Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado Integrado em Medicina Veterinária

Dissertação

Does the combination of tramadol with methadone provide better analgesic effect than methadone alone, in dogs undergoing routine ovariectomy?

Patrícia Isabel Pereira Vieira

Orientador(es) | David Orlando Ferreira

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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ABSTRACT

This study aims to evaluate tramadol's effect during elective ovariectomy in female dogs under general anaesthesia with isoflurane, premedicated with 0.025 mg/kg medetomidine IM, 0.2 mg/kg methadone IM and 2 mg/kg ketamine IM. Seventeen female dogs were divided into control (CG) and tramadol (TG) groups. Dogs in the TG also received a 4 mg/kg IV tramadol bolus one minute prior to skin incision. Heart rate (HR), systolic blood pressure, and mean blood pressure were recorded at baseline, skin incision, traction (T1) and clamping (T2) of the right ovarian pedicle, and traction and clamping of the left ovarian pedicle. Tramadol caused a 39.8% ($p<0.05$) and a 31.9% ($p<0.05$) HR increase at T1 and T2, respectively, when compared to the CG, which lasted a maximum of eight minutes. Although tramadol has a potential positive chronotropic effect, it can safely provide additional intraoperative analgesia before intense noxious stimuli, for at least seven minutes.

Keywords: Tramadol; Methadone; Ovariectomy; Analgesia; Isoflurane.

SERÁ QUE A COMBINAÇÃO DO TRAMADOL COM A METADONA PROPORCIONA UM EFEITO ANALGÉSICO MELHOR DO QUE A METADONA SOZINHA, EM CADELAS SUJEITAS A OVARIECTOMIA DE ROTINA?

RESUMO

Este estudo tem como objetivo avaliar o efeito do tramadol durante a ovariectomia eletiva de cadelas anestesiadas com isoflurano, pré-medicadas com 0.025 mg/kg de medetomidina IM, 0.2 mg/kg de metadona IM e 2 mg/kg de cetamina IM. Dezassete cadelas foram divididas em grupo de controlo (GC) e tramadol (GT). No GT receberam também um bólus IV de tramadol a 4 mg/kg, um minuto antes da incisão cutânea. A frequência cardíaca (FC), pressão arterial sistólica e pressão arterial média foram registadas na "baseline", incisão cutânea, tração (T1) e pinçamento (T2) do pedículo ovárico direito, e tração e pinçamento do pedículo ovárico esquerdo. O tramadol causou um aumento de 39.8% ($p<0.05$) e 31.9% ($p<0.05$) da FC em T1 e T2, respetivamente, comparando com o GC, que durou no máximo oito minutos. Apesar do potencial efeito cronotrópico positivo do tramadol, este proporciona analgesia intraoperatória adicional, de forma segura, antes de estímulos nóxicos intensos.

Palavras-chave: Tramadol; Metadona; Ovariectomia; Analgesia; Isoflurano.

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LIST OF ABBREVIATIONS

5-HT – Serotonin/5-hydroxytryptamine
ASA – American Society of Anaesthesiologists
Bas - Baseline
CCL – Cranial Cruciate Ligament
CI – Cardiac Index
Cmax – Peak Plasma Concentration
CNS – Central Nervous System
CYP450 – Cytochrome P450
DBP – Diastolic Blood Pressure
DOR – δ Opioid Receptors
EP – Epidural
$EtCO_2$ – Expired Carbon Dioxide
GABA – Gamma-Aminobutyric Acid
HR – Heart Rate
IM – Intramuscular
Iso% - Isoflurane Concentration
IV – Intravenous
IVLD50 – Median Intravenous Lethal Dose
KOR – ƙ Opioid Receptors
M1 – O-desmethyltramadol
M2 – N-desmethyltramadol
M5 – N,O-didesmethyltramadol
MBP – Median Arterial Blood Pressure
MOR – μ Opioid Receptors
NE - Norepinephrine/noradrenaline
NMDA – N-methyl-D-aspartate
NSAID – Non-steroidal anti-inflammatory drug
OE – Ovariectomy
OHE – Ovariohysterectomy
RR – Respiratory Rate
SBP – Systolic Blood Pressure
SpO_2 – Peripheral Oxygen Saturation
SVRI – Systemic Vascular Resistance Index
TºC – Body Temperature in ºC
T0 – Skin Incision
T1 – Traction of the Right Ovarian Pedicle
T2 – Clamping of the Right Ovarian Pedicle

