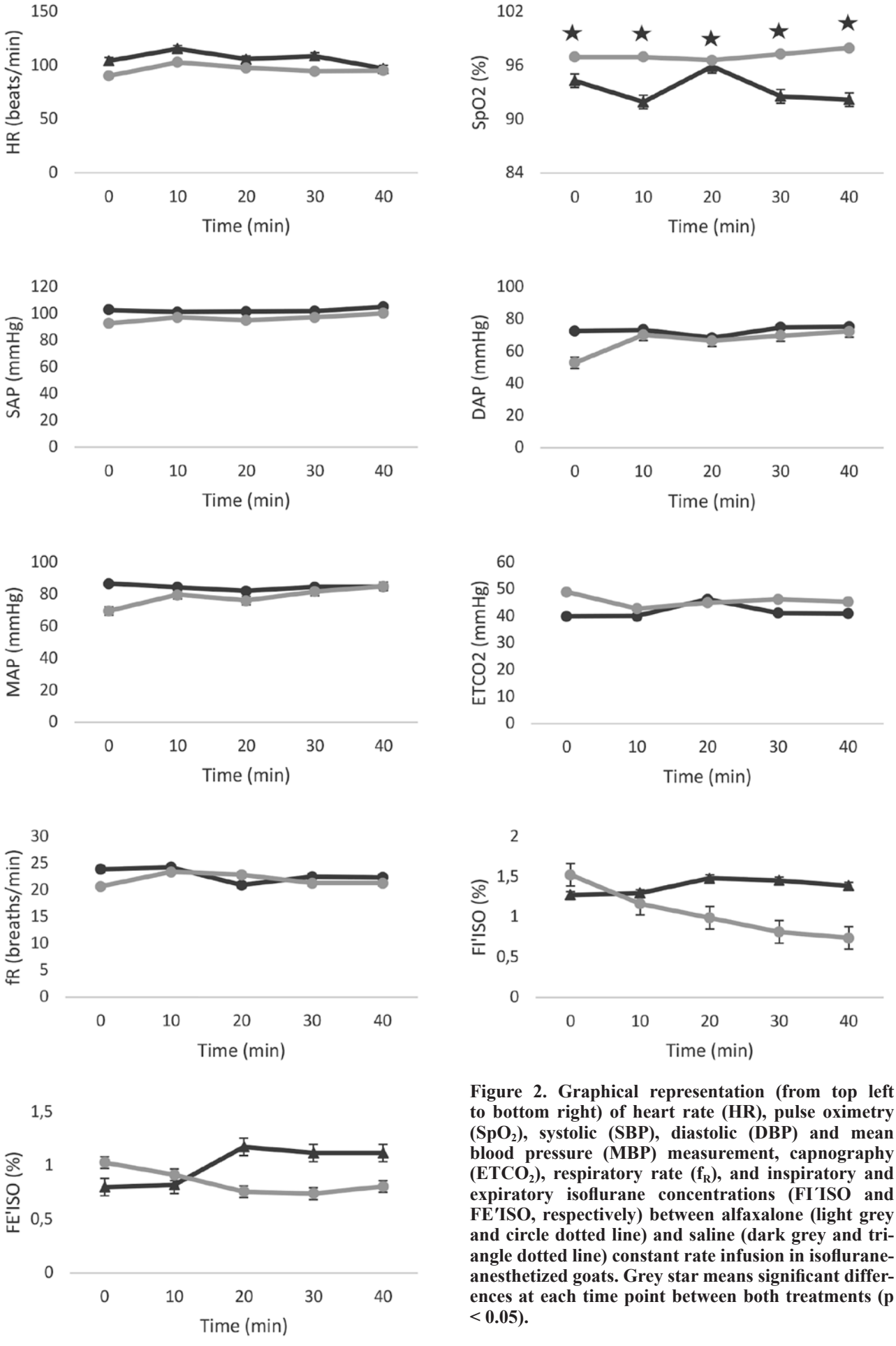

Figure 2. Graphical representation (from top left to bottom right) of heart rate (HR), pulse oximetry (SpO_2), systolic (SBP), diastolic (DBP) and mean blood pressure (MBP) measurement, capnography ($ETCO_2$), respiratory rate (f_R), and inspiratory and expiratory isoflurane concentrations (FI′ISO and FE′ISO, respectively) between alfaxalone (light grey and circle dotted line) and saline (dark grey and triangle dotted line) constant rate infusion in isoflurane-anesthetized goats. Grey star means significant differences at each time point between both treatments ($p < 0.05$).

improve pulse oximetry readings in isoflurane-anesthetized goats. Although no significant differences were observed for the other variables (most likely due to the small sample size), the results suggest that an alfaxalone CRI may have some isoflurane-sparing effects. However, further studies are needed to confirm this.

