PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

ISABELA PÍCOLO GUIMARÃES ALVES NICÁCIO

INSTILAÇÃO INTRAPERITONEAL DE ROPIVACAÍNA ISOLADA E ASSOCIADA À DEXMEDETOMIDINA PARA O CONTROLE DA DOR APÓS OVARIOSALPINGOHISTERECTOMIA EM GATAS

Presidente Prudente - SP
2018

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

ISABELA PÍCOLO GUIMARÃES ALVES NICÁCIO

INSTILAÇÃO INTRAPERITONEAL DE ROPIVACAÍNA ISOLADA E ASSOCIADA À DEXMEDETOMIDINA PARA O CONTROLE DA DOR APÓS OVARIOSALPINGOHISTERECTOMIA EM GATAS

Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientador:
Profa. Dra. Renata Navarro Cassu

Presidente Prudente - SP
2018

636.8
N582i

Nicácio, Isabela Pícolo Guimarães Alves.
Instilação intraperitoneal de ropivacaína isolada e associada à dexmedetomidina para o controle da dor após ovariosalpingohisterectomia em gatas / Isabela Pícolo Guimarães Alves Nicácio. – Presidente Prudente, 2018.
59f.: il.

Dissertação (Mestrado em Ciência Animal) - Universidade do Oeste Paulista – Unoeste, Presidente Prudente, SP, 2018.
Bibliografia.
Orientador: Renata Navarro Cassu

1. Ropivacaína. 2. Dexmedetomidina . 3. Instilação intraperitoneal . I. Título.

Catalogação na Fonte: Michele Mologni – CRB 8/6204

ISABELA PÍCOLO GUIMARÃES ALVES NICÁCIO

INSTILAÇÃO INTRAPERITONEAL DE ROPIVACAÍNA ISOLADA E ASSOCIADA À DEXMEDETOMIDINA PARA O CONTROLE DA DOR APÓS OVARIOSALPINGOHISTERECTOMIA EM GATAS

Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal – Área de concentração: Fisiopatologia Animal.

Orientador:
Profa. Dra. Renata Navarro Cassu

BANCA EXAMINADORA

_____
Profa. Dra. Renata Navarro Cassu - Orientadora
Universidade do Oeste Paulista – Unoeste
Presidente Prudente - SP

_____
Prof. Dr. Stelio Pacca Loureiro Luna
Universidade Estadual Paulista - Unesp
Botucatu - SP

_____
Dra. Lidia Mitsuko Matsubara
Médica Veterinária Autônoma

DEDICATÓRIA

Dedico este trabalho a minha mãe, irmã e afilhada, por todo incentivo, amor, confiança, dedicação e por serem a razão da minha vida. Sem vocês eu nada seria.

AGRADECIMENTOS

Agradeço primeiramente a Deus, pois sem Ele nada seria possível.

Agradeço a minha orientadora Profa Dra Renata Navarro Cassu, por estar ao meu lado em mais uma etapa profissional, pela disponibilidade, confiança, compreensão, apoio, bom humor e amizade durante a realização deste trabalho.

Ao PROSUP CAPES pelo apoio financeiro, que me proporcionou dedicação exclusiva ao andamento da pesquisa; o crescimento tanto profissional quanto pessoal foi imensurável.

Aos animais, que inconscientemente me ajudaram e aos seus proprietários que facilitaram o processo, bem como as ONG´s Associação Protetora de Cães e Gatos (Pirapozinho - SP) e Ajude um Focinho (Rancharia - SP) que depositaram plena confiança em nossa equipe.

Ao meu marido Gabriel Montoro Nicácio pelo esforço realizado ao me ajudar como professor, cirurgião, amigo e companheiro; também pelo incentivo, pelos conselhos e pelas noites não dormidas devido aos horários das aferições.

Ao aprimorando de anestesiologia, José Sergio Costa Junior pela ajuda indispensável, compreensão, incentivo, paciência e longas horas de convivência.

As alunas de iniciação científica, Tatiane dos Santos Bruno e Ana Beatriz Flávio Stelle, pelas horas dedicadas ao andamento desta pesquisa, pelas noites em claro, pelos finais de semana longe da família, pelas longas conversas, carinho e paciência.

A professora de diagnóstico por imagem, Rejane Brinholli e as suas aprimorandas Luísa e Sheila, por toda a paciência ao adaptarem a rotina e ao carinho dedicado tanto a nós quanto aos pacientes.

Ao professor Rogério Giuffrida por toda paciência ao colaborar com as análises estatísticas.

A aprimoranda de clínica médica de pequenos animais, Mayra, por ser a responsável pelo recrutamento de grande parte dos pacientes através da divulgação do projeto.

As professoras de cirurgia de pequenos animais, Camila Angela Bernardi, e anestesiologia Glaucia Prada Kanashiro, bem como os aprimorandos desses dois setores (Gustavo, Andressa, Camila, Rafaela, Murilo e Letícia), estagiários e alunos,

pelo carinho e paciência ao ajudarem no que fosse preciso e adaptarem a rotina para facilitar o andamento desta pesquisa.

Ao amigo Denis Robison Gomes, pelo bom humor, conselhos, longas conversas e por não medir esforços ao me ajudar.

Agradeço a todos os amigos que tive o prazer de conhecer nesse período, que ajudaram a tornar essa jornada mais leve e feliz, cada um a sua maneira, por me ajudarem e me apoiarem, em especial: Matheus Rocha Ribeiro, Felipe Franco Nascimento, Carolina Bacarin e João Victor Passareli.

A todos os professores, funcionários e colegas da Pós Graduação e do Hospital Veterinário da UNOESTE que contribuíram de forma direta e indireta para a conclusão dessa jornada.

RESUMO

Instilação intraperitoneal de ropivacaína isolada e associada à dexmedetomidina para o controle da dor após ovariosalpingohisterectomia em gatas

Objetivou-se avaliar a eficácia analgésica e a segurança da instilação intraperitoneal (IP) da ropivacaína isolada e associada à dexmedetomidina para o controle da dor pós-operatória em gatas. Em estudo encoberto, foram avaliadas 45 gatas encaminhadas para OSH eletiva. Os animais foram sedados com a associação de acepromazina (0,05 mg/ kg) à meperidina (6 mg/kg), por via IM. A indução anestésica foi feita com propofol IV em dose suficiente para a intubação endotraqueal, seguindo-se a manutenção com isofluorano/O_2. Após a estabilização da anestesia geral, foi feita a incisão ventral da linha média, seguindo-se a distribuição aleatória dos animais em três tratamentos (n = 15), que consistiram na instilação IP de solução salina 0,9% (grupo S), ropivacaína 0,25% (1 mg/kg, grupo R), ropivacaína 0,25% (1 mg/kg) associada à dexmedetomidina (4 µg/kg, grupo RD). Durante as primeiras 24 horas após a extubação traqueal, o grau de analgesia foi mensurado utilizando-se IVAS e a Escala Composta Multidimensional-UNESP-Botucatu (ECM). Nas gatas com ECM > 6, morfina (0,1 mg/kg, IM) foi administrada como analgesia de resgate. Empregou-se teste qui-quadrado, ANOVA com teste de Tukey e teste de Kruskall-Wallis e Friedman para dados paramétricos e não paramétricos, respectivamente ($p < 0,05$). Os escores de dor não diferiram entre os grupos ($p > 0,05$). Analgesia de resgate foi necessária a partir de 2, 4 e 8 horas nos grupos S, R e RD, respectivamente. A incidência de suplementação analgésica não diferiu entre os grupos, sendo efetuados 7 resgates no grupo S e um resgate nos grupos R e RD ($p > 0,05$). Conclui-se que a instilação IP de ropivacaína isolada e associada à dexmedetomidina resultou em analgesia semelhante, e que a adição de dexmedetomidina não prolongou a duração da analgesia em gatas após OSH.

Palavras-chave: ropivacaína, dexmedetomidina, instilação intraperitoneal.

ABSTRACT

A comparison between intraperitoneal instillation of ropivacaine alone and in combination with dexmedetomidine for analgesia in cats following ovariohysterectomy

The aim of this study was to investigate the analgesic effects and adverse events of the intraperitoneal (IP) instillation of ropivacaine alone and in combination with dexmedetomidine in cats undergoing ovariohysterectomy (OHE). Prospective, randomized, blinded, positively controlled clinical study. Forty-five cats aged (mean ± standard deviation) 17 ± 9 months and weighing 2.6 ± 0.5 kg. The cats were sedated intramuscularly (IM) with meperidine (6 mg kg^{-1}) combined with acepromazine (0.05 mg kg^{-1}). Anesthesia was induced with intravenous propofol to effect, and maintained with isoflurane in oxygen. After the establishment of general anesthesia, the cats were distributed into three treatment groups (n = 15) which consisted of the IP instillation of saline solution 0.9 % (group S), ropivacaine 0.25 % alone (1 mg kg^{-1}, group R), and combined with dexmedetomidine (4 µg kg^{-1}, group RD). Postoperative analgesia was assessed for 24 hours post-extubation using an Interactive Visual Analog Scale (IVAS) and the UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS). Rescue analgesia was provided with IM morphine (0.1 mg kg^{-1}) if the MCPS ≥ 6. Data were analyzed using the chi-square test, Tukey test, Kruskal-Wallis test, and Friedman test ($p < 0.05$). The pain scores did not differ between groups at any time point ($p > 0.05$). Rescue analgesia was required in 4/15, 1/15, and 1/15 of the cats in the S, R, and RD groups, respectively ($p > 0.05$). Intra-operative bradycardia was more frequent in the RD and R groups compared to the S group ($p = 0.04$). As part of a multimodal pain therapy, IP ropivacaine or its combination with dexmedetomidine produced similar analgesic effects and apparently neither treatment improved analgesia compared to saline in cats undergoing ovariohysterectomy.

