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Comparison of Propofol and Alfaxalone as Anesthesic Drugs in Bitches Undergoing Ovariohysterectomies (Health Bitches and with Pyometra) and Cesarean Sections

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Simple Summary: This study compared the effectiveness of two anesthesia drugs, alfaxalone and propofol, for female dogs undergoing ovariohysterectomy (n= 69) or cesarean sections (n= 28). Maternal parameters were monitored during surgery, and neonatal viability was assessed post-delivery. Results showed no significant differences in maternal parameters between the two drugs, except for transient tachycardia with alfaxalone. Propofol required more additional doses for induction. Neonatal mortality rates were similar, but alfaxalone was associated with better neonatal viability and required less neonatal care compared to propofol. In summary, both drugs were equally effective for ovariohysterectomy, while alfaxalone showed potential benefits for neonatal health after cesarean sections.

Abstract: This study compared the efficacy of alfaxalone and propofol to induce anesthesia in bitches undergoing an ovariohysterectomy or cesarean sections. 34 healthy and pyometra-affected females were induced with IV propofol administration, 35 healthy and pyometra-affected bitches were induced with the IV administration of alfaxalone. For the cesarean sections, females were induced with propofol (n=14) or alfaxalone (n=14). Maternal physiological parameters (heart rate, blood pressure, temperature) were recorded throughout surgeries; the viability and a modified Apgar score was recorded in the neonates at 5, 60 and 120 minutes after delivery. The use of propofol and alfaxalone did not show relevant differences over parameters assessed, when ovariohysterectomy was performed in healthy bitches or females with pyometra, as well as when cesarean sections were compared; only, a transient tachycardia appeared after alfaxalone administration. However, a higher percentage of bitches needed extra-doses of propofol. Neonatal mortality was similar between both groups, but when alfaxalone was used a higher neonatal viability was observed. Propofol and alfaxalone showed similar efficacy to perform the ovariohysterectomy in all bitches. Both protocols were optimal for cesarean sections, but alfaxalone was associated with a higher neonatal viability and a higher neonatal survival rate.

Keywords: propofol; alfaxalone; ovariohysterectomy; cesarean section; Apgar test

1. Introduction

In small animals, surgeries of the female reproductive tract are common procedures in veterinary practice. Routine ovariohysterectomy involves the removal of both ovaries and the uterine horns [1] and is usually performed via a ventral midline approach [2]. Pyometra is a hormone-

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mediated disorder in which the uterine endometrium is altered due to a secondary bacterial infection; surgical treatment of pyometra includes an ovariohysterectomy similar to the developed in healthy bitches, although it must be done carefully due to the greater fragility of the uterine tissues. Finally, the cesarean section section includes a medial ventral approach and once the uterine horns have been exteriorized the uterine body is incised and puppies delivered as fast as possible [3]. For all the about procedures, preoperative and postoperative factors must be considered and the anesthetic protocol should be optimized to minimize neurological and cardiorespiratory depression in females.

Premedication should provide sedation and analgesia prior to facilite anesthesic induction. Propofol and alfaxalone are two drugs commonly used to perform anesthetic induction. Propofol is an alkylphenol-based intravenous anesthetic agent, known for its rapid anesthesic induction [4] and short recovery times [5]. Alfaxalone is a synthetic neuroactive steroid which interacts with the y-aminobutyric acid (GABA) receptors of the central nervous system, producing anesthesia and muscle relaxation [6]. When comparing their effects, propofol is associated with decreases in heart rate and mean arterial pressure, and also promote respiratory depression [7]; alfaxalone does not accumulate in tissues after repeated doses due to its rapid metabolism and clearance [7], and also has been reported that cardiovascular and respiratory parameters remained remarkably stable during general anesthesia [8].

Different studies have reported the use alfaxalone for total intravenous anesthesia in healthy bitches undergoing ovariohysterectomy [6,9,10]. In addition, the effectiveness of propofol or alfaxalone in anesthesic induction during cesarean sections have been also evaluated [11,12] and the influence over neonatal viability was determined, showing best results when alfaxalone was applied. In addition, only one study [13] has assessed the effectivenes of alfaxalone for total intravenous anaesthesia in bitches undergoing elective cesarean section. Other studies have described an anesthetic protocol consisting of pre-medication with an opioid followed by induction and maintenance with propofol until delivery of neonates [3,14]. However, no studies have been performed in cesarean sections, comparing the efficacy of propofol or alfaxalone to perform the anesthesic induction and maintenance until neonates delivery. Finally, to our knowledge, no studies have been done assessing the efficacy and safety of propofol versus alfaxalone in bitches with pyometra.

