UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

SUELEN LORENA DA SILVA

Evaluation of the analgesic effect of an ultrasound-guided transverse abdominus plane block with 0.25% bupivacaine in bitches undergoing ovariohysterectomy

Dissertação apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Mestra em Anestesiologia.

Orientador(a): Prof. Dr. Francisco José Teixeira Neto

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FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Silva, Suelen Lorena da.
Evaluation of the analgesic effect of an ultrasound-guided transverse abdominus plane block with 0.25% bupivacaine in bitches undergoing ovariohysterectomy / Suelen Lorena da Silva. - Botucatu, 2023

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina de Botucatu
Orientador: Francisco José Teixeira Neto
Capes: 40102130

1. Cães. 2. Bupivacaína. 3. Ultrassom. 4. Disforia. 5. Dor pós-operatória. 6. Escala de resultado de Glasgow. 7. Ovários - Cirurgia. 8. Analgésicos - Efeito fisiológico.

Palavras-chave: Dysphoria; Glasgow scale; Postoperative pain; Ultrasound-guided block.

Suelen Lorena da Silva

Evaluation of the analgesic effect of an ultrasound-guided transverse abdominus plane block with 0.25% bupivacaine in bitches undergoing ovariohysterectomy

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Botucatu, 02 de março de 2023.

AGRADECIMENTOS

As Universidades públicas pelas quais passei e carrego comigo. A Unesp – Botucatu, pela grandiosidade do que me proporcionou.

Ao querido orientador, professor Chico, pelos ensinamentos, conhecimento compartilhado durante esses anos, por todo apoio e empenho neste projeto. Tenho você como exemplo de excelência na docência e na pesquisa, além da excelência, guarda o entusiasmo por tudo que faz.

A minha família por se fazer sempre presente, por me lembrar da minha força, capacidade e luz nos dias de escuridão.

A todos do grupo de pesquisa, impecáveis nesta parceria, Carol Hagy, Diego, Gustavo “Empinadinho”, Mayara “Moita”, Renatinha, Tábata.

A Natache, pela amizade, ensinamentos e parceria. Você possui um papel fundamental no meu crescimento.

Aos funcionários da FMVZ por auxiliarem no desenvolvimento do projeto, Ed, Clô, equipe da esterilização.

Ao pessoal da FMB, principalmente a Tatiane, pela paciência, auxílio e empenho.

A todos os meus amigos de Goiânia e de Botucatu por entenderem minha ausência, por serem um porto seguro em meio há algumas tempestades.

Aos tutores que confiaram no nosso trabalho e possibilitaram o desenvolvimento deste estudo.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) pela bolsa de estudo.

“E uma das condições necessárias a pensar certo é não estarmos demasiado certos de nossas certezas”

Paulo Freire

RESUMO

SILVA, SL. Avaliação da duração do efeito analgésico do bloqueio local ecoguiado TAP block com bupivacaína 0,25% em cadelas submetidas a ovariohisterectomia. Botucatu, 2023. 33 p. Dissertação (Mestrado) – Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Campus de Botucatu.

Este estudo objetivou avaliar a eficácia analgésica do bloqueio do músculo transverso abdominal utilizando-se bupivacaína 0,25%. Em um estudo prospectivo, duplo-cego, cadelas anestesiadas com isoflurano receberam aleatoriamente uma infiltração TAP bilateral guiada por ultrassom de dois pontos com soro fisiológico 0,9% (Controle, n = 15) ou bupivacaína a 0,25% (TAPbupi, n = 15) antes da ovariohisterectomia. Os escores de sedação e a forma abreviada da escala composta de dor de medida de Glasgow (CMPS-SF) foram avaliados por 3 observadores por 24 horas após a cirurgia. Analgesia de resgate foi fornecida se o Glasgow CMPS-SF excedesse o limite de acordo com pelo menos 2 avaliadores. A concordância entre os 3 observadores foi classificada como boa para sedação (kappa ponderado: 0,61-0,80) e Glasgow CMPS-SF (correlação intraclasse: 0,61-0,80). Observou-se alta incidência de analgesia de resgate nos grupos Controle (13/15) e TAPbupi (14/15) (p = 0,54). O número de intervenções analgésicas pós-operatórias não diferiu entre os grupos Controle (2,73 ± 1,71) e TAPbupi (3,53 ± 1,88) (p = 0,23). A incidência de comportamentos que podem estar associados à disforia ou dor induzida por opioides não diferiu entre os grupos: choramingar [11/15 (Controle) e 14/15 (TAPbupi), p = 0,14); ansioso/nervoso/com medo [4/15 (Controle) e 8/15 (TAPbupi), p = 0,13]. A infiltração incompleta do anestésico no plano interfascial foi relatada em um animal do grupo TAPbupi. Concluiu-se que a falha do bloqueio TAP em fornecer analgesia/antinocicepção superior pode ter sido causada por reações disfóricas aos opioides (choramingamento e ansiedade/nervosismo) ou, menos provavelmente, por infiltração incompleta do plano interfascial.

Palavras-chave: Disforia, escala de Glasgow, dor pós-operatória, bloqueio guiado por ultrassom.

ABSTRACT

SILVA, SL. Evaluation of the analgesic effect of an ultrasound-guided transverse abdominus plane block with 0.25% bupivacaine in bitches undergoing ovariohysterectomy. 2023. Botucatu, 2023. 33 p. MSc Thesis – Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Campus de Botucatu.