Keywords: $alpha_2$ adrenergic agonists, intraperitoneal, local anesthetic, pain.

SUMÁRIO

A comparison between intraperitoneal instillation of ropivacaine alone and in combination with dexmedetomidine for analgesia in cats following ovariohysterectomy

Abstract

Objective To investigate the post-operative analgesia and both intra-operative and post-operative adverse effects of the intraperitoneal (IP) ropivacaine and its combination with dexmedetomidine, as an adjunctive analgesic method, in cats undergoing ovariohysterectomy.

Study design Prospective, randomized, blinded, positively controlled clinical study.

Animals Forty-five client-owned cats.

Methods The cats were sedated intramuscularly (IM) with meperidine (6 mg kg^{-1}) combined with acepromazine (0.05 mg kg^{-1}). Anesthesia was induced with propofol and maintained with isoflurane. Meloxicam (0.2 mg kg^{-1}) was subcutaneously administered in all cats after intubation. After opening of the abdominal cavity, the cats were randomly distributed into three treatment groups (n = 15) which consisted of the IP instillation of saline solution 0.9% (group Control), ropivacaine 0.25% alone (1 mg kg^{-1}, group R), and combined with dexmedetomidine (4 μg kg^{-1}, group RD). Intraoperatively, electrocardiography, non-invasive arterial blood pressure and respiratory variables were monitoring. Pain was assessed preoperatively, and 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours post-extubation, using an Interactive Visual Analog Scale (IVAS) and the UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS). Sedation was assessed at

the same time points. Rescue analgesia (morphine IM, 0.1 mg kg^{-}) was administered if the MCPS ≥ 6. Data were analyzed using the chi-square test, Tukey test, Kruskal-Wallis test, and Friedman test ($p < 0.05$).

Results The pain scores, and the sedation scores did not differ between groups at any time point. The requirements for postoperative analgesia did not differ between groups. Intra-operative bradycardia was more frequent in the RD and R groups compared to the Control group ($p = 0.04$).

Conclusion and clinical relevance As part of a multimodal pain therapy, IP ropivacaine or its combination with dexmedetomidine produced similar analgesic effects and apparently neither treatment improved analgesia compared to saline in cats undergoing ovariohysterectomy.

Keywords alpha$_2$ adrenergic agonists, cat, intraperitoneal, local anesthetic, pain

Introduction

In recent years an increasing number of clinical studies have shown the benefits of intraperitoneal (IP) instillation of local anesthetic agents for immediately post-operative pain relief following laparoscopic cholecystectomy in humans (Shukla et al. 2015; Gupta et al. 2016; Das & Deshpande 2017). In veterinary medicine, positive analgesic effects, such as a decreased need for supplemental systemic analgesics and reduced pain scores, have been reported with IP analgesia using bupivacaine and lidocaine in dogs (Campagnol et al. 2012; Kim et al. 2012; Morgaz et al. 2014; Kalchofner Guerrero et al. 2016) and cats (Benito et al. 2016). However, recent studies demonstrated that the IP instillation of ropivacaine is an attractive option due to the prolonged analgesic effects

and low risk of cardiac and systemic toxicity (Gupta et al. 2016; Das & Deshpande, 2017; Lambertini et al. 2018). While bupivacaine consists of a mixture of equimolar amounts of both dextrorotatory and levorotatory enantiomers, ropivacaine consists of the pure S (-) isomer. Additionally, this local anesthetic is less lipophilic and less potent than bupivacaine (Zink & Graf. 2008). These physicochemical characteristics have been closely related to less risk of cardiac and systemic toxicity (Graf et al. 2002). In animal models, the intravenous administration of ropivacaine provided a wider safety margin than bupivacaine at equivalent and equipotent doses (Dony et al. 2000).

In humans, a recent clinical study reported that the IP instillation of ropivacaine provides more pronounced and prolonged analgesia when compared with IP bupivacaine after laparoscopic cholecystectomy (Das & Deshpande 2017). On the other hand, Lambertini et al. (2018) reported comparable analgesic effects following IP administration of bupivacaine or ropicavaine for dogs undergoing ovariohysterectomy. The divergence in the results of these studies might be attributed to the different doses of ropivacaine administered. In the human study the dose of ropivacaine used was higher compared to bupivacaine, while in the canine study the same doses were used of both local anesthetics.

In addition, improved quality and duration of analgesia have been demonstrated following the combination of local anesthetics with alpha$_2$ adrenergic agonists (Shukla et al. 2015; Acharya et al. 2018). Dexmedetomidine is a potent and selective alpha$_2$ adrenergic agonist that has been added to bupivacaine and ropivacaine in different techniques of local anesthesia (Brummett et al. 2011; Evangelista et al. 2017). In human clinical reports, the IP administration of dexmedetomidine with bupivacaine resulted in lower post-operative pain scores and longer analgesic duration compared to local anesthetic alone after laparoscopic surgery (Shukla et al. 2015; Oza et al. 2016).

The mechanism of action of alpha$_2$ adrenergic agonists in combination with local anesthetics is not well elucidated. Evidence suggests that the local analgesic effect of dexmedetomidine could be attributed to the direct inhibition of the Aδ and C fibers and to the blockade of the hyperpolarization-activated cationic current (Brummett et al. 2011). Furthermore, the vasoconstrictor potential of the dexmedetomidine may delay the absorption and increase the terminal elimination half-life of the local anesthetic (Benito et al. 2018).

Nevertheless, cardiovascular changes such as bradycardia, cardiac arrhythmias, and increased systemic vascular resistance (SVR) have been associated with intramuscular and intravenous dexmedetomidine administration in both cats and dogs, leading to concerns with respect to its use in small animal practice (Murrell et al. 2005; Monteiro et al. 2009; Pypendop et al. 2011).

To date, few studies have investigated the post-operative analgesic effects of IP local anesthetics in cats (Benito et al. 2016, Benito et al. 2018). In addition, there are no published studies regarding the IP instillation of ropivacaine in cats.

The aim of this study was to investigate the analgesic effects and adverse events of the IP instillation of ropivacaine alone and in combination with dexmedetomidine in cats undergoing ovariohysterectomy. The hypothesis was that the addition of dexmedetomidine to ropivacaine would decrease the post-operative pain scores and delayed the time to rescue analgesia.

Material and Methods

Animals

After obtaining informed consent, 45 crossbreed client-owned cats admitted for elective ovariohysterectomy were enrolled. The study was approved by the Institutional Animal

Care Committee (protocol 3843/2017 CEUA). Only cats with normal complete blood count and serum chemistry, aged ≥ 6 months, and with an American Society of Anesthesiologists physical status I (ASA I) were included in the study. The exclusion criteria were: pregnancy, lactation, extreme aggression, body weight < 2 kg, body condition score greater than 6 or less than 3 on a nine-point scale, and systemic diseases. The cats arrived at the hospital at least 48 hours prior to surgery to allow the observer to become familiar with each cat. Preoperatively, all cats were evaluated by abdominal ultrasonography for confirmation of the absence of pregnancy. Before each experiment, the cats were fasted overnight with free access to water.

Study design

In a prospective, randomized, blinded, positive-controlled clinical study, the cats were randomly assigned using an online software program (Research Randomizer, Computer software, http://www.randomizer.org/, Pennsylvania, USA) to receive one of the three treatments: saline (Control group, n = 15), ropivacaine 0.25% alone (group R, n = 15), and combined with dexmedetomidine (group RD, n = 15).

Anesthesia and surgery

All anesthetic procedures were performed by the same anesthetist who was blinded to the group allocation. The cats were sedated intramuscularly (IM) with meperidine (6 mg kg^{-1}; Dolosal, Cristália Produtos Químicos e Farmacêuticos, SP, Brazil) in combination with acepromazine maleate (0.05 mg kg^{-1}; Acepran 0.2%, Vetnil, SP, Brazil). Fifteen minutes later, the cephalic vein was aseptically catheterized with a 24 gauge catheter (Angiocath; Becton Dickinson Indústrias Cirúrgicas Ltda, SP, Brazil) and anesthesia was induced by intravenous (IV) administration of propofol (Propovan; Cristália Produtos Químicos e Farmacêuticos, SP, Brazil) for sufficient relaxation to achieve endotracheal intubation, and maintained by isoflurane at an end-tidal concentration of

126 1.66% ± 0.25 (Gas analyzer module VAMOS plus; Dräger, SP, Brazil) in oxygen, using
127 a non-rebreathing system (SAT 500; Takaoka, SP, Brazil). The gas analyzer was
128 calibrated before each anesthesia with a standard gas mixture (CO_2: 5 vol %, N_2O: 70
129 vol %, O_2: 24 vol % and isoflurane: 1 vol %) (White Martins Gases Especiais, SP,
130 Brazil). Lactated Ringer's solution (JP Indústria Farmacêutica, SP, Brazil) was
131 administered IV at 10 mL $kg^{-1}hour^{-1}$ until extubation. Subcutaneous meloxicam (0.2 mg
132 kg^{-1}; Maxicam, Ouro Fino Pet Saúde Animal, SP, Brazil) was administered to all cats
133 after the induction of anesthesia.