Several studies have considered the use of an Apgar score and short-term survival prognosis in evaluating neonatal viability [15–19]. Neonatal parameters assessed in APGAR are heart rate, mucous membrane color, respiratory rate, stress-induced reflexes, and neonatal activity [3,17]. Temperature, glucose, and lactate plasmatic concentration are also variables to consider in neonates [20–22]. The present study was performed to assess the efficacy and reliability of alfaxalone in comparison with propofol to induce anesthesia in healthy bitches and bitches with pyometra, undergoing an ovariohysterectomy. In addition, the use of propofol and alfaxalone as as anesthetic induction agents for cesarean sections in bitches and its influence over neonatal viability were also defined.

2. Materials and Methods

2.1. Animals

Ninety-nine privately owned females and 124 puppies were enrolled in the current study. .All patients were identified by their history number, breed, and age, and they underwent a physical examination (heart rate, glucose, rectal temperature, breathing frequency, and mucus color) and blood analysis before surgery. Sanitary status (properly vaccinated and dewormed) was very similar. Bitches were categorized according to the type of surgery: ovariohysterectomy (health females, pyometra) and cesarean section. All females underwent a cesarean section were categorized as brachycephalic breeds (Amenrican Bully, English Bulldog; French Bulldong), Surgeries were performed at the Veterinary Hospital at the University of Las Palmas de Gran Canaria between 2020 and 2022. This study was developed according to the European laws for animal welfare

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2.2. Experimental design

The bitches were assigned to three experimental groups according the type of surgery performed: ovariohysterectomy in health bitches (OVH), ovariohysterectomy in bitches with pyometra (OVHP) and cesarean section (CS). All females were premedicated with the IM administration of methadone and after 10-15 minutes, anesthesia was induced. In group OVH (n= 42), 20 females were induced with IV propofol administration (OVHP) and 22 females were induced with the IV administration of alfaxalone (OVHA); in bitches with pyometra (OVHP), 14 females were induced with IV propofol (OVHPP) and 13 females with the IV alfaxalone (OVHPA). In both groups, after induction, the maintenance of the anesthesic plan was performed with sevoflurane. The females (n=28) underwent to an cesarean section were subdivided in two groups: in group CSP (n=14) induction was performed with propofol and additional boluses of propofol were administered to maintain the anesthesia until the complete delivery of puppies and then anesthesia was maintained afterwards with sevoflurane; in group CSA (n=14), induction was completed with alfaxalone, additional boluses of alfaxalone were administered to maintain the anesthesia and once the puppies were delivered, the anesthesia was maintained with sevoflurane. Physiological parameters (heart rate, breathing frequency, blood pressure, temperature, oxygen saturation) were recorded during surgeries in the dams of the different groups; in addition, the viability and a modified Apgar score were recorded in the neonates at 5, 60 and 120 minutes after birth. In all surgery groups, the bitches were assigned uniformly according age, weight and breed.

2.3. Pre-anaesthetic evaluation and pre-surgical assessment

A clinical examination was conducted to measure the respiratory rate, heart rate, mucous membrane condition and temperature before females were premedicated. A preanesthetic assessment was done, including blood test (Procyte dx, IDEXX, Laboratorios S.L, Spain), biochemistry analysis (Catalyst Dx, IDEXX Laboratorios, S.L, Spain), thorax radiography and electrocardiogram. Regarding, cesarean sections, plasmatic progesterone levels were determined before surgery and a transabdominal ultrasound (C4-1 Curved Array, ZONE Sonography®, Mindray Zonare Z.One PRO) was performed in the dams, and the neonatal heart rates were measured to check for fetal stress. All C-sections were scheduled between days 59-61 after ovulation.

Premedication included the IM administration of methadone (0,04 mg/kg; Semfortan 10 mg/ml. CIMAvet, Spain). After the application, bitches for ovariohysterectomies (healthy and with pyometras) and cesarean sections were preoxygenated for 10 minutes and catheters were applied in the cephalic vein. Prior to induction, the operating room was prepared, particularly for cesarean sections.

