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RESEARCH PAPER

Alfaxalone for total intravenous anaesthesia in bitches undergoing elective caesarean section and its effects on puppies: a randomized clinical trial

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Abstract

Objective To evaluate the effects and reliability of alfaxalone constant rate infusion (CRI) in comparison to isoflurane to maintain anaesthesia in bitches undergoing elective caesarean section.

Study design Prospective, randomized, 'blinded' clinical trial.

Animals Twenty-two client-owned bitches and 94 puppies.

Methods Bitches were randomly assigned to receive an alfaxalone CRI [0.2 mg kg^{-1} $minute^{-1}$ intravenously (IV), and once the last puppy was delivered, the dose was halved; $n = 11$] or 2% (vaporizer dial setting) isoflurane ($n = 11$) for maintenance of anaesthesia. All dogs were induced with alfaxalone (3 mg kg^{-1}) IV. Additional alfaxalone (0.3 mg kg^{-1} IV) was administered if the depth of anaesthesia was inadequate and the total dose was calculated. Bitches were mechanically ventilated. Analgesia was administered after the delivery of puppies. Physiological variables were recorded every 5 minutes. The bitches' recovery times were also recorded. Quality of induction and recovery were evaluated. Puppies' vigour was evaluated with a modified Apgar score at 5 and 60 minutes after birth. Puppies' survival rates at 24 and 48 hours and at 15 days were recorded. Data were analysed using an ANOVA, Student's *t*-test or Wilcoxon rank-sum test.

Results The rescue dose of alfaxalone was higher ($p = 0.01$); bitches' recoveries were longer ($p < 0.001$) and puppies' Apgar scores were significantly lower at 5 and 60 minutes ($p < 0.001$ and $p = 0.003$, respectively) with alfaxalone than with isoflurane. However, no significant differences were found for puppies' survival between groups.

Conclusions and clinical relevance Alfaxalone CRI seems to be a possible protocol for puppies and bitches undergoing elective caesarean sections. However, bitches recovered more slowly and puppy Apgar scores were lower in comparison to isoflurane.

Keywords alfaxalone, Apgar score, bitch, caesarean section, total intravenous anaesthesia.

Introduction

During caesarean section, the anaesthetic technique must provide optimal conditions both for the dam and for the foetus. Unfortunately, all anaesthetics cross the placenta and the foetus blood–brain

barrier, leading to foetal depression and may result in poor survival (Clarke et al. 2014).

Several studies have been performed to determine the optimal anaesthetic protocol during caesarean sections. Luna et al. (2004) compared the effects of four anaesthetic protocols on the puppies' neurological and cardiorespiratory variables and concluded that the best anaesthetic technique was epidural local anaesthesia. Nevertheless, epidural injection may require some degree of sedation for administration and can be sometimes contraindicated. When epidural anaesthesia was unsuitable, of the protocols studied, propofol appeared to be the most appropriate induction agent prior to maintenance with an inhlation agent, to maintain puppy neurological reflexes of the protocols studied (Luna et al. 2004)[1].

Isoflurane commonly is used to maintain anaesthesia for caesarean section in several species. In dogs, it was positively associated with puppies' vocalization, a sign of vigour and good survival score (Moon-Massat & Erb 2002). Nevertheless, in sheep, volatile agents may produce maternal and foetal hypotension and acidosis (Palahniuk & Schnider 1974; Okutomi et al. 2009). In addition, besides the environmental pollution that inhalant anaesthetics may produce (Zuccherelli 2007), chronic exposure to halogenated agents at low dose might affect the staff's health (Shirangi et al. 2008).

The new water-soluble formulation of alfaxalone was successfully proposed as an alternative to propofol to induce anaesthesia in bitches and produced better Apgar scores in puppies (Metcalfe et al. 2008; Doebeli et al. 2013). Alfaxalone in combination with alfadolone (Althesin; Glaxo, UK) was used to maintain anaesthesia in 90 women undergoing caesarean section (Gulotta et al. 1980). This steroid anaesthetic had no effect on the Apgar score, uterine tone, maternal–foetal metabolism, cardiocirculatory and respiratory stability. The authors also considered using Althesin as an alternative anaesthetic in order to avoid chronic staff exposure to volatile anaesthetics.

Alfaxalone CRI in dogs results in a rapid recovery and a good muscle relaxation (Ambros et al. 2008; Suarez et al. 2012). It is rapidly metabolized and eliminated by the body (clearance rate = 59 mL minute^{-1} kg^{-1}; Ferré et al. 2006). It has a high margin of safety and minimal cardiovascular effects (Morgaz Rodríguez et al. 2012). Although it was used for induction of anaesthesia in dogs undergoing caesarean section, alfaxalone has not yet been evaluated to maintain anaesthesia during this procedure.