134 Electrocardiography, heart rate (HR), respiratory rate (RR), end-tidal carbon
135 dioxide concentration ($ETCO_2$), end-tidal isoflurane concentration (ET_{ISO}), oxygen
136 saturation of hemoglobin (SpO_2%), were continuously measured during anesthesia,
137 using a multi-parametric monitor (VAMOS plus; Dräger) and recorded every 5 minutes.
138 Arterial blood pressure was monitored indirectly by sphygmomanometry, with a
139 Doppler ultrasound device (Doppler 841-A; Parks Medical Electronics), using an
140 appropriately sized cuff, between 40 and 50% of the circumference of the thoracic limb,
141 with the probe placed over the metacarpal artery on the plantar surface. The end-tidal
142 concentration of isoflurane was adjusted based on non-invasive blood pressure (NIBP),
143 HR, and conventional signs of anesthesia (rotation of the eyes, loss of palpebral reflex
144 and loss of jaw tone).

145 Ovariohysterectomy was performed using a standard technique through median
146 laparotomy access in supine cats. All surgical procedures were performed by the same
147 surgeon using a 3-cm ventral midline approach and 3-clamp technique (Fossum 2018).

148 The anesthesia time (time elapsed from the administration of propofol to
149 discontinuation of isoflurane), surgery time (time elapsed from the first incision until
150 placement of the last suture), time to extubation (time elapsed from termination of

isoflurane until extubation), and recovery time (time elapsed from the time of discontinuation of isoflurane to voluntary movement into a sternal position) were recorded for each cat. Extubation was performed when the cat recovered the swallowing reflex.

Study groups

After the establishment of general anesthesia, the cats were distributed into three treatment groups (n = 15) which consisted of the IP instillation of saline solution 0.9% (0.4 mL kg^{-1}, Control group), ropivacaine alone (1 mg kg^{-1}; 0.25%, Ropi; Cristália Produtos Químicos e Farmacêuticos, SP, Brazil; group R), and combined with dexmedetomidine (4 μg kg^{-1}; Dexdomitor; Zoetis, SP, Brazil; group RD). In the R and RD groups, the ropivacaine 1% was diluted in a saline solution 0.9 % to obtain a concentration of 0.25%, achieving a final volume of 0.4 mL kg^{-1}. After the abdomen was surgically opened and the uterus and ovaries were exposed the local anesthesia or saline solution were instilled into the peritoneal space before performing any ligation of the ovarian pedicles or uterus, administering an equivalent volume on the ovarian pedicles (left and right) and uterine cervix, using a 3 mL syringe attached to a 22-gauge catheter, as reported by Benito et al. (2016). Five minutes later, the excisions of the pedicles and uterus were initiated.

Post-operative monitoring

The same single observer, unaware of the treatment groups, was responsible for the pain and sedation assessments, which were performed 24 hours prior to surgery (baseline), and 0.5, 1, 2, 4, 6, 8, 12, 18, and 24 hours after extubation. The observer was a veterinary post-graduate student, with experience in the assessment of pain in cats using behavioral indices. Pain was assessed by two different pain scoring systems, including the Interactive Visual Analogue Scale (IVAS, from 0 mm = no pain to 100 mm =

maximum pain) and UNESP-Botucatu Multidimensional Composite Pain Scale (MCPS, from 0 = no pain to 24 = maximum pain). The MCPS pain scoring involved only two domains (pain expression, scale range = 0-12 points; psychomotor change, scale range = 0-12 points) (Brondani et al. 2013). For scoring, each cat was initially evaluated for 1 minute in its cage. Following this, the cat was stimulated to move around, for observation of reactions and behavior. Finally, the incision and surrounding area of the abdomen was palpated using 2-3 digits, and the reaction of the cat was assessed and recorded (Benito et al. 2016).

Additionally, the monitoring of HR was continued into the post-operative period using a stethoscope, at the same moments at which pain and sedation scores were assessed.

Morphine was administered (0.1 mg kg^{-1} IM; XX) as rescue analgesia if the MCPS scores were ≥ 6 (0-24 points), as reported by previous studies (Benito et al. 2016, Benito et al. 2018). The number of cats requiring rescue analgesia and the number of morphine doses were recorded.

A numerical rating score was used for the assessment of the degree of sedation, where: 0 = Completely awake, able to stand and walk; 1 = Stands, but staggers when attempting to walk; 2 = With encouragement is unable to stand but laying in sternal recumbency with head elevated; 3 = Able to lift head with encouragement, but resting head down, sternal recumbency; 4 = Responsive to light stroking, lateral recumbency; 5 = Unresponsive to light stroking, lateral recumbency (Dobbins et al. 2002).

Adverse events

The occurrence of adverse events during the study period such as seizures, nausea, and cardiovascular effects (bradycardia, arrhythmias, hypertension, or hypotension) were recorded. Bradycardia, hypertension, and hypotension were defined as a HR < 120

beats minute^{-1}, NIBP < 90 mmHg, and NIBP > 140 mmHg, respectively for longer than 5 minutes consecutively (Trim 1994).

Outcome measures

The primary outcome measures were the pain scores assessed by the IVAS and MCPS pain scales and the requirement for the rescue analgesia. Secondary outcome measures included the sedation scores and adverse effects.

Statistical analysis

A sample size of at least 15 cats per group was estimated to achieve 80% statistical power to detect a mean pain difference of 30% between the treated groups (R and RD, mean MCPS expected of 2.7) and the Control group (mean MCPS expected of 4.0), and a standard deviation (SD) of 1.3, at an overall alpha level of 0.05. Mean MCPS pain scores and SD were estimated from a pilot study.

A Kolmogorov–Smirnov test was performed to assess the normality of the variables. Data are expressed as mean ± standard deviation (parametric variables) or median (range) (non-parametric variables) as appropriated.

Bodyweight, age, time to extubation, and surgical, anesthetic, and recovery times were compared between groups using one-way ANOVA followed by a Tukey´s test.

The incidence of adverse events in the three groups was compared using the Fisher exact probability test. A Kruskal-Wallis test was used to compare pain and sedation scores between groups. A Friedman test was used to compare differences in pain and sedation scores over time within each group. Corresponding areas under the curves (AUCs) of IVAS and MCPS were calculated from baseline until 24 hours using the trapezoidal method and compared between groups using a Kruskal-Wallis test.

Survival analysis was used to compare the probability of use of rescue medication in the three groups.

The number of cats that required rescue analgesia was compared between groups using the Fisher´s exact test. A Kruskal-Wallis test was used to compare the number of morphine doses administered post-operatively in the groups. Data from pain scores obtained after the first dose of rescue analgesia were removed from the statistical analyzes. All analyses were performed using GraphPad Prism7.0 (GraphPad Software Inc., CA, USA). Differences were considered significant when $p < 0.05$.

Results

Fifty cats were initially enrolled in the study, however only 45 of these met the inclusion criteria. Five cats were excluded (three cats exhibited aggressive behavior, one cat was diagnosed with pyometra, and one cat was pregnant).

There were no significant differences with respect to age, weight, and surgery, anesthesia, and extubation times between groups ($p > 0.05$). The recovery time was longer in the RD ($p = 0.011$) compared with the Control and R groups (Table 1).

In the intraoperative period, the incidence of bradycardia was higher in the R ($p = 0.043$) and RD groups ($p = 0.006$) compared with the Control group. Bradycardia was detected at 10 minutes after the IP instillation and lasted approximately 40 minutes, with an incidence of 53.3% (8/15 cats) and 33.3% (5/15 cats) in the RD and R groups, respectively. First degree heart block was observed in one and two cats in the R and RD groups, respectively.

For the MCPS scores, no significant differences were observed for either treatment or time ($p > 0.05$). Regarding the IVAS, there was no significant effect of treatment ($p > 0.05$), but a significant effect of time was found in all groups ($p < 0.05$). Compared to the baseline values, significantly higher IVAS scores were observed from 1 to 24 hours

in the R ($p < 0.0001$) and S ($p < 0.0001$) groups, and from 1 to 8 hours in the RD ($p < 0.0001$) group (Table 2). In the analysis of AUC, comparable results were detected between groups based on both IVAS and MCPS scores ($p > 0.05$).

Compared to the baseline, higher sedation scores were detected at 0.5 hour in the R ($p < 0.0001$) and Control ($p < 0.0001$) groups, and from 0.5 to 1 hour after extubation in the RD group ($p < 0.0001$) (Table 2).

The number of cats ($p = 0.17$) that required rescue analgesia and the number of morphine doses ($p = 0.15$) administered throughout the study period did not differ statically between groups. Four cats (26.6%) in the Control group required rescue analgesia between 1 to 2 hours after extubation. One cat (6.6%) in each of the R and RD groups received rescue analgesia at 4 and 8 hours after extubation, respectively. In the Control group, three cats needed two doses of rescue analgesia, and one cat required only one dose (total of 7 doses of morphine). In the R and RD, none of the cats needed rescue analgesia more than once (Table 3).