T3 – Traction of the Left Ovarian Pedicle

T4 – Clamping of the Left Ovarian Pedicle

T_{max} – Time of Peak Plasma Concentration

1 – INTRODUCTION

Domestication of wolves occurred 10 000 to 12 000 years ago, and since then, dogs have been part of human life. Despite initially being used for hunting activities, nowadays, they are considered family members [1; 2]. Due to the dogs' role in modern society, population control and prophylaxis of different affections are of significant concern for tutors. Hence elective surgeries have gained popularity in the last decades. Elective surgery can have multiple natures, including orthopaedic or dermatological, but typically, it is related to gonadectomy, making it one of the most performed surgeries in Veterinary Medicine. [3; 4]

Ovariectomy (OE) or ovariohysterectomy (OHE) are surgeries performed mostly for population control but also to prevent disorders related to the reproductive system, such as ovarian and mammary gland neoplasia, and cystic endometrial hyperplasia. If the ovaries are removed before the first oestrus, the risk of developing a mammary gland tumour decreases to 0.5%. The relative risk increases to 8% if ovaries are removed between the first and second oestrus, and to 26% if removed between the second and third oestrus. After the third oestrus cycle, there is no reported benefit in removing the ovaries for reducing the risk of developing malignant mammary gland tumours. Yet, it is still beneficial to perform OE/OHE to prevent cystic endometrial hyperplasia that usually leads to pyometra, ovarian and uterine tumours, and other miscellaneous genital tract pathologies. [3; 4] These procedures are also suggested in the treatment of diabetes and epilepsy and may be beneficial to reduce undesirable behaviours related to sex hormones [3; 5].

The OE procedure has been gaining relevance in the last decades and has become the standard technique in the Netherlands and other European countries, as this technique has fewer potential complications than OHE[4; 5]. This last procedure involves a more extensive incision of the skin and abdominal fascia, and more tissue trauma due to the clamp and severance of the uterus and its vessels, creating a higher risk for intraabdominal haemorrhage. [3; 4] Also, post-surgical vaginal bleeding may occur after an OHE procedure associated with uterine ligatures or perforation/opening of the uterus [4].

On the other hand, after the proestrus and oestrus phases of the oestrus cycle, in which the risk for haemorrhage is higher due to the enlargement of the surrounding blood vessels, OE has no other advantage over OHE, apart from less tissue trauma [6]. Additionally, OE does not prevent possible affections such as pyometra or other genital tract pathologies, in the case of ovarian remnant tissue [7]. The OE/OHE procedure may also contribute to the development of other pathologies such as urinary incontinence (with a higher risk for large and giant breeds, mainly if performed under three months of age), obesity, cranial cruciate ligament (CCL) disease, transitional cell carcinoma, diabetes mellitus, osteosarcoma and hemangiosarcoma. The CCL disease, osteosarcoma and hemangiosarcoma cause substantial morbidity, but due to their low reported incidence (1.8%, 0.2% and 0.2%, respectively), OE/OHE is still recommended due to the above-reported advantages. [5; 8]

1.1 – Anatomy of the canine female reproductive system

The internal female reproductive system consists mainly of the ovaries, the uterine tubes and the uterus, all connected by a variety of ligaments, as illustrated in Figure 1 [5; 9].