The objectives of this study were to compare isoflurane and alfaxalone administration in client-owned bitches undergoing elective caesarean sections by evaluating the maintenance of anaesthesia (quality and cardiorespiratory functions), recovery from anaesthesia (duration and quality) and the effects on puppies (Apgar, survival and mortality scores).

We tested the hypothesis that, for maintenance of anaesthesia, alfaxalone CRI (0.2 mg kg^{-1} minute^{-1}, and, after puppies' delivery, 0.1 mg kg^{-1} minute^{-1}) would result in better scores (see earlier) than isoflurane (2%) anaesthesia in bitches as well as in their puppies.

Material and methods

Animals

This randomized (1:1), blinded, parallel-group clinical study has been approved by the Ethic Committee of VetAgro Sup, France (no. 1302). Legal and ethical requirements have been met with regard to the humane treatment of animals in accordance with the Euroguide, and good clinical practice of veterinary care was respected. Informed client consent was obtained for each animal included in the study.

Eligible dogs were healthy bitches enrolled to undergo elective caesarean sections. Ultrasound examinations were performed and progesterone venous concentrations were measured daily from 59 days post-ovulation. Aglepristone 15 mg kg^{-1} (Alizine; Virbac, France) was administered if progesterone concentrations were between 6 and 15 nmol L^{-1} and if there was no evidence of foetal distress (puppies' heart rate >200 beats minute^{-1}, calculated using the M-mode in the ultrasound machine). The surgery was then planned for the following day. Criteria for exclusion from the study were complications related to surgery or anaesthesic administration occurring during the procedure.

Bitches' recovery time was the primary outcome on which the sample size calculation was based. R statistical software (R Core Team; R Foundation for Statistical Computing, Austria; http://www.R-project.org/) was used to calculate it. To detect a difference of recovery time of 20 minutes between the two groups (time empirically chosen based on our experience; SD = 15 minutes) with a two-sided

[1][Correction added on 7 March 2016, after first on-line publication: Part of the Introduction has been removed on this version]

5% significant level and a power of 90%, a sample size of 12 patients per group was necessary. As a result, 24 bitches were enrolled in the study. They were presented to the service of anaesthesia of the veterinary campus of VetAgro Sup, from January 2013 to April 2014, for elective caesarean sections. They were assigned randomly to receive alfaxalone CRI (group A) or isoflurane (group I) during the maintenance phase of anaesthesia.

Simple randomization, using sequentially numbered, opaque, sealed envelopes, was performed by an anaesthesia technician and a fifth-year student, who were the only people aware of treatments and were in charge of theatre and drug preparation.

Study design

Once the bitch arrived, a 22, 20 or 18 gauge catheter (BD Insyte-W, UT, USA), depending on the dog's size, was introduced into the cephalic vein and the surgical region was clipped. The patient was then moved to the theatre.

The surgical table was tilted (13 ° to the horizontal in reverse Trendelenburg position). The bitch was preoxygenated for 5 minutes with oxygen 100% (100 mL kg^{-1} $minute^{-1}$) administered via a face mask.

Anaesthesia was induced with alfaxalone (Alfaxan; Jurox-Dechra, UK) 3 mg kg^{-1} injected intravenously (IV) over 60 seconds. Additional boluses (0.3 mg kg^{-1} every 15 seconds) were administered, when judged necessary by the anaesthetist, to allow tracheal intubation (with an appropriately sized tube). The total dose of alfaxalone to allow endotracheal intubation (defined as time from the first attempt to successful intubation) and the total time for intubation were recorded.

Oxygen (100%) was delivered via a Bain or a circle circuit and an anaesthetic machine (Moduflex Optimax; Dispomed, France). Mechanical ventilation (Hallowell EMC 2000; Hallowell, Pittsfield, MA, USA) was started immediately after intubation [peak inspiratory pressure = 20 cmH_2O, respiratory rate (f_R) adjusted to maintain end-tidal carbon dioxide ($P_E'CO_2$) between 35 and 45 mmHg (4.7–6.0 kPa)].

In group A, anaesthesia was maintained with an alfaxalone CRI (0.2 mg kg^{-1} $minute^{-1}$ IV) using a syringe driver (PerfusorSpace; Braun, France). The rate of infusion was halved after the last puppy's delivery. In the control group (group I) anaesthesia was maintained with 2% isoflurane (vaporizer dial setting) (Isoflo; Axience, France) administered from a previously calibrated vaporizer (Ohmeda Isotec 3; Ohmeda, UK). The rate of alfaxalone infusion was halved after the last puppy's delivery. In both groups additional rescue boluses of alfaxalone (0.3 mg kg^{-1} IV) were administered if the depth of anaesthesia was judged to be inadequate by the anaesthetist. The total rescue dose of alfaxalone was calculated.