Regarding the post-operative adverse events, vomiting was observed in one cat of the RD group in the first hour after extubation. This cat did not receive morphine at any time point of the study.

Discussion

The results of this study demonstrated little or no benefits of administering IP ropivacaine alone or in combination with dexmedetomidine for post-operative pain management in cats undergoing ovariohysterectomy. Moreover, IP ropivacaine and its combination with dexmedetomidine provided comparable analgesic effects. Thus, the initial hypothesis that the addition of dexmedetomidine to ropivacaine could result in a more pronounced and prolonged analgesic effect was denied.

Previous studies have reported that IP instillation of local anesthetics, as part of a multimodal analgesia protocol, contributed to the decrease in post-operative pain scores and analgesic requirements in both dogs (Campagnol et al. 2012; Kim et al. 2012) and cats (Benito et al. 2016). Although the frequency of rescue analgesia did not differ significantly between groups, the R and RD groups demonstrated a trend towards lower requirement for rescue analgesia, suggesting that IP analgesia provided more effective control of post-operative pain compared to the control treatment. As in the current study, Benito et al. (2018) also reported satisfactory analgesic effects following IP administration of bupivacaine 0.25% plus epinephrine, and bupivacaine 0.25% plus dexmedetomidine (1 μg kg^{-1}) for cats undergoing ovariohysterectomy. The authors found low MCPS pain scores with minimal need for post-operative analgesic supplementation. Although the local anesthetic administered in the current study was not the same as reported by Benito et al. (2018), the similarity of the results suggests that the IP administration of ropivacaine 0.25% was as effective as bupivacaine 0.25% for post-operative pain relief in cats. Moreover, the higher dose of dexmedetomidine (4 μg kg^{-1}) administered in the current study seems to have produced analgesic effects equivalent to those reported by Benito et al. (2018), suggesting that the intensity of analgesia was not influenced by the dose. Previous studies have shown variable analgesic responses using thermal threshold testing in cats administered dexmedetomidine (Slingsby & Taylor 2008; Pypendop et al. 2014). In cats, comparable antinociceptive effects were found after dexmedetomidine administration of doses ranging from 5μg kg^{-1} to 20 μg kg^{-1} (Pypendop et al. 2014). Nevertheless, despite thermal nociceptive thresholds having been widely used to assess pain, they do not translate directly to clinically observable analgesia.

Despite our findings being comparable to those reported by Benito et al. (2018), it is important to emphasize that in their study, the cats received only meloxicam as a preventive analgesia, while in our study the same AINE was combined with meperidine. The decision to use meperidine was based on its short duration of action, approximately 1-2 hours in cats (Mathews et al. 2014), aiming to provide intraoperative analgesia with little influence on post-operative pain. For ethical concerns, due to the inclusion of a saline treated group, and in order to approximate this experimental design with daily clinical situations, the decision was made to administer meloxicam to all cats prior to surgery.

In contrast to our results, Lambertine et al. (2018) reported a high prevalence of rescue analgesia (41%) following ovariohysterectomy in dogs treated with IP ropivacaine 0.5% (3 mg/kg) in combination with morphine IM and carprofen SC. Additionally, the majority of dogs received rescue analgesia at 8 hours after extubation, while in the current study only one IP ropivacaine treated cat (6.6 %) required supplemental analgesia at 4 hours after extubation. Although our results cannot be directly compared with the study of Lambertine et al. (2018), since the species are not the same, the divergences might be explained by the particularity of each experimental design, such as different systemic analgesics used in combination with the IP block, concentrations of ropivacaine, and pain scoring systems. Besides this, it is likely that differences in the elimination half-life of ropivacaine between dogs and cats could justify the delayed time for rescue analgesia found by Lambertine et al (2018). However, until now, there are no published studies describing the pharmacokinetics profile of ropivacaine in cats.

In humans, clinical studies reported that the addition of dexmedetomidine to local anesthetics increased the duration of IP analgesia compared to the local anesthetic

alone (Oza et al. 2016; Elnabtity & Ibrahim 2018). Evidence suggests that local vasoconstriction induced by the addition of dexmedetomidine to peripheral nerve blocks plays an important role in the duration of analgesia (Ouchi et al. 2014). In cats, the maximum plasma concentration of bupivacaine was achieved at 30 ± 24 and 123 ± 59 minutes with a terminal elimination half-life of 4.79 ± 1.7 and 10.5 ± 10.3 hours, after the IP administration of bupivacaine alone and combined with dexmedetomidine, respectively (Benito et al. 2016; Benito et al. 2018). In the current study, the provision of rescue analgesia was needed four hours later in the RD in relation to the R, suggesting that the addition of dexmedetomidine increased the duration of analgesia. However, due to the small number of the cats receiving rescue analgesia, this effect cannot be confirmed.

In the present study the degree of pain was evaluated by two scoring systems, the IVAS, which has been widely employed for post-operative pain assessment (Benito et al. 2016; Ribeiro et al. 2017) and the MCPS which is a valid and reliable method for assessing acute pain in cats (Brondani et al. 2013). In agreement with our results, other studies also did not find significant differences between groups using IVAS and MCPS pain scores in cats undergoing ovariohysterectomy treated with IP analgesia (Benito et al. 2016; Benito et al. 2018). The IVAS is a subjective method based on visual observation, interaction with the assessor, and palpation of the wound. The MCPS involves the evaluation of the three domains identified as “pain expression”, “psychomotor changes” and “physiologic variables”. In the current study the MCPS assessment was based only on the first two domains, due to the difficulty in measuring arterial pressure using a vascular Doppler in cats with restless behavior. Thus, given the subjectivity of both pain scales, the previous training of the assessor was indispensable for the adequate recognition of pain. In addition, the adaptation period of 48 hours prior

350 to surgery was essential for the assessor to become familiar with the behavior of each
351 cat. In addition, low pain scores (mean IVAS below 20 mm, and mean MCPS below 2
352 points) were identified during the study period which could make the detection of
353 significant differences between groups difficult. Particular experimental conditions,
354 including an experienced surgeon, minimal tissue trauma, and the provision of
355 preoperative analgesia could explain the low pain scores found in all treatment groups.

356 The classical signs of toxicity of local anesthetics are related to the activation of
357 the central nervous system, including shivering, muscle twitching, seizure, and tremor
358 (Mathews et al. 2014). In addition, cardiovascular dose-dependent effects can also be
359 identified, such as direct myocardial depression, arrhythmias, prolonged conduction,
360 and total cardiovascular collapse (Zink & Graf 2008).

361 Although ropivacaine treated cats did not exhibit clinically significant signs of
362 local anesthetic toxicity, cardiac side effects were detected in the current study.
363 Intraoperatively, transitory bradycardia was the most frequent adverse event observed in
364 both groups treated with IP analgesia. Moreover, three cats treated with IP analgesia
365 presented first degree heart block, which is defined as a PR interval > 90 ms (Côté
366 2010). Evidence has suggested that ropivacaine may increase PR intervals, resulting in
367 first degree heart block and bradycardia (Borgeat et al. 2004). In addition, stimulation of
368 the peripheral alpha$_2$ receptors by the dexmedetomidine determines an increase in the
369 vagal tonus which can also contribute to the occurrence of these events (Murrell et al.
370 2005). Both the bradycardia and first degree heart block were considered minor
371 complications and did not require specific treatment. In view of the high incidence of
372 bradycardia (53%) in the RD group, it seems that the vagolytic effects of meperidine did
373 not interfere in the changes induced by the dexmedetomidine on heart rate. This result is
374 supported by a previous study that reported that the IM administration of

375 dexmedetomidine alone or in combination with meperidine resulted in comparable
376 decreases in heart rate in cats (Nagore et al. 2013). Overall, arterial blood pressure
377 remained stable with some fluctuations, during the intraoperative period, independent of
378 the treatment administered.

379 In contrast from other studies that reported a high incidence of vomiting (> 50%)
380 following IM dexmedetomidine in cats (Monteiro et al. 2008; Nagore et al. 2013), in the
381 current study this effect was observed in only one cat (6.6%) of the RD group. As this
382 cat did not receive morphine, it is likely that the vomiting was induced by
383 dexmedetomidine. The low incidence of vomiting observed in the current study may be
384 attributed to the dose of dexmedetomidine administered. Recently, a study showed that
385 emesis in cats was induced with a mean dose of 7.0 μg/kg of dexmedetomidine
386 (Thawley et al. 2015).

387 In the post-operative period, the recovery time was longer in the RD group,
388 suggesting a residual sedative effect of dexmedetomidine. In the present study, the time
389 elapsed from dexmedetomidine instillation until the highest sedative effect was 56 ± 7
390 minutes (30 minutes after extubation). This result is in agreement with Pypendop et al.
391 (2014) who reported a sedative effect for 60 minutes after intravenous administration of
392 5 μg kg^{-1} of dexmedetomidine in cats. As behavior responses can be affected by the
393 degree of sedation, it is possible that the high sedation scores impaired the pain
394 assessments between 0.5 and 1 hour post-extubation. The animal´s reaction to von Frey
395 thresholds and to palpation of surgical incision, abdomen, and flank could be impaired
396 by a profound degree of sedation, increasing the MNT and decreasing the pain scores.
397 On the other hand, through MCPS assessment, a high degree of sedation could interfere
398 in the psychomotor responses, increasing the pain scores. Besides the residual
399 dexmedetomidine sedative effect, the preoperative administration of acepromazine may

400 also have contributed to the post-operative sedative effects observed in all treatment
401 groups. However, it is difficult to quantify whether the sedation interfered in the pain
402 assessment.