2.4. Anesthetic induction and patient preparation

Each dose of propofol and alfaxalone was divided into four portions and administered for 1 minute at least. Propofol was administered at a dose of 4 mg/kg (Propovet, 10 mg/ml, Esteve, Spain), while for alfaxalone was used at 1 mg/kg dose (Alfaxan, 10 mg/ml, Jurox, Ireland). All potential adverse effects following the application were recorded, as well as whether a higher dose of anesthetic induction was necessary to complete the intubation. Immediately after induction, endotracheal induction was performed and then connected to an automatic respirator that delivered 3–4% oxygen, and sevoflurane at 2-3%. However, for cesarean sections, after endotracheal intubation, bitches were connected to an automatic respirator that delivered 3–4% oxygen and instead sevoflurane, maintenance doses of propofol or alfaxalone were administered. After the removal of the puppies, 2-3% sevoflurane was administered to maintain anesthesia until the end of the surgery.

From induction to the end of the surgery, the same control equipment (Ventilator GE Datex Ohmeda Anesthesia Machine, Vetmat, Bizkaia) monitored continuously the patients' heart rate, breathing frequency, blood pressure, temperature, oxygen saturation, and capnography. If the female dog experienced pain during the surgery, Fentanyl (Fentadon 50 micrograms/mL, Dechra, Spain) was

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administered. To maintain blood pressure before and after surgery, crystalloid fluids (Ringer Lactate® 3 mL/kg/h, Braun, Rub, Spain) were administered.

2.5. Surgical procedure

Ovariohysterectomies (healthy bitches and bitches with pyometra) were performed using a conventional approach. An incision was performed in the skin and subcutaneous tissues to expose the linea alba (which was slightly raised) and an incision puncture was made to gain access to the abdominal cavity. The uterine horns and ovaries were located and exposed and a ligature was made in the mesovarian ligament, caudal to the ovarian pedicle, including the ovarian vessels. Finally, a double ligature was done in the uterine body, with a transfixing suture in the middle of both. Next, the closure was made through plans; first, the abdominal wall was sutured with a 0-1 monofilament absorbable suture (Monosyn®, B. Braun Surgical SA, Rubí, Spain) in a discontinuous pattern, and then, the subcutaneous tissue was closed in a continuous pattern. Finally, the skin was closed with an intradermal suture (Monosyn® 3/0, B. Braun Surgical SA, Rub, Spain) to facilitate post-surgical care.

A standard surgical technique was used for the cesarean section. The uterine body and horns were carefully exposed, and a 4-10 cm incision was made in the uterine body. The neonates were swiftly extracted and transferred to the neonatal resuscitation area. After removal of the puppies, the uterine mucous membrane and submucosa were closed using a monofilament absorbable suture (Monosyn® 3/0, HR22, B. Braun Surgical SA, Rubí, Spain) in a single continuous pattern and then an inverting pattern (Cushing) Was done. Afterwards, intravenous oxytocin (1-4 IU Oxiton®, Ovejero, León, Spain) was administered to facilitate the expulsion of the placental membrane. Finally, the closure was performed by plans, in the same manner as the ovariohysterectomies.

2.6. Neonatal resuscitation

All cesarean sections followed the same protocol once the puppies reached the neonatal resuscitation area. Electric blankets were used to regulate the temperature. There were enough personnel in the resuscitation area to care for up to two puppies.

The ABC protocol (airway/breathing/cardiac) was used to properly manage newborns. The airways were cleaned by suctioning the mouths and noses with a bulb syringe, removing any fluids or meconium, and ensuring that the mouth and nose were devoid of fetal membrane. The newborns were rubbed vigorously with a towel, and the animal's head was tipped downward to allow fluids to drain from the airway. The pups did not swing, thus avoiding excessive intracranial pressure, subdural hemorrhage, or stomach contents getting into the airway.

Oxygenation and a heat source were available during the process. The respiratory rate was closely monitored and if the neonates were not breathing properly, they were placed on an oxygen mask. If newborns were auscultated and did not have a heartbeat, emergency neonatal resuscitation began with heart compressions (2-3 ppm/second). If the newborn's vitality did not improve after several minutes of resuscitation, a catheter was inserted into the jugular vein, and drugs were administered intravenously. Medicaments used were: naloxone (0.05–0.1 mg/kg) to reverse the effects of opiods given to the mother during premedication; heptaminol (0.1 mg/kg) to support systole, blood pressure, and heart rate. ABC was stopped if neonates did not show a favorable evolution after 45 minutes of resuscitation.