This study aimed to evaluate the analgesic efficacy of a *transversus abdominis* plane (TAP) block with 0.25% bupivacaine in dogs undergoing ovariohysterectomy**.** In a prospective, double blinded study, 30 animals were premedicated with intramuscular dexmedetomidine (3 µg/kg) and morphine (0.5 mg/kg), anesthesia was induced with propofol and maintained with isoflurane. Animals randomly received a four-point ultrasound-guided TAP infiltration with 0.3 mL/kg per site of 0.9% saline (Control, n = 15) or 0.25% bupivacaine (TAP_{bupi}, n = 15). Intravenous fentanyl (5 µg/kg) was administered before surgical incision in both groups. The short form of the Glasgow composite measure pain scale (CMPS-SF) was assessed by 3 observers for 24 hours. Rescue analgesia (methadone 0.2 mg/kg, intravenously) was provided if Glasgow CMPS-SF exceeded the threshold according to at least 2 observers. A high incidence of rescue analgesia was observed in the Control (13/15) and TAP_{bupi} (14/15) groups (p = 0.54). The number postoperative analgesic interventions did not differ between the Control (2.73 ± 1.71) and TAP_{bupi} (3.53 ± 1.88) groups (p = 0.23). Incidence of behaviors associated with opioid-induced dysphoria or pain did not differ between groups: whimpering [11/15 (Control) and 14/15 (TAP_{bupi}), p = 0.14); anxious/nervous/fearful [4/15 (Control) and 8/15 (TAP_{bupi}), p = 0.13]. Incomplete infiltration of the interfascial plane was reported in one animal of the TAP_{bupi} group. With the liberal use of opioids in the present study, failure of the TAP block to provide superior analgesia may have been biased by the high incidence of dysphoric reactions associated with the use of high cumulative doses of opioids.

Keywords: Dysphoria, Glasgow scale, postoperative pain, ultrasound-guided block,

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LISTA DE ABREVIATURAS E SIGLAS

CMPS- SF Glasgow Composite Measure Pain Scale – Short Form

et al – and others

FE´ISO - end-expired isoflurane concentration

f_R - respiratory rate

H- hora

HR –Heart rate

IM – intramuscular

IV – intravenous

Kg – kilogram

mg – milligram

ml – milliliter

MNT - mechanical nociceptive thresholds (MNT)

PE´CO_2 - end-expired carbon dioxide partial pressure

SAP – systolic arterial pressure

SpO_2- hemoglobin saturation

TAP *block* – *transversus abdominis* plane

$TAP_{bup}BUPI_{0,25\%}$ - bupivacaíne group

SUMÁRIO

1. INTRODUCTION

Acute postoperative pain after abdominal surgery may involve pain of somatic (abdominal wall) and visceral origin. Although incision of the abdominal wall could also result in somatic pain, the ovariohisterectomy surgery has been referred as a procedure that causes moderate visceral pain (Boscan et al., 2011).

First described in humans in 2006 (Odonnell, 2006), an ultrasound-guided *transversus abdominis* plane (TAP) block consists of injecting a local anesthetic into the interfascial plane between the *obliquus abdominis internus* and the *transversus abdominis* muscles with the aim of blocking the ventral branches of thoracic and lumbar nerves that provide sensitive innervation to the abdominal wall (Otero and Portela, 2019). To date, most veterinary studies evaluating the (TAP) block technique, have focused on the spread of dye (methylene blue) into the interfascial plane and on the number of thoracic and lumbar nerves stained in canine cadavers (Schroeder at al., 2011; Bruggink et al., 2012; Drożdżyńska et al., 2017; de Miguel Garcia et al., 2020; Freitag et al., 2021). Recent studies in dogs undergoing ovariohysterectomy surgery have shown that infiltration of the TAP with bupivacaine, combined or not with other adjuvant drugs (dexmedetomidine), resulted in superior analgesia compared to animals receiving a standard analgesic protocol (Campoy et al., 2022; Cavaco et al., 2022; Paolini et al., 2022).

For ethical reasons opioids are incorporated into pain studies to provide intraoperative and postoperative pain control. However, pure mu opioid analgesics have the potential to cause dysphoric reactions (e.g., whining, anxiety/fearfulness) which can be confounded as pain-like behaviors by the scores used to assess postoperative pain (Lucas et al., 2001; Troncy et al., 2002; Hofmeister et al., 2006; Becker et al., 2013). Therefore, opioid-induced dysphoria may cause an escalation in the use of rescue analgesia, biasing the results of studies evaluating alternative options to improve postoperative pain control.

The primary objective of this study was to evaluate the post-operative analgesic effectiveness of a two-point TAP block technique with 0.25% bupivacaine in female dogs undergoing ovariohysterectomy surgery. The hypothesis formulated was that, in dogs receiving an opioid-based analgesic protocol with morphine as premedication, fentanyl for intraoperative analgesia, and methadone for postoperative analgesia, a two-point TAP block technique with 0.25% bupivacaine would provide superior analgesia in comparison to a saline/placebo group receiving the same opioid-based analgesic protocol. As a second objective this study aimed to report the incidence of behaviors that could represent either opioid-induced dysphoria or pain.