A Ringer lactate IV infusion (Ringer Lactate Aguettant; Aguettant Laboratories, France) at 10 mL kg^{-1} $hour^{-1}$ was administered by an infusion pump (InfusomatSpace; Braun, France). Capillary blood (from the tongue) was sampled for blood gas analysis after induction of anaesthesia (T_0) and at the end of the surgery (T_1) (VetStat Idexx, France).

Surgeries were performed by the same experienced surgeon. Lidocaine 1 mg kg^{-1} (Lurocaïne; Vetoquinol, France) was injected subcutaneously along the incision line 7–10 minutes before the beginning of the surgery. Mean arterial blood pressure (MAP), heart rate (HR), f_R, oxygen saturation (SpO_2) and $P_E'CO_2$ were recorded every 5 minutes until recovery with a calibrated monitor BSM-2303K (Nihon Kohden, Germany).

Once the last puppy was delivered, the following drugs were administered IV: 0.2 mg kg^{-1} morphine (Morphine Aguettant 100 mg 10 mL^{-1}; Aguettant, France); 0.1 mg kg^{-1} meloxicam (Loxicom, 5 mg mL^{-1}; Norbrook, UK); 0.025 UI kg^{-1} ocytocine (Ocytovem; Ceva Sante Animale, France); 30 mg kg^{-1} cephalexine (Rilexine 1 g; Virbac, France); and 0.1 mg kg^{-1} metoclopramide (Emeprid; Ceva Sante Animale, France).

At the end of the surgery (T_1), the administration of alfaxalone or isoflurane was stopped and the bitches were allowed to breathe spontaneously. Times from the end of anaesthesia to extubation, head lifting, sternal position, standing and nursing were registered.

Implementation

To ensure that the anaesthetist was unaware of treatment allocation, the vaporizer was always hidden under a black bag. The CRI of alfaxalone or the same volume of saline was prepared, in an unidentified syringe, before the rest of the staff had access to the theatre. Once anaesthesia was induced, the CRI with alfaxalone (group A) or saline (group I) was started. Once the bitch was connected to the circuit and the endotracheal tube's cuff was inflated, the anaesthesia technician touched the vaporizer under the bag to start (or not) the inhalant anaesthesia. During this proce-

dure, the anaesthetist was not allowed to observe the anaesthetic machine.

Bitches' recovery

The quality of induction and recovery were video-recorded and scored by two experienced anaesthetists using a visual analogue scale (VAS; 0 cm, the worst recovery/induction possible; 10 cm, the best possible recovery/induction) and a numerical rating scale (NRS; quality of induction – 1, poor: inability to intubate, excitement; 2, fair: difficult intubation, mild excitement or both; 3, good: easy intubation but minimal reflex response or persistent jaw tone; 4, smooth: easy intubation, no reflexes; quality of recovery – 1, poor: marked excitement or struggling and need for restraint; 2, fair: minor excitement, restlessness but no need for restraint; 3, good: relatively smooth recovery and minimal vocalization; 4, smooth: smooth recovery) (Ambros et al. 2008).

Care of puppies

Once the puppies were delivered, fluid was suctioned from the upper airways. Puppies were stimulated with massage, blow-dried and once resuscitated they were placed in a warm incubator. All puppies were identified with a coloured collar and a number.

The number of puppies, the number of puppies born alive and the number of puppies born dead or euthanized (because of cleft palate or other malformations) were recorded on the day of the surgery. The puppies' vigour was scored using a modified Apgar score (see Appendix S1) within 5 minutes after birth, and also 60 minutes later.

Puppies' survival rates at 24 and 48 hours and at 15 days, were obtained from the owners.

Statistical analysis

The procedure was divided into three periods: P_1 was the time from induction (T_0) to skin incision; P_2 was the time from skin incision to last puppy delivery; and P_3 was the time between the last puppy delivery and the end of surgery (T_1).

Statistical analysis was performed with R statistical software. All data were tested for normal distribution using a Shapiro–Wilk test.

For serial measurement over time, a repeated measures ANOVA was used. Treatment and period were included in the tests. *Post hoc* *t*-tests for matched pairs were used for multiple comparisons between periods. A Bonferroni correction was then applied. Other data between groups were compared using Student's *t*-test or a Wilcoxon rank-sum test, when applicable. Differences were considered to be statistically significant if $p < 0.05$. Results are expressed as means ± SD for parametric variables, or median [min, max] for non-parametric variables.