After the puppies were stabilized, the placenta was removed, and blood was drawn to measure biochemical parameters. Also, the temperature was measured, and the puppies were weighed on the scale. Afterward, the newborns were placed in an incubator (Vetario© S40) at a temperature of 33–34°C and fed before 60 minutes after birth. Puppies with physical deformities such as anasarca, pronounced cleft palate, or wide fontanelle were registered. If the newborns' welfare was incompatible due to congenital malformations, they were euthanized.

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2.7. Apgar test evaluation

The Apgar test was performed to determine neonatal viability [3]. Apgar test included five parameters: heart rate, respiratory rate, mucosal appearance, neonatal mobility, and reflex/irritability status. The heart rate was measured using a stethoscope and an ultrasound scan; the respiratory rate was determined by counting the number of spontaneous breaths per minute; the color of the mucous membrane was evaluated when viewing the mucous directly; mobility was determined when observing the spontaneous movement; and reflex irritability was assessed through the response to external stimulation like compression of the tip of the paw. Each parameter was scored either as a 0, 1, or 2 (final score, ranging from 0 to 10). Therefore, puppies were classified into three categories according to their final Apgar score: normal neonates (score > 7), moderate viability neonates (score: 3-7) and critical neonates (score < 3). Apgar test was performed at 0 (Apgar 0), 60 (Apgar 60), and 120 (Apgar 120) minutes after the neonates were born.

2.8. Statistical analysis

The SPSS 10.0 software package was used to analyze the data (SPSS Inc., Chicago, IL, USA). The frequency and proportion of categorical variables, as well as the mean and standard deviation of scale variables, were calculated. Normal data (parametric) was examined with several repeated measures of analysis of variance (ANOVA); when statistical significance was found, the Bonferroni post-hoc test was conducted for multiple comparisons. The General Linear Model (GLM) was used to analyze all data pertaining to Apgar evaluation and puppy viability (puppies born alive, puppies with birth defects, and neonatal mortality). The GLM took into account the effects of the anesthetic protocol (2 protocols), Apgar scores (critical neonates, moderate viability neonates, and normal viability neonates), time of Apgar evaluation (Apgar 0, Apgar 60, Apgar 120), and the interactions between these. When $P < 0.05$ values were considered statistically significant.

3. Results

3.1. Ovariohysterectomies in healthy bitches and bitches showing pyometra

The mean weight and age of healthy females (n=42) were 14.34 kg and the age ranged between 8 months and 4 years, while bitches with pyometra (n=27) showed a mean weight of 17.12 kg and the age ranged between 7 and 11 years, without significant differences between propofol and alfaxane groups. Table 1 shows the percentage of females requiring additional doses of propofol/alfaxalone to complete the induction, side effects and the administration of fentanyl during surgery. When propofol was administered, a higher percentage of females (55.9%, 19/34) required more extra-doses to complete induction compared when alfaxalone was applied (28.6%, 10/35), with no significant differences between OVH and OVHP groups. However, side effects were observed in alfaxalone groups showing females a transient tachycardia (11.4% vs 0.0%, $p<0.05$) that normalized after 5 minutes. Finally, intraoperative fentanyl was necessary about 41% of females when propofol was administered, without significant differences compared with alfaxalone groups.

Table 1. Percentage of extra-doses of propofol/alfaxalone to complete induction, side effects after induction and fentanyl administration during surgery in OVHP, OVHA, OVHPP and OVHPA groups.

Groups	Extra doses	Side effects	Intraoperative fentanyl
OVH_P	55.0%^{a} (11/20)	0.0% (0/20)	40.0% (8/20)
OVH_A	31.8%^{b} (7/22)	4.5% (1/22)	27.3 % (6/22)
$OVHP_P$	57.1%^{a} (8/14)	0.0% (0/14)	42.8% (6/14)
$OVHP_A$	23.1%^{b} (3/13)	23.1% (3/13)	46.1% (6/13)
$OVH_P + OVHP_P$	55.9%^{a} (19/34)	0.0%^{a} (0/34)	41.1% (14/34)
$OVH_A + OVHP_A$	28.6%^{b} (10/35)	11.4%^{b} (4/35)	34.3% (12/35)

^{ab}: Different letters in the same row and category denote significant differences ($p<0.05$).