2- MATERIAL AND METHODS

2.1. Animals and experimental design

After approval by the Institutional Animal Care and Ethics Committee (CEUA protocol number 179/2020) and informed owner consent, a total of 30 healthy bitches scheduled for elective ovariohysterectomy were included in the study. Assessment of health status was based on clinical examination and complete blood cell count/serum biochemistry values within reference ranges. Animals that displayed fear or aggressive behavior while interacting with the individuals in charge of performing pain assessment were excluded from the study.

In a randomized, double blinded, prospective design, animals were assigned to 2 groups (Control and TAP_{bupi}). In the TAP_{bupi} group (n = 15), a bilateral two-point TAP block with 0.25% bupivacaine was performed under ultrasound guidance before surgery. In the control group (n = 15) the same technique was performed with an equal volume of physiological saline. Assuming that 70% and 20% of animals in the control and treated groups would receive rescue analgesia (treatment failure), a total 15 of animals per group was estimated to be necessary to demonstrate a treatment effect with a statistical power of 80% and an alpha level of 5%.

2.2 Anesthetic protocol and recovery from anesthesia

Animals were previous fasted for 12 hours with free access to water until 1 hour before anesthesia. Intramuscular dexmedetomidine (5 µg/kg) (Dexdomitor, Zoetis) morphine (0.5 mg/kg) (Dimorf, Cristália) were administered as premedication. After placement of a catheter in a cephalic vein, anesthesia was induced 15 minutes later with propofol (4.0 ± 1.5 mg/kg, intravenously) (Propovan, Cristália) and maintained with isoflurane (Propovan, Cristália) under volume-controlled mechanical ventilation (tidal volume 12 mL/kg and inspiration-to-expiration ratio of 1:2) (Dräger Primus, Drägerwerk AG & Co). The inspired oxygen concentration was set at 40% and the respiratory rate adjusted to maintain end-expired carbon dioxide partial pressure ($PE´CO_2$) between 35 and 45 mmHg (Dräger Primus, Drägerwerk AG & Co). Monitored intraoperative variables included heart rate (HR) registered by a lead II electrocardiogram; Doppler systolic arterial pressure

(SAP) obtained by placing the 9.0 mHz Doppler sensor over a digital artery (cuff width-to-limb circumference ratio close to 0.4) (Model 811B, Parks Medical); hemoglobin saturation (SpO_2) recorded by a clip-type sensor placed in the tongue; end-expired carbon dioxide partial pressure ($PE´CO_2$) and end-expired isoflurane concentration (FE´ISO) recorded by an infrared gas analyzer incorporated into the anesthesia workstation (Dräger Primus, Drägerwerk AG & Co). The FE´ISO was adjusted produce surgical depth of anesthesia, defined as immobility associated with absence of palpebral reflexes and of spontaneous breathing efforts during mechanical ventilation. The FE´ISO was further increased if a hypertensive response (SAP > 140 mmHg) or if patient-ventilator asynchrony coincided with surgical stimulus. Esophageal temperature was maintained between 37 and 38^{o}C by means of a warm air device (Bair Hugger™ 775, 3M Corporate). A lactated Ringer´s solution (Ringer Lactato; JP Indústria Farmacêutica) (5 mL//kg/hour) was administered intraoperatively through the cephalic catheter.

After induction of anesthesia, a single individual blinded to treatment (S.L.S.) groups performed infiltration of either physiological saline (Control group) or 0.25% bupivacaine (TAP_{bupi} group) (Neocaína, Cristália) according to a two-point TAP block technique described in dogs by Portela et al. 2014. This individual had one year of previous experience in performing this technique. Briefly, with the dogs positioned in lateral recumbency, after the skin was clipped and surgically prepared, a multifrequency (16-5 mHz) linear transducer (VF16-5, Siemens) was placed on the lateral abominal wall, perpendicular to the spine, to visualize target anatomic structures (Acuson NX3, Siemens) in two locations: 1) caudal to the last rib; 2) cranial to the iliac tuberosity. A 22 Gauge, 5 cm long, echogenic needle (Sonoblock, Pajunk) was inserted according to an “in plane” technique in a ventral-dorsal direction until its tip was positioned in the interfascial plane between the *obliquus internus abdominis* muscle and the *transversus abdominis* muscle. Correct location of the needle in the interfascial plane was confirmed by hydrodissection, with clear separation of these two muscles during slow injection of 0.3 mL/kg of either physiological saline or 0.25% bupivacaine in each of the four anatomical locations. In case of absence of hydrodissection/suspected intramuscular injection at the beginning of the injection, the needle was repositioned, and the remaining volume was injected into the correct interfascial plane. After infiltration of the two sites in one hemiabdomen, the animal was flipped to the other side and the procedure was repeated on the ipsilateral abdominal wall. Animals where there was partial infiltration of the interfascial plane due to difficulties in positioning the needle tip in the correct location were recorded.

Fifteen minutes after concluding the ultrasound-guided interfascial infiltration intravenous fentanyl (5 µg/kg) (Fentanest, Cristália) was administered one single time over 1 minute and surgery was commenced by an experienced surgeon (G.G.P.) unaware of treatment groups. After the end of surgery, isoflurane vaporization was interrupted, and the animal was allowed to breath spontaneously. The endotracheal tube was removed upon the return of the swallowing reflex. Surgical time, total anesthesia time (time elapsed from endotracheal intubation until interruption of isoflurane vaporization) recovery time (time elapsed between interruption of isoflurane vaporization until the animal was standing) were recorded for comparison between groups.