A constant rate of 0.05 mg/kg/min (or 3 mg/kg/h) of alfaxalone was used in the protocol for isoflurane-anesthetized experimental goats of the present trial. In the veterinary literature, a great variability between species can be found in terms of the infusion regimens. In earlier reports, it has been shown that alfaxalone administered in CRI at 0.07 and 0.11 mg/kg/min for total intravenous anesthesia (TIVA) produces a clinically acceptable anesthetic depth for surgical procedures in dogs (Ambros et al., 2008; Suarez et al., 2012). More recent studies have shown cardiovascular stability and a good plane of anesthesia when alfaxalone is continuously administered at 0.15 mg/kg/min in dogs (Dehuisser et al., 2017, 2019a). Schwarz et al., (2014) established a minimum infusion rate (MIR) of 10 mg/kg/h (0.18 mg/kg/min) for TIVA with alfaxalone as a sole agent in cats (Schwarz et al., 2014). Conversely, a lower infusion rate (3 mg/kg/h or 0.05 mg/kg/min) has been shown to maintain an adequate depth of anesthesia in horses (Goodwin et al., 2018). Nevertheless, Ndawana et al., (2014) showed that a MIR of 0.16 mg/kg/min (10 mg/kg/h) of alfaxalone was feasible to prevent gross movement of trunk, head or limbs after supramaximal noxious stimuli in goats.

In the present study, the FE′ISO required to maintain anesthesia was lower with treatment A, although the difference was not significant ($p = 0.195$). Similarly, in desflurane-anesthetized sheep, infusion of alfaxalone at a rate of 0.07 mg/kg/min results in a decrease of desflurane requirements to maintain a surgical depth of anesthesia (Granados et al., 2012). Also, co-administration of a fentanyl CRI (10 µg/kg/h) results in a significant decrease of the alfaxalone infusion rate in dogs (Dehuisser et al., 2017), while combinations with medetomidine (3 – 5 µg/kg/h) alone or with either butorphanol (30 µg/kg/h) or guaifenesin (80 mg/kg/h) have been demonstrated to reduce the alfaxalone dose regimen (1.5 – 2 mg/kg/h or 0.025 – 0.033 mg/kg/min) for TIVA in horses (Goodwin et al., 2013; Ohmura et al., 2016; Aoki et al., 2017). In goats, a significant reduction of the alfaxalone CRI down to 0.02 mg/kg/min has been observed after a combination with either midazolam (0.1 – 0.9 mg/kg/h) or fentanyl (5 – 30 µg/kg/h) (Dzikiti et al., 2015; Dzikiti et al., 2016). The results of the present report suggest that an alfaxalone CRI at 0.05 mg/kg/min, possibly in combination with other drugs, may reduce the required dose of inhalant anesthetics delivered to the patient, which warrants further research.

Although cardiovascular parameters were within normal ranges throughout anesthesia, BP values were lower in the initial phase of the procedure during treatment A, with minimal or no initial changes in the HR. Dose-dependent decrease in systolic arterial pressure and systemic vascular resistance, due to a vasodilatory effect, have previously been reported after clinical and supraclinical induction doses of alfaxalone in dogs and cats (Muir et al., 2008; Muir et al.,, 2009). Furthermore, alfaxalone administered in either boluses or CRI for TIVA may cause hypotension in cats (Beths et al., 2014; Schwarz et al., 2014). However, either no side effects or minimal changes in the hemodynamic parameters have been shown after alfaxalone CRI in dogs (Ambros et al., 2008) and horses (Goodwin et al., 2018). While balanced cardiovascular parameters have been recorded during TIVA with alfaxalone CRI in goats (Ndawana et al., 2014), a delayed hypotensive state (between 45 and 75 minutes after the start of the alfaxalone CRI) has been demonstrated in desflurane-anesthetized sheep (Granados et al., 2012). The results of the present study show that HR and BP were not affected by co-administration of 0.05 mg/kg/h alfaxalone CRI in isoflurane-anesthetized goats.

The SpO_2 readings were significantly higher during the co-administration of alfaxalone CRI than in the saline group. This may be an incidental finding, or may indicate better pulmonary gas exchange and/or lung perfusion with treatment A, resulting in improved arterial oxygenation. Alternatively, the authors hypothesize that the alfaxalone CRI could have improved the peripheral blood flow either by the aforementioned vasodilatory effect, or due to an increase in the cardiac index after improvement of the myocardial contractility, as previously reported in desflurane-anesthetized sheep (Granados et al., 2012). However, further studies are needed including blood gas analyses and invasive cardiovascular monitoring before final conclusions can be drawn.

The most important limitation of this research is the very small sample size. This study should therefore be regarded as a true pilot study. Based on the difference found in this study for FE′ISO, a sample size calculation was performed (www.powerandsamplesize.com), and it was shown that to achieve a power of 80%, with $p < 0.05$, thirty six goats are needed to find significant differences in FE′ISO between the control group and the alfaxalone CRI group.

In conclusion, the co-administration of alfaxalone at an infusion rate of 0.05 mg/kg/h may have a sparing effect in isoflurane requirements in goats. The cardiorespiratory function was well-maintained and no abnormalities were observed during either the maintenance with alfaxalone CRI or during recovery. Further investigations on the use of a constant rate infusion of alfaxalone as co-adjuvant in the maintenance of anesthesia in goats have to be performed. It should however be emphasized that alfaxalone can be used in goats under experimental conditions, but not in food-producing species as it is not included in EU Regulation 37/2010.

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