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Table 2 shows the mean blood pressure and heart rate in OVH and OVHP groups. Blood pressure did not show significant differences between groups, although bitches induced with afaxalone presented more uniform values throughout the surgery. In females induced with alfaxalone (OVHA and OVHPA), the heart rate was significantly higher ($p<0.05$) during the first 10 minutes of surgery, compared when propofol (OVHP and OVHPP) was administered, but in the second half of surgery, both groups had similar values. In addition, temperature showed very slight modifications throughout the surgery (range: 37.0-37.7 ºC) without observing significant differences between groups. Finally, after surgery, the heart rate and temperature were monitored and measured in minutes 0, 30, and 60; there were no significant differences between protocols, except for a slight decrease of the body temperature one hour after surgery in all groups.

Table 2. Mean (± sd) of blood pressure and heart rate throughout ovariohysterectomies in healthy bitches (OVH) and bitches with pyometra (OVHP).

	Blood pressure (mm Hg)				Heart rate (beats/min)			
Minutes	OVH_P	OVH_A	$OVHP_P$	$OVHP_A$	OVH_P	OVH_A	$OVHP_P$	$OVHP_A$
0	82.9 ± 2.1	89.0 ± 1.2	83.6 ± 3.2	85.6 ± 3.0	88.9 ± 5.1^a	117.5 ± 6.2^b	99.5 ± 4.1^a	122.1 ± 2.9^b
5	85.2 ± 2.3	80.5 ± 2.7	81.3 ± 4.2	82.2 ± 3.1	79.4 ± 4.4^a	110.7 ± 4.7^b	96.3 ± 3.2^a	111.5 ± 4.0^b
10	81.5 ± 3.1	72.5 ± 3.2	74.6 ± 2.2	75.4 ± 2.8	78.5 ± 3.0	92.6 ± 5.2	92.7 ± 6.0	102.5 ± 4.6
15	81.6 ± 2.5	75.6 ± 2.3	73.1 ± 3.4	73.2 ± 2.5	79.0 ± 3.9	85.7 ± 3.3	93.8 ± 3.5	97.5 ± 3.1
20	82.9 ± 2.1	89.0 ± 1.2	83.6 ± 3.2	85.6 ± 3.0	88.3 ± 4.2	96.6 ± 6.2	93.5 ± 3.4	90.0 ± 3.9
25	79.0 ± 3.3	86.2 ± 1.9	75.2 ± 2.9	79.6 ± 5.1	83.1 ± 4.3	81.5 ± 2.9	93.1 ± 2.1	94.1 ± 4.7
30	--	--	73.2 ± 2.3	70.6 ± 4.1	--	--	104.5 ± 5.3	106.3 ± 3.7

^{ab}: Different letters in the same file and parameter (blood pressure/heart rate) denote significant differences ($p<0.05$).

3.2. Cesarean sections

Bicthes underwent to cesarean section showed a mean weight and age of 23.5 kg and 3.4 years, without significant differences between propofol and alfaxane groups. A higher percentage of females required extra doses of propofol to complete the induction till the delivery of neonates (Table 3), compared when alfaxalone was used (64.3% vs 28.6%, CSP and CSA, respectively; $p<0.05$). No differences were observed between groups regarding the administration of fentanyl during surgery, with values ranging between 43% and 50%. Finally, side effects immediately after induction were basically recorded in CSA group (2/16) showing transient tachycardia.

Table 3. Percentage of extra-doses of propofol/alfaxalone, side effects after induction and fentanyl administration during cesarean sections in experimental groups.

	Extra doses	Side effects	Intraoperative fentanyl
CS_P	64.3%^{a} (9/14)	7.1% (1/14)	50.0% (7/14)
CS_A	28.6%^{b} (4/14)	14.2% (2/14)	42.8% (6/14)

^{ab}: Different letters in the same row denote significant differences ($p<0.05$).

Table 4 reports the mean blood pressure and heart rate recorded in bitches throughout the cesarean sections. Blood pressure did not show significant differences between groups, and only a slight decrease was observed in the mean values at the end of the cesarean sections. Similarly to ovaryohisterectomies, the heart rate showed higher values ($P<0.05$) in the first 10 minutes of surgery when alfaxalone was administered, but then heart rate was similar between both protocols. All

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females survived the cesarean section and had an appropriate post-operative recovery; bitches were consciousness within 30 minutes after surgery, being monitored for the next 3 hours.