2.3. Evaluation of sedation, pain, and nociception

Animals were admitted to the Veterinary Teaching Hospital on the day before surgery. Throughout the acclimatization period individuals responsible for evaluating postoperative pain positively interacted with the animals by providing snacks and taking them for strolling. A total of 3 observers (S.L.S, M.T.L., D.A.O.) unaware of treatment assignment evaluated sedation, pain, and nociception. All 3 observers had at least 2 years of experience with anesthesia and pain assessment in animals, and previously received a video training about pain behavior in dogs as a part of another study. A numeric rating scale was used to evaluate sedation; while pain and nociception were assessed by the short form of the Glasgow Composite Measure Pain Scale (Glasgow CMPS-SF) and by the mechanical nociceptive thresholds (MNT), respectively. Evaluations were performed at baseline (12 hours before surgery) and during the postoperative period for 24 hours of endotracheal tube removal (Figure 1).

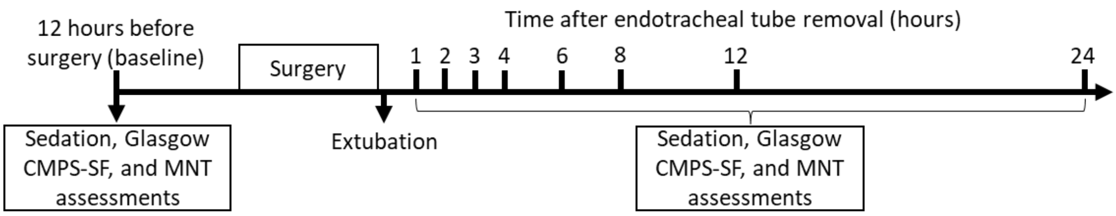

Figure 1: Timeline of sedation scores, short form of Glasgow composite measure pain score (Glasgow CMPS-SF), and of mechanical nociceptive thresholds (MNT) recorded during the study.

The numeric rating used to assess sedation scale ranged from zero to 10 where zero represents a fully conscious/alert animal and 10 denotes the highest level of sedation possible (animal in lateral recumbency not responsive to tactile, auditory, and visual stimulation). The Glasgow CMPS-SF has 5 behavioral descriptors, from which 2 involve analysis of behavior via interaction with the rater and 3 involve non interactive behavioral analysis. According to the Glasgow CPMS-SF pain intensity can range from 0 (absence of pain) to 24 (pain of highest intensity imaginable) (Reid et al. 2007). Animals received recue analgesia if the total pain score ≥ 6 according to at least 2/3 observers.

Because the Glasgow CMPS-SF can be biased towards rating pain of higher intensity in animals that are unable to ambulate because of deeper levels of sedation, the interactive descriptor “animal mobility” was disregarded if the sedation score as ≥ 3 according to at least 2/3 observers; the maximum pain intensity attributable to an animal under these circumstances was 20 and animals received rescue analgesia when pain scores were ≥ 5 according to 2/3 observers (Reid et al. 2007; Steagall et al. 2020).

After recording sedation and pain (Glasgow CMPS-SF) scores, nociception was evaluated by measuring MNT using an electronic von Frey device (Electrocnic von Frey, IITC Life Science, Woodland Hills, CA, USA). For measuring MNT each animal was petted and gently placed in lateral recumbency by one assistant. A second individual unaware to group assignment placed the von Frey filaments at a 0.5 cm distance from the skin incision and applied progressively increasing pressure to the skin until an avoidance response was observed (fight or flight response/trying to stand, crying, or trying to bite, looking at the wound) was recorded. The MNT was taken as amount of pressure (in grams) applied to the skin when the avoidance response was observed. A total of five sequential MNT measurements were performed at each time point; the highest and the lowest measurements were discarded, and the MNT representative each time point was taken as average of the remaining 3 measurements.

2.4. Rescue analgesia

Methadone (Mytedom, Cristália) (0.2 mg/kg, intravenously) was administered if Glasgow CMPS-SF exceeded thresholds for rescue analgesia according to at least 2/3 observers. Sedation and the Glasgow CMPS-SF was re-evaluated one hour after administration of rescue analgesia, regardless of the pre-defined time points considered during the postoperative period (Figure 1). If the pain score remained above the threshold for rescue analgesia, methadone was administered, and the response (sedation/Glasgow CMPS-SF) was reassessed one hour later. If the threshold for rescue analgesia was still exceeded for the 3rd time, methadone was combined with meloxicam (Flamavet, União Química) (0.2 mg/kg, intravenously) and dipyrone (D-500, Zoetis) (25 mg/kg, intravenously). In case of an animal was still refractory after the 3rd rescue analgesic, methadone was readministered as necessary. If the 6th rescue analgesia was administered based on Glasgow CMPS-SF, intravenous methadone was combined with 0.02 mg/kg of acepromazine (Acepran 0.2%, Vetnil) and no other treatment was provided.

2.5. Incidence of behavioral changes related to opioid-induced dysphoria

Behavioral changes associated with pain or opioid-induced dysphoria noted by at least 2 observers were recorded for later analysis. Dysphoric-like behavioral changes included: animals whimpering in the kennel (item A of the Glasgow CMPS-SF) or overall appearance of nervousness/anxiety or fear (item D of the Glasgow CMPS-SF).

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2.6. Statistical analysis

All analysis were performed using R software (v.4.2.1) and SAS for windows (v.9.4.) and Graphpad Prism (v9.0.2.). Variables with symmetric and asymmetric distribution according to a Shapiro Wilk test are represented as mean ± standard deviation and median (range). The significance level was fixed in < 5% for all tests.