Results

In all, two dogs were excluded from the study (Fig. 1) and 22 bitches (group I, $n = 11$; group A, $n = 11$) were thus included, with the following age and weight characteristics: age – group I, 3.5 [2, 4.5] years old; group A, 2.5 [1, 8] years old; weight – group I, 22 [13, 95] kg; group A, 13 [4, 82] kg. Bitches were of similar age in the two groups but were heavier in group I than in group A ($p = 0.016$). With regard to breed, group A comprised seven French bulldogs, a Saint Bernard, a Pappillon, a Beagle and a Lhasa Apso, and group I comprised three Bull Terriers, three French Bulldogs, a Boxer, a Border Collie, a Saint Bernard, a Basset Hound and a Newfoundland dog. The Border Collie was confirmed MDR1-positive and therefore the dose of morphine was reduced by 25%.

The time and quality of induction as well as the total dose of alfaxalone required for intubation and the intubation time were not significantly different between groups. The total rescue dose of alfaxalone was significantly higher in group A than in group I ($p = 0.01$). During time period P_3, bitches in group A needed significantly more rescue doses than bitches in group I ($p < 0.001$). Within group A, rescue dose requirements were significantly lower during time period P_2 than during P_1 ($p = 0.04$). Within group I, rescue bolus requirements were significantly lower during P_3 than during P_1 ($p = 0.04$) and P_2 ($p = 0.04$) (Table 1).

No difference was observed between or within groups in the cardiovascular parameters measured during the maintenance of anaesthesia except for MAP, which was significantly lower in group I than in group A during time periods P_2 and P_3 ($p = 0.02$ and $p = 0.009$, respectively) (Table 2).

Blood gas measurement showed that the capillary partial pressure of carbon dioxide (PCO_2) and $P_{E'}CO_2$ increased significantly in group I at the end of the surgery ($P_{E'}CO_2$, P_3 *versus* P_1, $p = 0.03$; PCO_2, T_1 *versus* T_0, $p < 0.001$). Nevertheless this increase was not clinically relevant. In group I at T_1, pH was significantly