403 One potential limitation of the current study is associated with the preoperative
404 provision of meloxicam which may have masked differences in the pain responses
405 between groups. Previous studies have reported satisfactory analgesic effects for up to
406 24 hours after administering a single dose of meloxicam (0.2 mg kg^{-1}) in cats
407 undergoing ovariohysterectomy (Gassel et al. 2005; Benito-de-la-Víbora et al. 2008).
408 Thus, the inclusion of a negative control group (no analgesic treatment) may have been
409 useful in detecting a treatment effect. However due to ethical concerns this group was
410 not included in the present study. Analysis bias may have occurred in the pain and
411 sedation assessments due to the exclusion of cats from the study after receiving rescue
412 analgesia. This approach may have reduced the power of our study and may have been
413 the reason for the lack of detection of major differences. Another limitation of this study
414 is the dose administered of both ropivacaine and dexmedetomidine. Due to the lack of
415 studies using dexmedetomidine as an adjunct of local anesthesia in small animals during
416 the experimental design of the current study, the dose at 4 μg kg^{-1} was based on a
417 previous study which investigated the effects of the combination of local anesthetic and
418 dexmedetomidine through an epidural route in cats (Sousa et al. 2010). Additionally,
419 until now, there are no studies concerning the IP instillation of ropivacaine for the relief
420 of post-operative pain in cats. Thus, the dose administered of ropivacaine was based on
421 the veterinary literature in order not to exceed the maximum recommended dose for
422 cats, especially because there is limited information regarding toxic dosing of this local
423 anesthetic in cats. Furthermore, the dilution of ropivacaine from 1% to 0.25% with
424 saline may have altered the physiochemical properties of the drug, as pH and pKa have

425 major effects on local anesthetic onset of action and duration of effect. Although
426 clinically relevant signs of systemic toxicity were not detected at the dose and
427 concentration of ropivacaine administered, the pharmacokinetic profile was not
428 evaluated in the current study. The comparison of the terminal half-life and time to peak
429 plasma concentration between the R and RD groups would have provided more power
430 to our study to determine if the addition of dexmeditomidine prolonged the duration of
431 action of ropivacaine or delayed the time to peak plasma concentration.
432

433 **Conclusions**

434 As part of a multimodal pain therapy, IP ropivacaine or its combination with
435 dexmedetomidine produced similar analgesic effects and apparently neither treatment
436 improved analgesia compared to saline in cats undergoing ovariohysterectomy. Further
437 studies are needed to determine the ideal dose and concentration, as well as the
438 pharmacokinetic profile of IP ropivacaine in cats.
439

440 **Acknowledgements**

441 This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de
442 Nível Superior – Brazil (CAPES) – Finance Code 001. The authors thank the owners of
443 the cats for their contributions and the clinicians in the Department of Veterinary
444 Surgery and Anesthesiology, University of Western São Paulo, for their support.
445

446 **Authors' contributions**

447 IPGAN: study design, perioperative care, behaviour scoring, rescue analgesia, drafting
448 of manuscript. ABFS: recruitment and enrolling study animals. TSB: data acquisition,
449 data management. JSCJ: anaesthesiologist, postoperative care. GMN: surgical

450 procedure. RBB: abdominal ultrasonography. RG: statistical analysis. RNC: study
451 design, data analysis, helped with statistical analysis, writing of manuscript. All authors
452 approved the final manuscript.
453

454 **References**

455 Acharya R, Baksi R, Mohapatra P (2018) Comparative analysis of duration of
456 postoperative analgesia between levobupivacaine and levobupivacaine with clonidine
457 after ultrasound-guided transversus abdominis plane block in patients undergoing
458 lower segment cesarean section. Anesth Essays Res 12, 943-948.
459 Benito J, Monteiro B, Beaudry F, Steagall P (2018) Efficacy and pharmacokinetics of
460 bupivacaine with epinephrine or dexmedetomidine after intraperitoneal
461 administration in cats undergoing ovariohysterectomy. Can J Vet Res 82, 124-130.
462 Benito J, Monteiro BP, Beaudry F et al. (2016) Pharmacokinetics of bupivacaine after
463 intraperitoneal administration to cats undergoing ovariohysterectomy. Am J Vet Res
464 77, 641-645.
465 Benito-de-la-Víbora J, Lascelles BD, García-Fernández P et al. (2008) Efficacy of
466 tolfenamic acid and meloxicam in the control of postoperative pain following
467 ovariohysterectomy in the cat. Vet Anaesth Analg 35, 501-510.
468 Borgeat A, Ekatodramis G, Blumenthal S (2004) Interscalene brachial plexus anesthesia
469 with ropivacaine 5 mg/mL and bupivacaine 5 mg/mL: effects on electrocardiogram.
470 Reg Anesth Pain Med 29, 557-63.
471 Brondani JT, Luna SPL, Padovani, CR (2013) Refinement and initial validation of a
472 multidimensional composite scale for use in assessing acute postoperative pain in
473 cats. Am J Vet Res 72, 174-183.

474 Brummett CM, Hong EK, Janda AM et al. (2011) Perineural dexmedetomidine added to
475 ropivacaine for sciatic nerve block in rats prolongs the duration of analgesia by
476 blocking the hyperpolarization-activated cation current. Anesthesiology 115,
477 836-843.
478 Campagnol D, Teixeira-Neto FJ, Monteiro ER et al. (2012) Effect of intraperitoneal or
479 incisional bupivacaine on pain and the analgesic requirement after
480 ovariohysterectomy in dogs. Vet Anaesth Analg 39, 426-430.
481 Côté E (2010) Feline arrhythmias: an update. Vet Clin North Am Small Anim Pract 4,
482 643-50.
483 Das NT, Deshpande C (2017) Effects of intraperitoneal local anaesthetics bupivacaine
484 and ropivacaine versus placebo on postoperative pain after laparoscopic
485 cholecystectomy: A randomised double blind study. J Clin Diagn Res 11, UC08-
486 UC12.
487 Dony P, Dewinde V, Vanderick B et al. (2000) The comparative toxicity of 230
488 ropivacaine and bupivacaine at equipotent doses in rats. Anesth Analg 91, 1489-
489 1492.
490 Elnabtity AM, Ibrahim M (2018) Intraperitoneal dexmedetomidine as an adjuvant to
491 bupivacaine for postoperative pain management in children undergoing laparoscopic
492 appendectomy: A prospective randomized trial. Saudi J Anaesth 3, 399-405.
493 Evangelista MC, Doodnaught GM, Fantoni DT, Steagall PVM (2017) Sciatic and
494 femoral nerve blockade using bupivacaine alone, or in combination with
495 dexmedetomidine or buprenorphine in cats. Vet Rec 180, 524-592.
496 Fossum TW (2018) Small animal surgery (5rd edn). Mosby, USA.

497 Gassel AD, Tobias KM, Egger CM (2005) Comparison of oral and subcutaneous
498 administration of buprenorphine and meloxicam for preemptive analgesia in cats
499 undergoing ovariohysterectomy. J Am Vet Med Assoc 227, 1937-1944.
500 Graf BM, Abraham I, Eberbach N et al. (2002) Differences in cardiotoxicity of
501 bupivacaine and ropivacaine are the result of physicochemical and stereoselective
502 properties. Anesthesiology 96, 1427-1434.
503 Gupta M, Naithani U, Singariya G, Gupta S (2016) Comparison of 0.25% ropivacaine
504 for intraperitoneal instillation v/s rectus sheath block for postoperative pain relief
505 following laparoscopic cholecystectomy: A prospective study. J Clin Diagn Res 10,
506 UC10-5.
507 Kalchofner Guerrero KS, Campagna I, Bruhl-Day R et al. (2016) Intraperitoneal
508 bupivacaine with or without incisional bupivacaine for postoperative analgesia in
509 dogs undergoing ovariohysterectomy. Vet Anaesth Analg 43, 571-578.
510 Kim YK, Lee SS, Suh EH et al. (2012) Sprayed intraperitoneal bupivacaine reduces
511 early postoperative pain behavior and biochemical stress response after laparoscopic
512 ovariohysterectomy in dogs. Vet J 191, 188-192.
513 Lambertini C, Kluge K, Lanza-Perea M et al. (2018) Comparison of intraperitoneal
514 ropivacaine and bupivacaine for postoperative analgesia in dogs undergoing
515 ovariohysterectomy. Vet Anaesth Analg 45, 865-870.
516 Mathews K, Kronen PW, Lascelles D et al. (2014) Guidelines for recognition,
517 assessment and treatment of pain: WSAVA Global Pain. Journal of Small Animal
518 Practice 55**,** E10-68.
519 Monteiro ER, Campagnol D, Parrilha LR, Furlan LZ (2009). Evaluation of
520 cardiorespiratory effects of combinations of dexmedetomidine and atropine in cats. J
521 Feline Med Surg 11, 783-792.