Demographic data and intraoperative variables with symmetric and asymmetric distribution were compared by an unpaired t test and a Mann-Whitney U test, respectively.

A descriptive analysis of sedation and Glasgow CMPS-SF was initially performed by calculating the mean (standard deviation), median (minimum/maximum value) stratified by groups and by observers. To evaluate the reliability among observers, weighted kappa coefficients were obtained for sedation, and intraclass correlation coefficients (ICC) where obtained for the Glasgow CMPS-SP (Altman, 1990).

The total number of rescue analgesic interventions was compared between groups by a Fisher´s exact test. The total number of animals that received rescue analgesia, and the total number of animals that displayed behaviors that might be associated with opioid-induced dysphoria (whimpering, anxiety/nervousness or fear) or pain was compared by a Chi-squared test.

3 - RESULTS

3.1. Demographic data and intraoperative variables

A total of 18 mongrel dogs were evaluated (Control, n = 11; TAP, n = 7), other breeds included in the study were Shih Tzu (Control, n = 1; TAP, n = 3), Maltese (Control, n = 1; TAP, n = 1), Dalmatian (TAP_{bupi} group, n = 1), Border Collie (TAP_{bupi} group, n = 1), Labrador (Control group, n =1), American Pit Bull Terrier (TAP_{bupi} group, n = 1), Poodle (Control, n = 1), Rough Collie (TAP_{bupi} group, n = 1).

There were no significant differences in body weight (p = 0.69) and age (p = 0.49). Pooled values of body weight and age [median (range)] were 6.7 (3.5–29) kg, 16 (5–72) months, respectively. Total surgery time [30 (21–85) min] and total anesthesia time [64.5 (56–133) min] did not differ between groups ($p = 0.07$ and $p = 0.21$, respectively). One animal of the Control group (Labrador retriever, 29 kg, 36 moth old) was an outlier for surgery time (85 min) and for total anesthesia time (133 min) because of accidental hemorrhage during the ligature of one ovarian pedicle. This animal recovered uneventfully from anesthesia. A large interindividual variability was observed in recovery times. Animals of the TAP_{bupi} group took longer to stand ($p = 0.016$) after interruption of isoflurane administration [25 (6–68) min] than animals of the Control group [16 (6–58) min].

3.2. TAP block technique and agreement among observers

In 3/15 animals of the Control group there was partial infiltration of the interfascial plane (animal #3: right cranial quadrant, animal #5: all 4 quadrants; animal #12: left cranial quadrant). In the TAP_{bupi} group, only one animal (animal # 14) presented partial/incomplete infiltration of interfascial plane located the right cranial and caudal quadrants.

Overall reliability among observers (pooled observations from the Control and TAP_{bupi} groups) was good for Sedation (weighted kappa between 0.61 and 0.80) and good for the Glasgow CMPS-SF (ICC between 0.61 and 0.80) (Table 1) (Altman 1990).

Table 1: Reliability* among observers 1, 2 and 3 evaluated by the weighted kappa coefficient (Sedation scores) and by the intraclass correlation coefficient (Glasgow CMPS-SF)

Scale	Observer 1 versus Observer 2	Observer 1 versus Observer 3	Observer 2 versus Observer 3
Sedation score	0.75 (0.69-0.82)	0.82 (0.77-0.86)	0.73 (0.66-0.81)
Glasgow CMPS-SF	0.83 (0.79-0.86)	0.77 (0.72-0.81)	0.77 (0.73-0.81)

*Degree of reliability: very good: 0.81–1.0; good: 0.61–0.80; moderate: 0.41–0.60; reasonable: 0.21–0.4; poor < 0.2 (Altman, 1990)

3.3. Rescue analgesic interventions, dysphoric-like behavior, postoperative sedation, analgesia, and antinociception

The total number and timing of rescue analgesic interventions per animal, and timing of behaviors that might be associated with opioid-induced dysphoria or pain is reported in Tables 2 and 3.

The total number of rescue analgesic interventions (mean ± SD) did not differ between the Control (2.73 ± 1.71) and TAP_{bupi} groups (3.53 ± 1.88) (Fisher´s exact test, $p = 0.23$). A total of 13/15 animals from the Control group and 14/15 animals from the Control and TAP_{bupi} groups, respectively, received at least one rescue analgesia, without difference in the number of rescued animals between groups (Chi-squared test, $p = 0.54$).

Table 2: Number and timing of rescue analgesic interventions per animal, and timing of behavioral changes (whimpering and a that might be associated with opioid-induced dysphoria or pain in the Control group.

Animal	Breed	Number of rescue analgesic	Timing of rescue analgesic interventions	Whimpering	Anxyous, nervous or fearfull
1	Maltese	3	1, 2, and 4h	1h	1h

2	Crossbreed	3	1 and 4h		
3	Poodle	5	1, 2, 3, 4, and 8h	1 and 12h	
4	Shih Tzu	4	1, 2, 3, and 4h	1, 2, 3, and 4h	3 and 24h
5	Labrador Retriever	4	1, 2, 3, and 6h	2 and 12h	1h
6	Crossbreed	3	1, 2, and 12h		
7	Crossbreed	1	1h		
8	Crossbreed	6	1, 2, 3, 4, 5, and 6h	1,2,3,5	
9	Crossbreed	2	2 and 6h	0, 1, 2, 5, and 24h	
10	Crossbreed	3	1,3, and 6h	0, 1 ,3, 6, and 24h	0, 6, 12, and 24h
11	Crossbreed	3	1, 3, and 6h	6h	
12	Crossbreed	3	3, 6, and 8h		
13	Crossbreed	1	3h	3h	
14	Crossbreed	0		1h	
15	Crossbreed	0		3h	

Table 3: Number and timing of rescue analgesic interventions per animal, and timing of behavioral changes (whimpering and a that might be associated with opioid-induced dysphoria or pain in the TAP_{bupi} group.