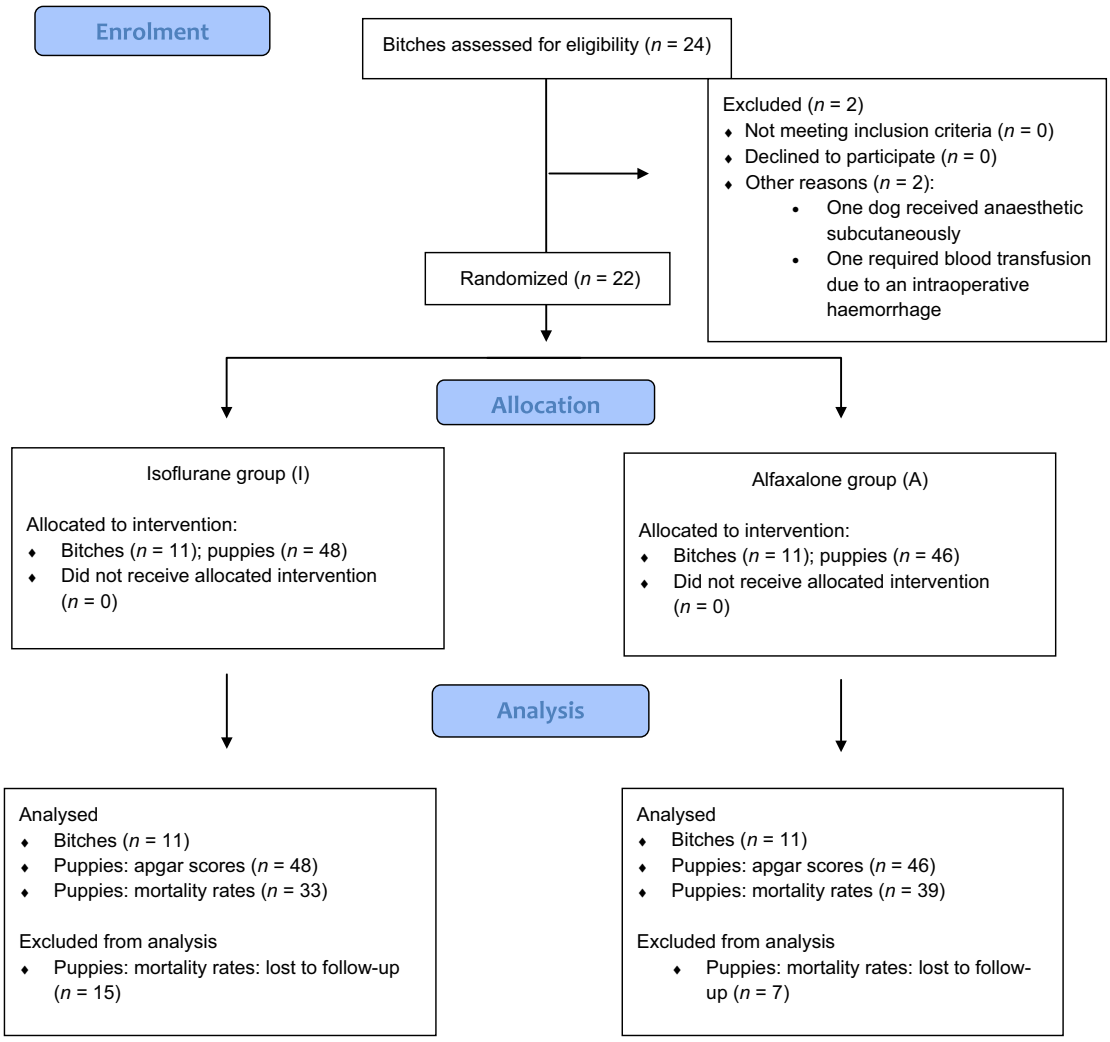

Figure 1 CONSORT flow diagram for this study. Bitches were randomly divided into two groups: group I, in which anaesthesia was maintained with isoflurane; and group A, in which a constant rate infusion of alfaxalone was used.

lower than at T_0 ($p < 0.001$) and significantly lower than in group A ($p = 0.01$) (Table 2).

No significant differences were found between groups concerning surgery and anaesthesia times. The time from induction to first puppy delivery was also the same between groups (Table 1).

The VAS showed a significantly better quality of recovery in animals that received isoflurane than in those that received alfaxalone ($p = 0.002$). No significant differences were found between groups when using the numerical rating scale. The recovery time and time for head lifting were significantly longer in group A than in group I ($p < 0.001$ and $p = 0.002$, respectively) (Table 1).

A total of 94 puppies were evaluated and there was no significant difference between groups in the total number of puppies (group I, $n = 48$; group A, $n = 46$) and in the number of puppies by litter (group I, 4 ± 3; group A, 4 ± 2). All puppies were born alive. Eight of them were euthanized because of congenital malformations. No significant differences were found between groups (Table 3). At 5 minutes after delivery, puppies' HR and f_R were significantly higher in group I than in group A ($p = 0.005$ and $p < 0.001$, respectively; Table 4). The Apgar scores were significantly higher in group I than in group A at 5 minutes [12 (5, 14) *versus* 9 (1, 14), respectively, $p < 0.001$] and 60 minutes after birth [13

Table 1 Surgical and anaesthesia times, alfaxalone induction and rescue doses, total recovery time, times to extubation, head lift, sternal position and suckling, and quality of induction and recovery in 22 bitches undergoing scheduled caesarean section. Anaesthesia was maintained with an alfaxalone constant rate infusion (group A, $n = 11$) or isoflurane (group I, $n = 11$)

	Group A	Group I
Induction time (seconds)	34 [22, 64]	40 [22, 64]
Intubation time (seconds)	93 [41, 225]	94 [57, 141]
Surgical time (minutes)	46 [35, 94]	50 [32, 71]
Anaesthesia time (minutes)	57 [45, 110]	61 [45, 81]
Time from induction to first puppy delivery (minutes)	16 [10, 31]	17 [10, 31]
Total dose for intubation (mg kg^{-1})	3.0 [3.0,3.6]	3.0 [3.0, 3.6]
Total alfaxalone rescue dose (mg kg^{-1})	1.2 [0.3, 3]	0.3 [0.0, 0.9]*
Alfaxalone rescue dose (μg kg^{-1}) during time periods		
P_1	33 [0, 125]	0 [0, 86]
P_2	0 [0, 100][a]	0 [0, 60]
P_3	15 [0, 42]	0 [0, 60][bc]*
Period duration (minutes)		
P_1	11 [7, 16]	12 [7, 13]
P_2	7 [3, 21]	10 [5, 14]
P_3	40 [28,73]	41 [27, 60]
Recovery time (minutes)	46 [17, 73]	22 [10, 31]*
Extubation time (minutes)	17 [1, 46]	6 [3, 25]
Head lifting time (minutes)	26 [5, 49]	6 [3, 21]*
Sternal position time (minutes)	25 [4, 49]	6 [3, 29]
Suckling time (minutes)	29 [7, 46]	15 [6, 23]
Quality of induction		
VAS (cm)	9.1 [6.1, 10.0]	9.8 [4.3, 10.0]
NRS	4 [3, 4]	4 [2, 4]
Quality of recovery		
VAS (cm)	5.9 [4.2, 7.6]	8.4 [6.9, 9]
NRS	4 [3, 4]	4 [2, 4]*

VAS, visual analogue scale; NRS, numerical rating scale. Data are reported as median [min, max]. P_1, time from induction (T_0) to skin incision; P_2, time from skin incision to last puppy delivery; P_3, time between the last puppy delivery and the end of surgery (T_1). *Significant difference between groups ($p < 0.05$) a, b, c superscripts indicate significant differences within the same group between P_1 and P_2, P_2 and P_3, and P_1 and P_3, respectively.