522 Morgaz J, Muñoz-Rascón P, Serrano-Rodríguez JM et al. (2014) Effectiveness of pre-
523 peritoneal continuous wound infusion with lidocaine for pain control following
524 ovariohysterectomy in dogs. Vet J 202, 522-526.
525 Murrell JC, Hellebrekers LJ (2005) Medetomidine and dexmedetomidine: a review of
526 cardiovascular effects and antinociceptive properties in the dog. Vet Anaesth Analg
527 32, 117-127.
528 Nagore L, Soler C, Gil L et al. (2013) Sedative effects of dexmedetomidine,
529 dexmedetomidine-pethidine and dexmedetomidine-butorphanol in cats. J Vet
530 Pharmacol Ther 36, 222-228.
531 Ouchi K, Koga Y, Nakao S, Sugiyama K (2014) Dexmedetomidine dose-dependently
532 enhances local anesthetic action of lidocaine. J Oral Maxillofac Surg 72, 474-480.
533 Oza VP, Parmar V, Badheka J et al. (2016) Comparative study of postoperative
534 analgesic effect of intraperitoneal instillation of dexmedetomidine with bupivacaine
535 and bupivacaine alone after laparoscopic surgery. J Minim Access Surg 12, 260-264.
536 Pypendop BH, Barter LS, Stanley SD, Ilkiw JE. (2011) Hemodynamic effects of
537 dexmedetomidine in isoflurane anesthetized cats. Vet Anaesth Analg 38, 555-567.
538 Pypendop BH, Ilkiw JE (2014) Relationship between plasma dexmedetomidine
539 concentration and sedation score and thermal threshold in cats. Am J Vet Res 75,
540 446-452.
541 Ribeiro MR, de Carvalho CB, Pereira RHZ et al. (2017) Yamamoto New Scalp
542 Acupuncture for postoperative pain management in cats undergoing
543 ovariohysterectomy. Vet Anaesth Analg 44, 1236-1244.
544 Shukla U, Prabhakar T, Malhotra K et al. (2015) Intraperitoneal bupivacaine alone or
545 with dexmedetomidine or tramadol for post-operative analgesia following

546 laparoscopic cholecystectomy: A comparative evaluation. Indian J Anaesth 59, 234-
547 239.
548 Slingsby LS, Taylor PM (2008) Thermal antinociception after dexmedetomidine
549 administration in cats: a dose-finding study. J Vet Pharmacol Ther 31, 135–142.
550 Souza SS, Intelisano TR, De Biaggi CP et al. (2010) Cardiopulmonary and isoflurane-
551 sparing effects of epidural or intravenous infusion of dexmedetomidine in cats
552 undergoing surgery with epidural lidocaine. Vet Anaesth Analg 37, 106-115.
553 Thawley VJ, Drobatz KJ (2015) Assessment of dexmedetomidine and other agents for
554 emesis induction in cats: 43 cases (2009-2014). J Am Vet Med Assoc 247, 1415-
555 1418.
556 Trim CM (1994) Monitoring the anaesthetized cat. In: Anaesthesia of the cat (1th end).
557 Hall LH and Taylor PM (eds). Balliere Tindal, USA. pp 194-223.
558 Zanuzzo FS, Teixeira-Neto FJ, Teixeira LR et al. (2015) Analgesic and antihyperalgesic
559 effects of dipyrone, meloxicam or a dipyrone-meloxicam combination in bitches
560 undergoing ovariohysterectomy. Vet J 205, 33-37.
561 Zink W, Graf BM (2008) The toxicity of local anesthetics: the place of ropivacaine and
562 levobupivacaine. Curr Opin Anaesthesiol 21, 645-650.
563

Table 1. Demographic data and procedural times (mean ± standard deviation) of cats undergoing ovariohysterectomy treated with IP instillation of saline solution (Control, n = 15), ropivacaine (R, n = 15) and ropivacaine/dexmedetomidine (RD, n = 15).

Variables	**Group**			
	Control	R	RD	p-value
Body weight (kg)	2.5 ± 0.4	2.4 ± 0.4	2.8 ± 0.7	0.11
Age (months)	15 ± 8	16 ± 7	20 ± 13	0.43
Surgery time (minutes)	21 ± 4	20 ± 5	19 ± 4	0.61
Anesthesia time (minutes)	38 ± 8	40 ± 9	40 ± 9	0.83
Extubation time (minute)	9 ± 7	6 ± 3	11 ± 8	0.13
Recovery time (minutes)	38 ± 13	42 ± 15	62 ± 33[†]	0.01

[†] Significantly different from Control and RD groups ($p < 0.05$)

Table 2. Pain and sedation scores [median (range)] measured prior to ovariohysterectomy (BL) and at 0.5, 1, 2, 4, 6, 8, 18 and 24 hours after extubation in cats treated with IP instillation of saline solution (Control, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/dexmedetomidine (RD, *n* = 15).

Scale	**Group**	**Time (hours)**									
		BL	0.5	1	2	4	6	8	12	18	24
IVAS	Control	0 (0-0)	5 (0-10)	5 (0-35)*	5 (5-35)*	10 (0-25)*	5 (0-20)*	5 (0-20)*	5 (0-15)*	5 (0-10)*	5 (0-10)*
	R	0 (0-0)	5 (0-15)	10 (0-20)*	10 (5-20)*	10 (0-20)*	5 (5-15)*	5 (5-15)*	5 (0-15)*	5 (0-15)*	5 (0-15)*
	RD	0 (0-0)	5 (0-15)	5 (0-20)*	5 (0-25)*	5 (0-15)*	5 (5-20)*	5 (0-15)*	5 (0-10)	5 (0-10)	5 (0-5)
MCPS	Control	0 (0-9)	0 (0-2)	1 (0-15)	2 (0-8)	1 (0-12)	1 (0-6)	1 (0-6)	1 (0-4)	1 (0-2)	1 (0-2)
	R	0 (0-5)	1 (0-4)	1 (0-4)	1 (0-5)	2 (0-10)	1 (0-4)	1 (0-5)	1 (0-3)	1 (0-4)	1 (0-4)
	RD	0 (0-6)	0 (0-2)	0 (0-5)	0 (0-5)	1 (0-4)	1 (0-4)	1 (0-6)	1 (0-4)	1 (0-3)	0 (0-3)
Sedation Score	Control	0 (0-0)	1 (0-3)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	R	0 (0-0)	1 (0-3)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	RD	0 (0-0)	2 (0-5)*	1 (0-2)*	0 (0-1)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)

*Significantly different from baseline values ($p < 0.05$).

IVAS = Interactive Visual Analogue Scale; MCPS = UNESP-Botucatu Multidimensional Composite Pain Scale

Table 3. Number of rescue doses administered over time following ovariohysterectomy in cats treated with IP instillation of saline solution (Control, *n* = 15), ropivacaine (R, *n* = 15) and ropivacaine/dexmedetomidine (RD, *n* = 15).

	Post-operative time (hours)										
Group	0.5	1	2	4	6	8	12	18	24	Total number of rescue doses	Total number of rescued cats
Control	0	2	4	1	0	0	0	0	0	7	4/15
R	0	0	0	1	0	0	0	0	0	1	1/15
RD	0	0	0	0	0	1	0	0	0	1	1/15

ANEXO (VETERINARY ANAESTHESIA AND ANALGESIA – NORMAS)

GUIDE FOR AUTHORS

INTRODUCTION

Veterinary Anaesthesia and Analgesia (VAA) publishes original, peer-reviewed articles covering all branches of anaesthesia and the relief of pain in animals. Articles concerned with the following subjects related to anaesthesia and analgesia are also welcome: the basic sciences, pathophysiology of disease as it relates to anaesthetic management, equipment, intensive care, chemical restraint of animals

including wildlife and exotic animals, welfare issues associated with pain and distress, education in veterinary anaesthesia and analgesia.

VAA is making an effort to avoid publication bias and will publish negative studies that have been well-designed and conducted. VAA uses plagiarism-detection software.

Types of article

Original Studies. These articles usually should aim to be approximately 3500 words with a maximum word count (after review) of 4000 words (introduction through discussion). Normally there should not be more than 30-40 references and 4-6 tables and/or figures. These articles include original experimental or clinical research and meta-analyses. They require a structured abstract with a maximum of 300 words containing the following headings: Objective, Study design, Animals or Animal population, Methods, Results, Conclusions and clinical relevance.

Review articles. Review articles are papers which clarify, summarize and critically evaluate the current literature and should usually have <5000 words. These will normally be invited by the Editors or a member of the Editorial Board, although unsolicited, acceptable material will be considered for publication. Databases and

literature search strategy used should be defined in the Material and methods. The abstract should contain no more than 300 words and be structured with the following headings: Objective, Databases used, Conclusions.

Short Reviews--"What is the Evidence?" These are short review articles designed as a platform for discussion and debate of a specific topic or question. They should be from 1500 - 3500 words with approximately 20 references and up to four tables and/or figures (if needed). The abstract should contain no more than 300 words and be structured with the following headings: Objective, Databases used, Conclusions.

Short communications. Short communications describe small or preliminary experiments and the results. They should have a maximum of 2000 words; have ten or fewer references and no more than one figure or table. They require a structured abstract with a maximum of 300 words containing the following headings: Objective, Study design, Animals or Animal population, Methods, Results, Conclusions and clinical relevance. Case reports (case-based studies; either single or multiple animals). In general, VAA is no longer publishing case reports. In exceptional circumstances, they may be considered. Please contact the Editors prior to submission.