Animal	**Breed**	**Number of rescue analgesic**	**Timing of rescue analgesic interventions**	**Whimpering**	**Anxyous, nervous or fearfull**
1	Border Collie	3	2, 12, and 13h	2 and 6h	
2	Shih Tzu	1	4h	1, 2, 3, 4, and 12h	4h
3	Rough Collie	4	4, 5, 8, and 12h	3, 4, 8, and 12h	5h
4	Crossbreed	6	1, 4, 5, 6, 7, and 8h	1, 3, 4, 5, 6, 8, 12, and 24h	3, 4, 5, 6, 8, and 12h
5	Crossbreed	6	1, 2, 3, 4, 5, and 6h	1, 2, 3, 4, 5, 8, 12, and 24h	1,4, and 5h
6	Crossbreed	0		1 and 3h	
7	Crossbreed	5	1, 2, 3, 6, and 7h	0, 6, 7, and 24h	
8	Crossbreed	7	1, 2, 3, 4, 5, 6, and 7h	0, 4, 5, 6, 7, and 12h	4, 5, and 7h
9	Shih Tzu	3	1, 2, and 3h	0, 2, 3, 6, 8, 12, and 24h	1h
10	Shih Tzu	3	1, 4, and 6h	1, 6, 7, 8, 12, and 24h	
11	Crossbreed	1	4h		
12	Crossbreed	5	1, 3, 6, 7, and 8h	24h	
13	Dalmatian	4	1, 3, 4, and 6h	1, 4, 6, 12, and 24h	
14	American Pit Bull Terrier	4	4, 5, 8, and 12h	2, 3, and 12h	4h
15	Maltese	3	1, 2, and 4h	0, 1, 2, 4, 5, 6, 8, 12, and 24h	4h

Incidence of behaviors that could be related to both opioid-induced dysphoria and pain (whimpering and anxiety/nervousness or fearfulness) was high in both groups. The number of animals that were whimpering in the kennel during the postoperative period did not differ between the Control group (11/15) and the TAP_{bupi} group (14/15) (Chi-squared test, $p = 0.14$). The number of animals that were anxious/nervous or fearful did not differ between the control group (4/15) and the TAP group (8/15) (Chi-squared test, $p = 0.13$).

Sedation scores increased from baseline for 6 and 8 hours in the Control ($p < 0.0001$) and TAP_{bupi} groups ($p < 0.0001$), respectively, without significant differences between groups ($p = 0.25$) (Figure 2a). The Glasgow CMPS-SF, increased from baseline for 12 hours in the Control group (p <0.0001 to 0.0066) and for 24 hours in the TAP_{bupi} group (p <0.0001 to 0.0036), but did not differ between groups (p = 0.15 to 1.0) (Figure 2b).

The von Frey MNTs were lower than baseline at some time points in the Control (2 hours, $p =0.0355$; 24 hours, $p = <.0001$) and in the TAP_{bupi} groups (12 hours, $p = 0.0021$; 24 hours, $p = 0.0173$), but without difference between groups (p = 0.417 to 0.957)