(10, 14) *versus* 12 (4, 14), respectively, $p = 0.003$] (Fig. 2).

Discussion

The results of this study suggest that maintenance of anaesthesia with an alfaxalone CRI in bitches undergoing elective caesarean section is practically feasible, has similar cardiopulmonary effects to isoflurane but induces longer recoveries. In puppies, alfaxalone was associated with lower Apgar scores, but survival and mortality were similar to those obtained with isoflurane.

Bitches in the alfaxalone group needed more alfaxalone rescue doses than did bitches in the isoflurane group. The recommended dose of alfaxalone in unpremedicated dogs for induction of anaesthesia is 3 mg kg^{-1} and is 0.13–0.15 mg kg^{-1} $minute^{-1}$ for CRI (http://alfaxan.co.uk/dosage). Herbert et al. (2013) used a CRI of alfaxalone of 0.13 ± 0.07 mg kg^{-1} $minute^{-1}$ to maintain anaesthesia in nonpregnant bitches premedicated with acepromazine and undergoing ovariohysterectomy. The CRI was reduced to 0.09 ± 0.03 mg kg^{-1} $minute^{-1}$ when the bitches were previously premedicated with dexmedetomidine. Cats undergoing ovariohysterectomy and premedicated with acepromazine or medetomidine required higher doses of alfaxalone (0.19 and 0.18 mg kg^{-1} $minute^{-1}$, respectively) (Schwarz et al. 2014). During preliminary trials we observed that 0.1 mg kg^{-1} $minute^{-1}$ was too low to maintain anaesthesia in unpremedicated bitches. We therefore decided

Table 2 Cardiopulmonary variables, rectal temperature and capillary blood gas analysis in 22 bitches undergoing scheduled caesarean section. For group details, see Table 1

Variable	Period	Group A	Group I
HR (beats minute^{-1})	P_1	158 ± 23	146 ± 13
	P_2	147 ± 17	142 ± 12
	P_3	148 ± 20	133 ± 18
f_R (breaths minute^{-1})	P_1	16 ± 4	16 ± 3
	P_2	16 ± 5	15 ± 5
	P_3	15 ± 6	15 ± 6
$P_{E'}CO_2$ (mmHg)	P_1	39 ± 7	37 ± 5
	P_2	41 ± 6	41 ± 5
	P_3	41 ± 7	42 ± 4[c]
(kPa)	P_1	5.2 ± 0.9	4.9 ± 0.7
	P_2	5.5 ± 0.8	5.5 ± 0.7
	P_3	5.5 ± 0.9	5.6 ± 0.5[c]
SpO_2 (%)	P_1	98 ± 2	98 ± 1
	P_2	99 ± 1	98 ± 1
	P_3	99 ± 1	98 ± 1
MAP (mmHg)	P_1	96 ± 20	77 ± 21
	P_2	98 ± 17	85 ± 20*
	P_3	120 ± 17	85 ± 18*
pH	T_0	7.39 ± 0.03	7.37 ± 0.03
	T_1	7.39 ± 0.07	7.30 ± 0.04*†
PCO_2 (mmHg)	T_0	38 ± 5	38 ± 3
	T_1	43 ± 5	44 ± 4#
(kPa)	T_0	5.1 ± 0.7	5.1 ± 0.4
	T_1	5.7 ± 0.7	5.9 ± 0.5#
PO_2 (mmHg)	T_0	254 ± 61	317 ± 96
	T_1	240 ± 100	299 ± 114
(kPa)	T_0	33.9 ± 8.1	42.3 ± 12.8
	T_1	32.0 ± 13.3	33.9 ± 15.2

HR, heart rate; f_R, respiratory rate; $P_{E'}CO_2$, end-tidal carbon dioxide; SpO_2, oxygen saturation; MAP, mean arterial blood pressure; PCO_2, capillary partial pressure of carbon dioxide; PO_2, capillary partial pressure of oxygen. For definition of time periods $P_{1\text{-}3}$, see table 1., T_0 time point after anaesthetic induction, (T_1) the end of surgery. Data are reported as means ± SD. *Significant difference between groups ($p < 0.05$). a,b,c superscripts indicate statistically significant differences within the same group between P_1 and P_2, P_2 and P_3, and P_1 and P_3, respectively. †Significant difference within the same group at T_0 and T_1.

to double the dose during P_1 and P_2. Nevertheless, our results suggest that the depth of anaesthesia was still inadequate in the alfaxalone group, especially during P_3. The number of additional boluses needed during P_3 could have been reduced if the alfaxalone CRI had been maintained and not halved during this period.