Letters. Letters should not exceed 800 words or 5 references, with one figure or table. These may be descriptions of new equipment, clinical observations, short case reports or comments that the correspondent believes to be of general interest to the readership. VAA does not routinely accept letters for publication criticizing existing publications. Where a reader feels such criticism is justified, they should write (by e-mail) directly to the Editors and they should aim to make their point in an objective, positive and constructive manner. The Editors will decide if or what action is required. The Editors' decision is final.

Other types. Historical notes, editorials, obituaries and book reviews are also published. These are generally invited by the Editors. Editorials usually should contain no more than 2500 words, 25 references and one table and/or figure. Please contact the Editors for more information.

If you need any further help please contact the Editorial Office: VAA@elsevier.com.

Submission checklist

You can use this list, to download as a PDF here, to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

A manuscript will be considered for publication only if the work detailed therein: Adheres to the Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals developed by the International Association of Veterinary Editors; Follows international guidelines for humane animal treatment as outlined in the Guide for the Care and Use of Laboratory Animals (NRC 2011) and associated guidelines: the Euroguide, the U.K. Animals (Scientific Procedures) Act (1986), the EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) The authors should clearly indicate in the manuscript that such guidelines have been followed in addition to stating that the

experiment was conducted in accordance to national laws.

Human and animal rights

All animal experiments should comply with and be reported according to the ARRIVE guidelines. If the work involves the use of human subjects, the author should ensure that the work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Use of inclusive language

Inclusive language acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, culture or any other characteristic, and should use inclusive

language throughout. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (e.g. 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess').

Reporting clinical trials

Randomized controlled trials should be presented according to the CONSORT guidelines. At manuscript submission, authors must provide the CONSORT checklist accompanied by a flow diagram that illustrates the progress of patients through the trial, including recruitment, enrollment, randomization,

withdrawal and completion, and a detailed description of the randomization procedure. The CONSORT checklist and template flow diagram are available online.

Other reporting guidelines

Please refer to the STROBE statement for observational studies and PRISMA for systematic reviews and meta-analyses. Further information regarding reporting guidelines for specialized studies can be found on the website of the Equator Network.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If there are no conflicts of interest then please state this:

'The authors declare no conflict of interest' (if there are multiple authors) or 'The author declares no conflict of interest' if there is one author only. Please include this statement on the title page upon submission. View this link for more information: About Conflict of Interest Statements.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyrightholder.

Authorship

Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and/or article preparation, so roles for all authors should be described. The statement that all authors have approved the final article should be true and included in the disclosure.

VAA refers to The International Committee of Medical Journal Editors (ICMJE) for the definition of authorship. ICMJE defines authors as those who: Made substantial contributions to the conception and design of, or acquisition of data or analysis and interpretation of data; Drafted the article or revised it critically for important intellectual content; Approved the final version to be published.

Authors should meet conditions 1, 2 and 3. Otherwise they should be mentioned in acknowledgements. Participation in the acquisition of funding alone, translation and/or editing of the manuscript alone or data collection alone does not merit authorship. Except in the case of complex large-scale or multi-center research, the number of authors should normally not exceed six. Please provide a statement on the title page defining the role of each author. For example:

Authors' contributions MD: data interpretation, statistical analysis and preparation of manuscript; RG: design, data management, and preparation of manuscript.

Changes to authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the

addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor consider the addition, deletion or rearrangement of authors after the manuscript has been accepted. While the Editor considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor will result in a corrigendum.

Copyright

Upon acceptance of an article, authors will be asked to complete a 'Journal Publishing Agreement' (see more information on this). An e-mail will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement.

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases.

Author rights

As an author you (or your employer or institution) have certain rights to reuse your work. More information.

Elsevier supports responsible sharing

Find out how you can share your research published in Elsevier journals.

AUTHOR INFORMATION PACK 29 Aug 2018 www.elsevier.com/locate/vaa 7

Role of the funding source

You are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

For anonymous review, this information should appear on the title page.

Funding body agreements and policies

Elsevier has established a number of agreements with funding bodies which allow authors to comply with their funder's open access policies. Some funding bodies will reimburse the author for the gold open access publication fee. Details of existing agreements are available online.

Open access

This journal offers authors a choice in publishing their research:

Subscription

• Articles are made available to subscribers as well as developing countries and patient groups through our universal access programs.

• No open access publication fee payable by authors.

• The Author is entitled to post the accepted manuscript in their institution's repository and make this public after an embargo period (known as green Open Access). The published journal article cannot be shared publicly, for example on ResearchGate or Academia.edu, to ensure the sustainability of peerreviewed research in journal publications. The embargo period for this journal can be found below.

Gold open access

• Articles are freely available to both subscribers and the wider public with permitted reuse.

• A gold open access publication fee is payable by authors or on their behalf, e.g. by their research funder or institution.

Regardless of how you choose to publish your article, the journal will apply the same peer review criteria and acceptance standards.

For gold open access articles, permitted third party (re)use is defined by the following Creative

Commons user licenses:

Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

For non-commercial purposes, lets others distribute and copy the article, and to include in a collective work (such as an anthology), as long as they credit the author(s) and provided they do not alter or modify the article.

The gold open access publication fee for this journal is USD 3000, excluding taxes. Learn more about

Elsevier's pricing policy: https://www.elsevier.com/openaccesspricing.

Elsevier Researcher Academy

Researcher Academy is a free e-learning platform designed to support early and mid-career researchers throughout their research journey. The "Learn" environment at Researcher Academy offers several interactive modules, webinars, downloadable guides and resources to guide you through the process of writing for research and going through peer review. Feel free to use these free resources to improve your submission and navigate the publication process with ease.

Language (usage and editing services)

Please write your text in good English (American or British usage is accepted, but not a mixture of these). Authors who feel their English language manuscript may require editing to eliminate possible grammatical or spelling errors and to conform to correct scientific English may wish to use the English

Language Editing service available from Elsevier's WebShop.

Submission

Our online submission system guides you stepwise through the process of entering your article details and uploading your files. The system converts your article files to a single PDF file used in the peer-review process. Editable files (e.g., Word, LaTeX) are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for

revision, is sent by e-mail.

Submit your article

Please submit your article via http://mc.manuscriptcentral.com/vaa

PREPARATION

Double-blind review

This journal uses double-blind review, which means that both the reviewer and author name(s) are not allowed to be revealed to one another for a manuscript under review. The identities of the authors are concealed from the reviewers, and vice versa. More information is available on our website. To facilitate this, please include the following separately:

Title page (with author details): This should include the title, authors' names and affiliations, a complete address for the corresponding author including an e-mail address, Acknowledgements (including funding), Authors' contributions and Conflict of Interest Statement.

Blinded manuscript (no author details): The main body of the paper (including the references, figures, and tables) should not include any identifying information, such as the authors' names or affiliations.

Equipment sources should be included but may be removed for review at the Editor's discretion.

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - unnumbered sections

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when crossreferencing text: refer to the subsection by heading as opposed to simply 'the text'.

Sections in the body of the manuscript (introduction to discussion) should not be separated by page breaks.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described. Excessive recycling of words from previous manuscripts, including the methods section, will not be allowed. See Elsevier's Ethics in Research & Publication brochure.

Specify in Materials and methods the ethical review committee approval process and the international, national, and/or institutional guidelines followed. Provide evidence in Materials and methods that the principles of reduction, refinement, and replacement have been met.

Statistics

For help with statistical reporting please refer to the SAMPL Guidelines which are available on the Equator Network. Further useful information on best practices in reporting sample size calculations in Randomized Controlled Trials in the field of anaesthesia can be found in the following British Journal of Anaesthesia article: *'Pitfalls in reporting sample size calculation in randomized controlled trials published in leading anaesthesia journals: a systematic review'*.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section may be appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Essential title page information

Title. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. *Author names and affiliations.* Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names.

Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author. *Corresponding author.*

Clearly indicate who will handle correspondence at all stages of refereeing and publication, and which author will be the corresponding author post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the pre-publication corresponding author.

Present/permanent address. If the first author who is not the corresponding post-publication author has moved since the work described in the article was done, or was

visiting at the time, a 'Present address' (or 'Permanent address') may be indicated as a footnote to that author's name.

This information should be provided on the title page upon manuscript submission. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes. Acknowledgments (including sources of funding)

Authors' contributions Conflict of interest statement

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references should not be included in the abstract. Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. The abstract should be on a separate page and should not exceed 300 words. For original research articles and short communications a structured abstract should be used with the following titles: Objective, Study design, Animals or Animal population, Methods, Results, Conclusions and clinical relevance. For review articles and "What is the Evidence?" articles the abstract should be structured and usually should have the following headings: Objective, Databases used and Conclusions.

Keywords

Up to six keywords or phrases should be listed immediately after the abstract. Ideally they should be MeSH headings. Note: these keywords are not necessarily the same that you will assign to your article during the submission process on ScholarOne.

Please use either British English or American English spelling (ensuring that this is consistent with the spelling used throughout the body of your manuscript) and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Abbreviations

Define abbreviations at their first mention in the body of the manuscript (introduction through discussion). Ensure consistency of abbreviations throughout the article. A list of commonly used abbreviations is available here.