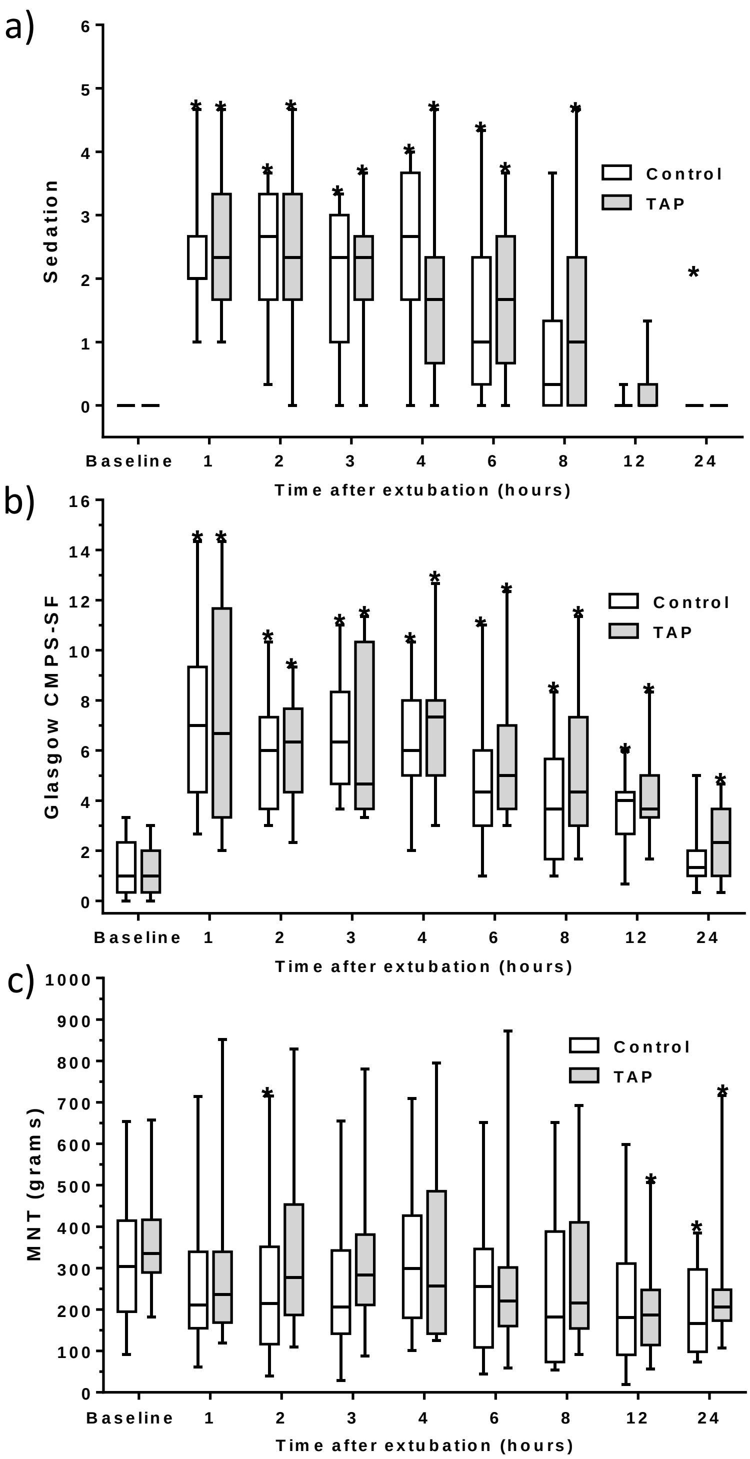

Figure 2: Sedation scores (numeric rating scale), the short form of the Glasgow composite measure pain score (Glasgow CMPS-SF), and electronic von Frey mechanical nociceptive thresholds (MNT) recorded before (baseline) and during the postoperative period of female dogs were physiological saline (Control) or 0.25% bupivacaine (TAP_{bupi}) were injected into the interfascial plane between the obliquus abdominis externus and the tranversus abdominis muscleunder ultrasound guidance (n = 15 per group).

4. DISCUSSION

This study showed the challenges involved in the assessment of postoperative analgesic effects of a TAP block technique in dogs undergoing abdominal surgery. Although the TAP block has been reported as an effective means of providing pain relief following ovariohysterectomy surgery in dogs (Campoy et al. 2022; Cavaco et al. 2022; Paolini et al. 2022), the present study failed to show a treatment effect. A high incidence of rescue analgesia was observed in the control (13/15 dogs) and TAP_{bupi} groups (14/15). One possible sources of bias that may explain differences between the present and previous reports was opioid-induced dysphoria. As opioid-induced dysphoric behavior may be difficult to differentiate from pain, this adverse reaction to opioid administration may explain the unexpected high incidence of rescue analgesic interventions in both control and treated groups. Dysphoria (which is the opposite of euphoria or a feeling of well-being) has been used in people to describe feelings of unpleasantness that may cause a patient to display aversion towards the environment (Starcevic 2007). In pain free dogs, dysphoria characterized by changes in normal behavior including restlessness, agitation, vocalization, whimpering, repeated head movements, paddling movements of the limbs has been reported after administration of a high intravenous dose of methadone (1 mg kg^{-1}) (Garofalo et al. 2012). The high incidence of whimpering and anxiousness/fearful behavior in animals of the Control (11/15) TAP_{bupi} (14/15) groups could be explained by opioid-induced dysphoria and not pain. In the present report, one animal from the control and one from the TAP_{bupi} group received a total of 6 consecutive rescues with intravenous methadone (from one to 6 hours after extubation), resulting in a cumulative dose of 1.2 mg/kg. Another animal form the TAP_{bupi} group receiver 7 consecutive rescue analgesic interventions at hourly intervals, totaling 1.4 mg/kg of methadone Because intravenous methadone has a terminal (elimination) half-life of approximately 4 hours in dogs (Ingvast-Larsson et al. 2010), it is likely that a carryover effect was present when relatively low doses of methadone were administered at hourly intervals to treat persistently high pain scores.

Two recent studies (Campoy et al. 2022, Cavaco et al. 2022) reported that the TAP block was effective to provide pain relief during the postoperative period of female dogs undergoing ovariohysterectomy surgery. In one of these studies, a two-point TAP block with 0.2 mL kg^{-1} of 0.25% bupivacaine in each hemiabdomen reduced the need for rescue analgesia (morphine 0.1 mg kg^{-1}, intravenously) in comparison to animals that received a TAP block with physiological saline as placebo (Cavaco et al. 2022). In this previous study, animals were premedicated with a relatively small dose of meperidine (2 mg kg intramuscularly), whose analgesic effects last approximately one hour, and intraoperative analgesia was provided by a constant rate infusion of an ultra-short acting opioid (remifentanil), whose analgesic action terminates within a few minutes after the end of an infusion. This is in contrast with the present study, where animals from both groups received a relatively large dose of morphine as premedication (0.5 mg kg^{-1}, intramuscularly), and fentanyl (5 µg kg^{-1}, intravenously) was administered before skin incision for intraoperative pain control. Because the elimination half-fifes of intravenous morphine and fentanyl (1.16 hour and 45.7 minutes, respectively) (Kukanich et al. 2005, Sano et al. 2006) it is likely that some residual effect of morphine was still present by the time animals recovered from anesthesia (median duration of anesthesia of 64 minutes). Therefore, pre, intra and postoperative opioid analgesic administration could have contributed to the dysphoric reactions that could have represented a confounder in the present study.