The rescue dose of alfaxalone required during time period P_1 was higher than during P_2 in both groups.

Table 3 Total number of puppies, euthanized puppies, dead puppies and further mortality rates at 24 and 48 hours and at 15 days born from 22 bitches undergoing scheduled caesarean sections. Anaesthesia was induced with alfaxalone and maintained with an alfaxalone constant rate infusion (group A, $n = 11$) or isoflurane (group I, $n = 11$)

	Group A	Group I
Total number of puppies	46	48
Number of puppies born alive	46	48
Number of puppies euthanized	4	4
Number of puppies dead at 24 hours	4	3
Number of puppies dead at 48 hours	1	0
Number of puppies dead at 15 days	0	2
NA* (*n*)	7	15
Ratio of puppies dead at 24 hours/ number of puppies born alive	0.09	0.08
Ratio of puppies dead at 48 hours/ number of puppies born alive	0.09	0.06
Ratio of puppies dead at day 15/ number of puppies born	0	0.04

*NA, number of puppies for which owner's information is not available.

Table 4 Distribution of puppies (*n*) depending on values obtained for the parameters heart rate (HR) and respiratory rate (f_R) in the Apgar score used in this study at 5 and 60 minutes after birth. For group details, see Table 3

	5 minutes after birth		60 minutes after birth	
	Group A	Group I	Group A	Group I
HR (beats minute^{-1})				
$n < 120$	6	1	0	0
$n = 120$–180	24	18	16	13
$n > 180$	13	25	27	31
NA	3	4	3	4
f_R (breaths minute^{-1})				
$n < 15$	7	1	0	0
$n = 15$–30	24	19	20	13
$n > 30$	13	24	23	30
NA	2	4	3	5

NA, number of puppies for which owner's information is not available.

Maney et al. (2013) showed that, in absence of premedication, the duration of anaesthesia in dogs after a single bolus of alfaxalone of 2.6 ± 0.4 mg kg^{-1} is around 11 minutes before extubation and 17–23 minutes before recovery. The additional boluses administered during P_1 (the length of which

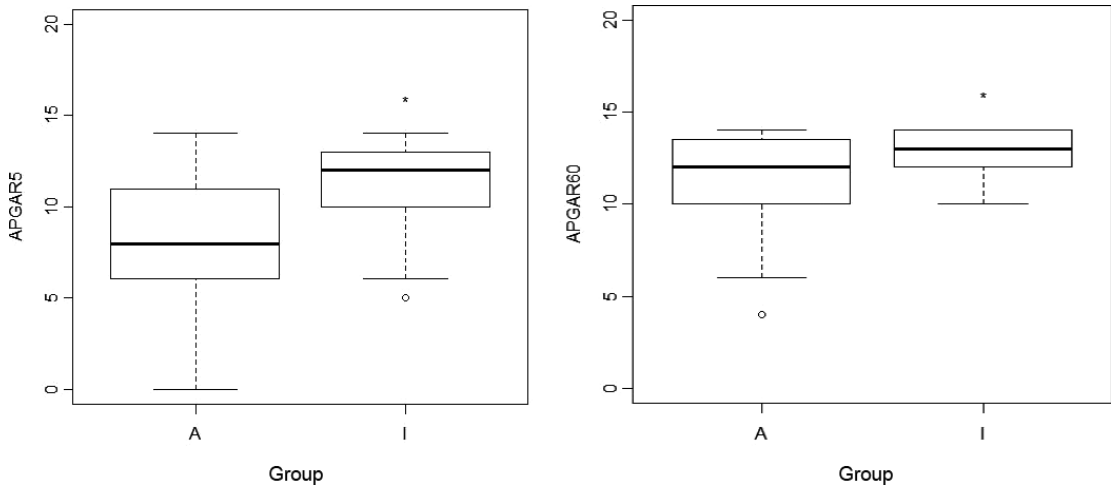

Figure 2 Apgar scores for puppies, born from 22 bitches undergoing scheduled caesarean sections. Anaesthesia was maintained with alfaxalone constant rate infusion [group A (bitches = 11), $n = 46$] or isoflurane [group I (bitches = 11), $n = 48$], at 5 (APGAR5) and 60 (APGAR60) minutes after delivery. The asterisk (*) indicates statistically significant difference ($p < 0.05$) between groups.

was 11 and 12 minutes, respectively, in groups A and I) may therefore have prolonged effects during P_2, contributing to the need for less alfaxalone during this period.