Table 4. Mean (± sd) of blood pressure and heart rate throughout cesarean section in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

	Blood pressure (mm Hg)		Heart rate (beats/min)	
Minutes	CS_P	CS_A	CS_P	CS_A
0	82.5 ± 3.1	88.4 ± 1.9	92.1 ± 4.1[a]	118.4 ± 3.9[b]
5	82.9 ± 4.1	80.1 ± 2.3	88.5 ± 4.5[a]	102.8 ± 5.1[b]
10	79.1 ± 5.3	81.5 ± 1.5	87.2 ± 4.9	94.6 ± 5.5
15	78.2 ± 2.5	80.8 ± 1.4	85.3 ± 3.8	91.4 ± 3.0
20	79.4 ± 2.8	81.2 ± 1,8	83.7 ± 4.0	88.3 ± 3.4
25	70.4 ± 3.7	72.5 ± 3.5	80.5 ± 3..4	84.7 ± 3.7
30	69.5 ± 5.2	70.1 ± 3.3	81.0 ± 3.7	84.5 ± 5.2
35	74.5 ± 3.3	72.7 ± 2.8	86.2 ± 4.0	89.1 ± 4.2

[ab]: Different letters in the same file and parameter (blood pressure/heart rate) denote significant differences ($p<0.05$).

The incidence of neonatal mortality is expressed in Table 5. Total neonatal mortality was 8.1% (10/124), but when stillborn puppies (6/10) were excluded, neonatal mortality after cesarean section dropped to 3.2% (4/124). No significant differences were observed between CSP and CSA, with similar percentages in the percentages of stillborn puppies and the puppies born alive but finally died, between 15-30 minutes after delivered. The percentage of bitches showing neonatal mortality was quite similar (35.7% vs. 28.6%, CSP, and CSA, respectively), and once excluded the surgeries with stillborn puppies, neonatal mortality was recorded in only 10.71% (3/28) of the cesarean sections.

Table 5. Neonatal viability after cesarean sections in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

	CS_P	**CS_A**	**Total**
Neonates stillborn	4.7% (3/64)	5.0% (3/60)	4.8% (6/124)
Neonates born alive that died	3.1% (2/64)	3.3% (2/60)	3.2% (4/124)
Total neonatal mortality	7.8% (5/64)	6.7% (4/60)	8.1% (10/124)

The mean average of the Apgar test increased slightly in each measurement in both protocols, and the difference between the Apgar 0 evaluation (7.0 ± 0.2 and 8.1 ± 0.2, CSP and CSA, respectively) and the Apgar 120 score (9.1 ± 0.2 and 9.7 ± 0.1, CSP and CSA, respectively) was markedly significant ($p< 0.01$). When both protocols were compared, it was observed that the alfaxalone protocol had higher Apgar test values ($p < 0.05$) in all three periods studied (Apgar 0: 7.0 ± 0.2 vs 8.1 ± 0.2; Apgar 60: 8.2 ± 0.2 vs 9.1 ± 0.1, Apgar 120: 9.1 ± 0.2 vs 9.7± 0.1, CSP and CSA, respectively). Finally, neonatal viability (critical, moderate, normal) for each period in both protocols is shown in Table 6. In the CSP group, immediately after birth, about 60% of the puppies were classified as normal neonates (score > 7), significantly higher ($P<0.01$) than recorded in moderate and critical neonates. In addition,

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neonates in critical condition decreased markedly at 60 and 120 minutes after birth, while the percentage of neonates in moderate or normal condition remained consistent. Regarding CSA group, the percentage of neonates with normal viability was notably higher ($p<0.005$) than the number of critical puppies in the Apgar 0 evaluation, and all neonates reached the best score at 120 minutes after birth. When both protocols were compared, it was observed that the percentage of normal neonates was higher in group CSA in Apgar 0, and this difference became more evident ($p<0.01$) at 60 and 120 minutes after birth, with values between 95-100 % of normal neonates in CSA group, while in CSP group were between 75-80%. Finally, in both protocols, no neonate decreased their score over time.

Table 6. Neonatal viability based on the Apgar score in in bitches induced with propofol (CS_P) or with alfaxalone (CS_A).