The use of smaller doses of morphine, fentanyl and methadone could have presented opioid-induced dysphoria. Dysphoric reactions have been identified in 23% of dogs that underwent stifle surgery and were receiving fentanyl infusions during recovery from anesthesia (Becker et al. 2013). Several publications have reported the occurrence of this adverse reaction to pure mu opioid agonists administered alone (Maiante et al. 2009; Garofalo et al. 2012) or during the perioperative period in dogs (Lucas et al. 2001; Troncy et al. 2002; Hofmeister et al. 2006; Becker et al. 2013). In the presence of suspected dysphoric-like behaviors, a useful assessment is to apply pressure close to the surgical wound. If the animal presents and aversive response, then pain is the likely cause and additional analgesia should be provided. If animal does not display an aversive behavior to palpation near the surgical wound, or the behavioral change persists or gets worse with additional opioid administration, the animal might be dysphoric and drugs such as acepromazine or dexmedetomidine can be administered to provide sedation (Hofmeister et al. 2006; Jarosinski et al. 2021). Alternatively, if dysphoria persists or gets worse with additional opioid administration, partial mu opioid reversal can be provided by a mu opioid antagonist (e.g., naloxone). If dysphoria and not pain is present, intravenous naloxone (10 µg kg^{-1}) has been reported be effective in inducing a calming effect in dogs (Hofmeister et al. 2006). Because there is the concern that naloxone may also reverse opioid-induced analgesia, some clinicians titrate naloxone in small incremental doses (1 µg kg^{-1} every 2-3 minutes) until behavioral changes are reversed.

Although mongrel dogs prevailed, there was a wide variation of dog breeds enrolled in the present study, from small breed dogs (Maltese and Shih Tzu) to large breed animals (Labrador Retriever). There are some dog breeds that might be more sensitive to pain than others. Objective studies evaluating the effect of breed on the response nociception reported that one breed (New Zealand Huntaway) was less sensitive to a thermal nociceptive stimulus than two other breeds (Harrier Hound, Greyhound) (Bowden et al. 2017). In a recent online survey performed among 1078 veterinarians, 28 dog breeds were rated in terms of their sensitivity to pain from 0 (not sensitive at all) to 100 (highest sensitivity imaginable). The lowest sensitivity to pain (median pain score < 30/100) was attributed to large animal Breeds (Mastiff, Labrador and Pit Bull), while highest sensitivity to pain (> 70/100) was associated with small animal breeds (Maltese, Chihuahua, Pomeranian, and Dachshund) (Gruen et al. 2020). However, these are clinical impressions and there is a need for studies with enough statistical power to show if there are breed-related differences in pain sensitivity.

In the present study the avoidance response to pressure applied by the von Frey filaments close to the surgical wound reflected nociception (neural process of encoding a noxious or potentially noxious stimuli) and not analgesia (unpleasant sensory and emotional experience associated with actual or potential tissue damage). The use of rescue analgesia in both groups represented a confounding factor that could explain the absence of difference in MNTs between groups. The lack of a treatment effect (increased MNT in the TAP_{bupi} group compared to controls) could also be explained by the amount of pressure applied by the von Frey filaments, which could have elicited an avoidance response visceral nociception that is unlikely to be altered by the TAP block. In bitches undergoing ovariohysterectomy, superficial and somatic pain, evaluated by the animal´s response to pinching the skin with a tissue forceps and to the pressure applied to the abdominal wall with a blunt object, respectively, did not differ between control animals, and animals receiving a single point TAP block with bupivacaine/dexmedetomidine or liposomal bupivacaine (Campoy et al. 2022).

With the TAP block technique effective somatic analgesia of the abdominal wall is achieved by reversibly blocking the conduction of the ventral branches of the spinal nerves, extending from the 11th thoracic nerve until the 3rd lumbar nerve (Schroeder et al.2011). Failure to correctly position the needle between the *transversus abdominis* muscle and the *obliquus internus abdominis* muscles could result in decreased success rate. Since this technique is operator dependent, the level of training of the individual responsible for performing the TAP block (1 year of experience) could have influenced the success rate of this interfascial block in the present report. However, incomplete infiltration of the interfascial plane occurred in only 1 animal of the TAP_{bupi} group, which is insufficient to bias the results.

In summary, failure of the TAP block to provide superior analgesia could have been explained by the dysphoric reactions to opioid administration masking the effectiveness of the interfascial block technique. Considering this hypothesis as true, pain assessment could have been biased by the use of larger doses of longer acting of opioids as premedication (morphine) and during the intra/postoperative period (methadone). This raises the question on whether the standard analgesic protocol in placebo-control studies should incorporate lower doses of opioids or drugs with short duration of action (e.g., meperidine and remifentanil) to provide analgesia only during the intraoperative period and avoid the confounding effect of opioid-induced dysphoria (Cavaco et al. 2022). This procedure is ethically acceptable if rescue analgesia is provided in case of signs of unacceptable postoperative pain.

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