Bitches in the alfaxalone group had slower and poorer quality recoveries than those in the isoflurane group. Recoveries from caesarean sections should be as short as possible, because the time intervals between delivery and first breath and the time of first contact with the bitch and its acceptance are critical moments for puppy survival. Pregnancy may produce alterations in uptake, distribution and disposition of anaesthetic agents (Raffe & Carpenter 2007). A single bolus of alfaxalone produced longer recoveries in pregnant than in non-pregnant ewes (Andaluz et al. 2013). As a consequence, it has been recommended to reduce drug dosage by 25% in pregnant animals. In our study, the dose of alfaxalone was even higher than the recommended dose in non-pregnant dogs. This may have resulted in prolonged recovery. Boluses of alfaxalone received during the third period of anaesthesia may also have contributed to longer recovery times. Alfaxalone clearance is dose-dependent (Ferré et al. 2006), and incremental bolus administration results in higher peak plasma concentrations. A CRI of about 0.18 mg kg^{-1} $minute^{-1}$ in cats corresponds to an alfaxalone plasma concentration of between 2 and 6 μg mL^{-1}. This plasma concentration was increased to 10.76 μg mL^{-1} in a cat that had received a rescue bolus in addition to the CRI (Schwarz et al. 2014). Pasloske et al. (2009) showed that morphine and acepromazine administered during premedication in Greyhounds increased half-lives and decreased clearance rates of alfaxalone. In our study, morphine was administered at the beginning of P_3 and then may have decreased the alfaxalone elimination rate. This could further explain prolonged recoveries in group A.

In our study, time to suckling was not significantly different between groups. Prolonged recovery after caesarean section may not be detrimental, as long as the puppies are well cared for and fed. In addition, bitches that do not undergo natural whelp may develop an abnormal maternal behaviour (which may go from indifference to aggressiveness; Greer 2014). Owners are therefore encouraged to take care of the litter to avoid aggressiveness from the mother after caesarean section. If the recovery is a little prolonged and the bitch is sedated, it may be easier to first feed the puppies.

Although all bitches recovered uneventfully and NRS scores were the same, the VAS score was better in the isoflurane group. Prolonged recovery in group A may have negatively affected the VAS score. In addition, alfaxalone has been described as producing an increase in dog sensitivity to noises, leading to poorer recovery qualities (Jiménez et al. 2012). Recoveries were performed in a stimulating and noisy environment, which could have affected their quality in the alfaxalone group. The NRS score may

lack sensitivity which could explain the different result from the VAS score.

The MAP was lower in the isoflurane group during P_3, perhaps due to inhalant anaesthetic-induced vasodilation. Regarding the light anaesthetic plane of bitches in group A, this may also be a reason for a higher blood pressure in this group.

Lower MAP values had no consequences for puppies' survival rates. This is in accordance with Palahniuk & Schnider's (1974) study, which showed that anaesthesia maintained with isoflurane in pregnant ewes at 1.0 and 1.5 minimum alveolar concentration decreased utero-placental blood flow without any repercussion on the foetus.

In our study, Apgar scores were significantly higher in the isoflurane group than in the alfaxalone group. In addition, at 5 minutes, HR and f_R were significantly higher in the isoflurane group. These differences had no effect on the survival scores, which were the same in both groups. Early evaluation of puppies' vigour could contribute to reduced mortality at birth. The Apgar scoring system is an easy and reliable method for evaluating the viability of both human and animal neonates (Veronesi et al. 2009). In their study, Veronesi et al. (2009) found that the highest mortality rates occurred during the first 2 hours and that Apgar score was a reliable method of assessing puppy survival. We supposed that lower Apgar scores, HR and f_R in group A resulted from puppies' sedation. Even though clearance of alfaxalone is high (59 mL kg^{-1} $minute^{-1}$; Ferré et al. 2006), hepatic function in neonates is decreased and may prolong sedation. Because isoflurane is mainly eliminated by the lungs, recovery occurs sooner.

The bitches' weight difference between groups is a limitation of our study. This might affect pharmacokinetics, pulmonary ventilation and recovery time. The fact that bitches were heavier in the isoflurane (mainly eliminated by the lungs) group and were mechanically ventilated might have limited the consequences of this difference. This problem could have been avoided using stratification during randomization.

Another limitation is related to the dose of alfaxalone CRI, which was halved once all puppies were delivered. This may have been responsible for the higher alfaxalone rescue does required and could have affected recovery time and quality.

This study was performed in bitches undergoing elective caesarean section, and hence the results may not be applicable to caesarean sections performed in emergency conditions and associated with foetal distress.

Conclusion

This study shows that an alfaxalone CRI can be used to maintain anaesthesia in bitches undergoing elective caesarean sections and does not seem to affect puppies' survival in comparison to isoflurane.

Clinical relevance

Alfaxalone could be an alternative to isoflurane for maintaining anaesthesia during caesarean section in dogs, also resulting in less atmospheric pollution and less chronic staff exposure.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Apgar score used in this study (Groppetti et al. 2010).