		Neonatal score		
	Groups	Critical (score < 3)	Moderate (score: 3-7)	Normal (> 7)
Apgar 0	CS_P	9.3%^{a,1} (5/54)	33.3%^{b} (18/54)	57.4%^{c} (31/54)
	CS_A	1.7%^{a,2} (1/58)	25.9%^{b} (15/58)	65.5%^{c} (38/58)
Apgar 60	CS_P	1.8%^{a} (1/54)	22.2%^{b,1} (12/54)	75.9%^{c,1} (41/54)
	CS_A	0%^{a} (0/58)	3.4%^{b,2} (2/58)	96.6%^{c,2} (56/58)
Apgar 120	CS_P	0%^{a} (0/54)	20.4%^{b,1} (11/54)	79.6%^{c,1} (43/54)
	CS_A	0%^{a} (0/58)	0%^{b,2} (0/58)	100%^{c,2} (58/58)

^{abc} Different letters in the same file and Apgar score denote significant differences ($p<0.01$); ^{12} Different numbers in the same row and Apgar score denote significant differences ($p<0.01$).

4. Discussion

Different studies have assessed the use of alfaxalone for the induction and maintenance of anesthesia in bitches. However, to our knowledge, this study describes for the first time the comparison between propofol and alfaxalone to induce and the anesthetic maintenance in all the basic reproductive surgical procedures (ovariohysterectomies and cesarean sections) usually performed on the bitch.

The use of propofol and alfaxalone did not show relevant differences over the maternal parameters assessed, when ovariohysterectomy was performed in healthy bitches (OVH) and bitches with pyometra (OVHP), as well as when cesarean sections were compared. However, when propofol was used, a higher percentage of bitches needed extra-doses to complete the induction, compared with alfaxalone. Taking into account that the infusion rate was similar in both protocols, it could indicate that alfaxalone produces deeper anesthesic induction than propofol. Different studies have concluded that the doses of drugs can be adjusted depending on the premedication used [23] and a slower infusion rate resulted in lower propofol and alfaxalone induction doses. Several studies have reported, when alfaxalone is used, an increase in heart rate as well as hypotension, especially at high doses; while with propofol, only hypotension is observed [24,25]. In our study, a transient tachycardia appeared in 9.09% of the female dogs treated with alfaxalone, but heart rate was similar between protocols less than 10 minutes after the anesthesic induction. During surgery, mean arterial pressure (MAP) showed the same behavior in both protocols. A mild to moderate decrease in MAP has been described in dogs after anesthetic induction using therapeutic doses [24,25] and other study [9] reported significant changes of the MAP when the suspensory ligament was pulled in female dogs when the induction and anesthesic maintenance was performed with propofol. In our study, no significant differences were observed between protocols, probably because sevoflorane was used to maintein the anesthesia. Intraoperative pain was basically monitored controlling the heart rate and MAP; when heart rate increased, a microdose of fentanyl was administetrated to bitches with slightly

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higher values in the propofol group. This finding agrees with the reported results when alfaxalone is used instead of propofol for induction and/or maintenance [9].

No studies have compared anesthetic parameters in female dogs with pyometras using propofol or alfaxalone. Our study showed that alfaxalone and propofol can be used as induction agents in hemodynamically unstable dogs. In the present study, bitches with pyometra (classified as unstable dogs) responded correctly to both agents when different parameters such as temperature, heart rate, and MAP were measured during surgery. In both groups (alfaxalone and propofol), female dogs experienced transient tachycardia after induction, but never showed apnea. Temperature decreased in both groups during surgery, which was associated with the influence of the anesthesic agents [24]. When the values were compared when ovariohysterectomy was performed in healthy bitches, no significant differences in heart rate, temperature, or MAP were found. Some studies have reported a harder recovery when alfaxalone was used for induction and maintenance, but may be related to the level of sedation prior to surgery [26]. In the present study, the recovery degree was practically similar between protocols, both in healthy bitches and in bitches with pyometra.

Several studies have found that neonates delivered by cesarean sections showed a mortality rate ranging from 4 to 15% [3,16,27–29]. In the present study, neonatal mortality was 8.1% and after removing the stillborn neonates, only 3.1% (4/124) of the newborns were born alive but died within 2 hours; 1 neonate had a severe congenital pathology (anasarca), and the other three came from cesarean sections with fetal stress. Some studies [3] reported lower neonatal mortality in caesarean sections with smaller litters (< 4 puppies), but other studies reported greater neonatal viability in caesarean sections of large litters [29]. Our study showed that neonatal mortality was more frequent in bitches with large litters and belong to brachycephalic breeds. This finding has been reported in other studies that show a high neonatal mortality rate (immediately after birth) associated with brachycephalic breeds [29,30].