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a

horizontal line for small fractional terms, e.g., X/Y. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should rarely be used. If used, indicate the position of the footnote in the text and present the footnote separately at the end of the article.

Artwork

Electronic artwork

General points

Make sure you use uniform lettering and sizing of your original artwork. Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier. Number the illustrations according to their sequence in the text. Use a logical naming convention for your artwork files. For Word submissions only, you may still provide figures and their captions, and tables within a single file at the revision stage. Please note that individual figure files larger than 10 MB must be provided in separate source files.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

Formats

Regardless of the application used, when your electronic artwork is finalized, please 'save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings. Embed the font or save the text as 'graphics'.

TIFF (or JPG): Color or grayscale photographs (halftones): always use a minimum of 600 dpi.

TIFF (or JPG): Bitmapped line drawings: use a minimum of 1000 dpi.

TIFF (or JPG): Combinations bitmapped line/half-tone (color or grayscale): a minimum of 600 dpi is required.

Please do not:

Supply files that are optimized for screen use (e.g., PowerPoint, GIF, BMP, PICT, WPG); the resolution is too low. Supply files that are too low in resolution. Submit graphics that are disproportionately large for the content.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. Please indicate your preference for color: in print or online only. Further information on the preparation of electronic artwork.

Illustration services

Elsevier's WebShop offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used in the legend.

Tables

Please submit tables as editable text and not as images, Excel files, or embedded in Word files. Tables should be placed on separate page(s) at the end after the references list or in a separate file. Number tables consecutively in accordance with their appearance in the text. The caption should be placed above the table and explain the origin of the data and any table notes should be placed below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

Reference links

Increased discoverability of research and high quality peer review are ensured by online links to the sources cited. In order to allow us to create links to abstracting and indexing services, such as Scopus, CrossRef and PubMed, please ensure that data provided in the references are correct. Please note that incorrect surnames, journal/book titles,

publication year and pagination may prevent link creation. When copying references, please be careful as they may already contain errors. Use of the DOI is encouraged.

A DOI can be used to cite and link to electronic articles where an article is in-press and full citation details are not yet known, but the article is available online. A DOI is guaranteed never to change, so you can use it as a permanent link to any electronic article. An example of a citation using DOI for an article not yet in an issue is: Clutton RE (2017) Recognizing the boundary between heroism and futility in veterinary intensive care. Vet Anaesth Analg, http://dx.doi.org/10.1016/j.vaa.2016.11.004 Please note the format of such citations should be in the same style as all other references in the paper.

Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Reference Style

Please follow the following guidelines when formatting the reference list in your manuscript: Abstracts that are more than two years old should not be used as references. Avoid abstracts as references when at all possible. Proceedings should not be used as references. References cited within the text that are 'unpublished observations' or 'personal communications' should not be included in the reference list.

Authors are responsible for verifying that the information provided under these headings is accurate and approved by the persons concerned. Information from manuscripts that have been submitted but not accepted should be cited as unpublished

observations. A modified Harvard style should be used in the reference list. Cite the author names followed by year of publication: (Jones 1997; Gregory 1999). Where there are two authors they should both be included with an ampersand: (Pascoe & Bennett 1999) Where there are three or more authors, the first author's name followed by et al. should be used: (Williams et al. 2016). If there is more than one reference per year from an author then distinguish with a letter: (Williams et al. 2016a) (Jones et al. 2016a,b) A detailed reference list should be supplied on a separate page, listed in alphabetical order of first author names. Journal titles should be abbreviated according to the standard forms in the National Library of Medicine, USA, database (MEDLINE or PubMed). Book titles should be written out in full. An EndNote style download is available here. The following are examples of style: Journal

Argraves WS, Suzuki S (1987) Amino acid sequence of the human fibronectin receptor. J Cell Biol 105, 1183-1190.

Andrade C, Sandarsh S, Chethan KB, Nagesh KS (2010) Serotonin reuptake inhibitor antidepressants and abnormal bleeding: a review for clinicians and a reconsideration of mechanisms. J Clin Psychiatry 71, 1565-1575.

Young LE, Blissitt KJ, Clutton RE et al. (1998) Temporal effects of an infusion of dobutamine hydrochloride in horses anesthetized with halothane. Am J Vet Res 59, 1027-1032.

Campagna I, Schwarz A, Keller S et al. (2015) Comparison of the effects of propofol or alfaxalone for anaesthesia induction and maintenance on respiration in cats. Vet Anaesth Analg 42, 484-492.

Larenza MP, Ringer SK, Kutter AP et al. (2009a) Evaluation of anesthesia recovery quality after lowdose racemic or S-ketamine infusions during anesthesia with isoflurane in horses. Am J Vet Res 70, 710-718.

Larenza MP, Peterbauer C, Landoni MF et al. (2009b) Stereoselective pharmacokinetics of ketamine and norketamine after constant rate infusion of a subanesthetic dose of racemic ketamine or Sketamine in Shetland ponies. Am J Vet Res 70, 831-839.

Conde Ruiz C, Del Carro A, Rosset E et al. (2015) Alfaxalone for total intravenous anaesthesia in bitches undergoing elective caesarean section and its effects on puppies: a randomized clinical trial. Vet Anaesth Analg. doi: 10.1111/vaa.12298 [Epub ahead of print].

Moher D, Liberati A, Tetzlaff J et al. (2009) Preferred reporting items for systematic reviews and metaanalyses: the PRISMA statement. PLoS Med 6, e1000097.

[No authors listed] (2013) Notice of formal retraction of articles by Dr. Y. Fujii. Br J Anaesth 110, 669. Book

Hall LW, Taylor PM (1994) Anaesthesia of the Cat (1st edn), Balliere Tindall, London, UK, pp. 189-193.

Pascoe PJ, Bennett RC (1999) Thoracic Surgery. In: Manual of Small Animal Anaesthesia and Analgesia (1st edn). Seymour C, Gleed R (eds). BSAVA, UK. pp. 183-196. Abstract

Portela D, Campoy L, Otero P et al. (2015) Ultrasound-guided thoracic paravertebral injection in dog cadavers. Vet Anaesth Analg 42, A55 (abstract).

Web address Seeler DC, Turnwald GH, Bull KS (1999) From teaching to learning:Part III. Lectures and approaches to active learning. J Vet Med Educ 21 http://scholar.lib.vt.edu/ejournals/JVME/V21-1/Seeler1.html Last accessed 1 January 2017.

Thesis Smith GY (1978) Title of thesis. PhD thesis, University. pp. 97-112.

Journal abbreviations source Journal names should be abbreviated according to the List of Title Word Abbreviations.

Video

Elsevier accepts video material and animation sequences to support and enhance your scientific research. Authors who have video or animation files that they wish to submit with their article are strongly encouraged to include links to these within the body of the article. This can be done in the same way as a figure or table by referring to the video or animation content and noting in the body text where it should be placed. All submitted files should be properly labeled so that they directly relate to the video file's content. . In order to ensure that your video or animation material is directly usable, please provide the file in one of our recommended file formats with a preferred maximum size of 150 MB per file, 1 GB in total. Video and animation files supplied will be published online in the electronic version of your article in Elsevier Web products, including ScienceDirect. Please supply 'stills' with your files: you can choose any frame from the video or animation or make a separate image. These will be used instead of standard icons and will personalize the link to your video data. For more detailed instructions please visit our video instruction pages. Note: since video and animation cannot be embedded in the print version of the journal, please provide text for both the electronic and the print version for the portions of the article that refer to this content.

Supplementary material

Supplementary material can support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, high-resolution images, background datasets, sound clips and more. Please note that such items are published online exactly as they are submitted; there is no typesetting involved (supplementary data supplied as an Excel file or as a PowerPoint

slide will appear as such online). Please submit the material together with the article and supply a concise and descriptive caption for each file. If you wish to make any changes to supplementary data during any stage of the process, then please make sure to provide an updated file, and do not annotate any corrections on a previous version. Please also make sure to switch off the 'Track Changes' option in any Microsoft Office files as these will appear in the published supplementary file(s). For more detailed instructions please visit our artwork instruction pages.

AFTER ACCEPTANCE

Online proof correction

Corresponding authors will receive an e-mail with a link to our online proofing system, allowing annotation and correction of proofs online. The environment is similar to MS Word: in addition to editing text, you can also comment on figures/tables and answer questions from the Copy Editor.

Web-based proofing provides a faster and less error-prone process by allowing you to directly type your corrections, eliminating the potential introduction of errors.

If preferred, you can still choose to annotate and upload your edits on the PDF version. All instructions for proofing will be given in the e-mail we send to authors, including alternative methods to the online version and PDF.

We will do everything possible to get your article published quickly and accurately. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. It is important to ensure that all corrections are sent back to us in one communication. Please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility.

Offprints

The corresponding author will, at no cost, receive a customized Share Link providing 50 days free access to the final published version of the article on ScienceDirect. The Share Link can be used for sharing the article via any communication channel, including email and social media. For an extra charge, paper offprints can be ordered via the offprint order form which is sent once the article is accepted for publication. Both corresponding and co-authors may order offprints at any time via Elsevier's Webshop. Corresponding authors who have published their article gold open access do not receive a Share Link as their final published version of the article is available open access on ScienceDirect and can be shared through the article DOI link.

AUTHOR INQUIRIES

Visit the Elsevier Support Center to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also find out when your accepted article will be published.