Similar to ovariohysterectomies, when propofol was used in caesarean sections, it was necessary to apply extra doses to complete the induction up to 4 times higher than that registered in bitches induced with alfaxalone. This result seems to confirm that, in all the surgical procedures in the study, the efficacy of alfaxalone was higher to induce a greater degree of anesthesia than that observed with propofol. Our findings agree with different studies [12] which confirm a greater efficacy of alfaxalone to induce a correct anesthetic plan to subsequently carry out cesarean sections, using practically similar induction doses, either propofol (4 mg/kg) or alfaxan (2-3 mg/kg). In the same way, the physiological parameters of the mother during surgery were similar in both surgical protocols, indicating that anesthetic maintenance (till puppy delivered) can be carried out interchangeably with propofol or alfaxalone, without evident changes in temperature, heart rate or MAP of the dam. In addition, both protocols presented the same degree of neonatal viability, similar to different studies that did not detect differences in the rate of neonatal mortality, comparing both anesthetic protocols [12,29].

Apgar test is a useful tool to assess the neonatal viability immediately after birth or a cesarean section [17]. In our study, a lower score was measured immediately after birth (Apgar 0) and increased 120 minutes following delivery (Apgar 120), similar to the results described in different studies [3,31] reporting mean values between 7.5 to 8.8. The anesthesic protocol showed influence over the Apgar test, with a higher proportion of neonates in optimal condition (score 7–10) immediately after delivery when alfaxalone was used and these results are in agreement with previous studies [11]. Furthermore, alfaxalone reduced the duration of apnea in the mother, and as a result, the neonatal score at birth was higher when compared to propofol [12] and a better viability was observed in neonates born of mothers where alfaxalone was used [11].

Based on the results of this study, the influence of the anesthetic protocol can be linked to neonatal viability. Mortality was observed in puppies showing a lower Apgar score at birth, but 100% survival was found when neonates had an Apgar score > 5, independently of the anesthesic protocol. However, in the alfaxalone group, neonates presented a better Apgar test immediately after birth, confirming this trend in the following measurements. Some studies have reported that the neonates awaken from anesthesia faster and with greater vitality [11] when alfaxalone is used. This could

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explain that neonates born via caesarean section with propofol had a lower mean score in the first Apgar measurement when compared to the alfaxalone group. All anesthesic drugs can cross the placenta, so propofol or alfaxalone used to perform induction and anesthesic maintenance in dams, may affect to the fetuses. As a side note, the placental transfer of propofol is quick [32] and intravenous propofol applied until the removal of all neonates could produce the same effect (respiratory depression) on the neonates as it did on the mother. Furthermore, propofol promotes the appearance of hypotension and decreases the resistance in the circulatory system and blood flow from the heart; these effects can also be observed in neonates. However, when alfaxalone was used, maternal cardiovascular depression was minimal, resulting in a compensatory increase in heart rate. If these same effects appear in neonates, it may explain because puppies born via caesarean section with propofol had a lower Apgar score and a lower vitality immediately after birth. Regardless of the anesthesic protocol, viability improved in neonates 120 minutes after birth, most likely because puppies had metabolized the anesthetic drugs received through the placenta, normalizing their vital functions. Some authors have suggested that once hypoxia is removed, the respiratory and cardiovascular functions quickly return to normality, improving neonatal viability [33,34].

5. Conclusions

Based on the findings of this study, propofol and alfaxalone shown to be safe for healthy bitches and bitches with pyometra, undergoing an ovariohysterectomy, without significant differences regarding the hemodynamic behavior during surgery. In addition, both anesthetic protocols were optimal for performing cesarean sections, but alfaxalone was associated with a higher neonatal viability and, presumably, a higher neonatal survival rate.

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Institutional Review Board Statement: The present study did not require ethical approval. All and all the procedures were carried out according to the Spanish legislation about animal care (L7/2023, 28 March 2023) and the European Guidelines on Animal Welfare (Directive 2010/63/EU).

Informed Consent Statement: A written informed consent was signed by each owner to submit the bitches to ovariohysterectomy and cesarean section, to allow all the needed clinical procedures on females and newborns and use the clinical records for research purposes.

Data Availability Statement: Data will be available for all readers upon request.

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