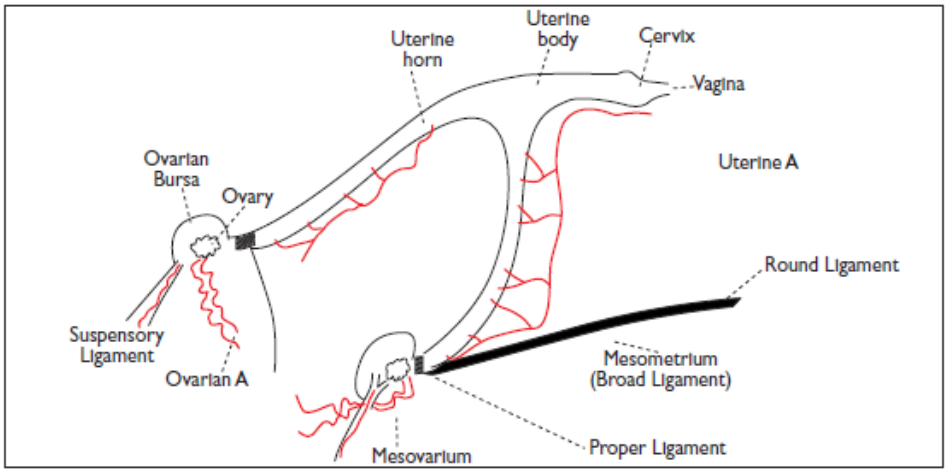


Figure 1 - Anatomy of the canine female reproductive system, focusing on the ligaments [5].

The ovaries are two oval structures located close to the abdominal wall and the caudal pole of the kidneys. As a result, the left ovary is more caudal than the right ovary. The uterine tubes are located between the ovary and the uterine horn, to which they transport the oocytes in each oestrus cycle. The uterus is divided into three parts, the neck (or cervix), the body and the two horns, which are ligated to the ovaries at their cranial end by the proper ligament of the ovary [5; 9].

All the structures mentioned above are connected between them and to the abdominal walls through the right and left uterine broad ligaments. The uterine broad ligament contains three portions: *mesovarium*, *mesosalpinx* and *mesometrium*. [5; 9]

The *mesovarium* connects the ovary to the dorsolateral region of the abdominal wall and contains the blood vessels and nerve fibres of the ovary. It begins cranially with the suspensory ligament of the ovary and ends caudally to the ovary. The suspensory ligament of the ovary is attached cranially to the abdominal wall near the twelfth or thirteenth rib, ending between the opening of the ovarian *bursa* and the ascending portion of the uterine tube. [9]

The *mesosalpinx* surrounds the ovary, creating the ovarian *bursa* with only one opening whose purpose is to lead the oocytes through to the uterine tube (which is entirely inside the *mesosalpinx*). In proximity to the opening, it is located the beginning of the proper ligament of the ovary and the end of the suspensory ligament of the ovary. [9]

The *mesometrium* extends from the proper ligament of the ovary to the peritoneal folds between the bladder and the descending colon, spreading toward the inguinal ring. The

mesometrium holds all the vasculature and nerve fibres of the uterus, including the uterine artery and vein and the uterine ramifications of the ovarian artery and vein [9].

The ovarian blood supply is carried out by the ovarian artery which arises from the aorta. The ovarian arteries, besides emitting branches to the adipose and fibrous tissues of the kidneys, also irrigate the uterine tubes and the cranial part of the uterine horns, through anastomosis with the uterine arteries. The right ovarian vein drains to the caudal vena cava and the left ovarian vein drains to the left renal vein. Similarly to the ovarian arteries, the ovarian veins also anastomose with the uterine veins. The uterine vein also receives a branch from the suspensory ligament of the ovary and the renal surface. [5; 9]

All the vasculature and nerve fibres that enter the ovary, as well as the ligaments that attach the ovary to the abdominal wall, are commonly called the ovarian pedicle, as a way of describing a group of structures that hold the ovary in its place (Figure 2) [10].

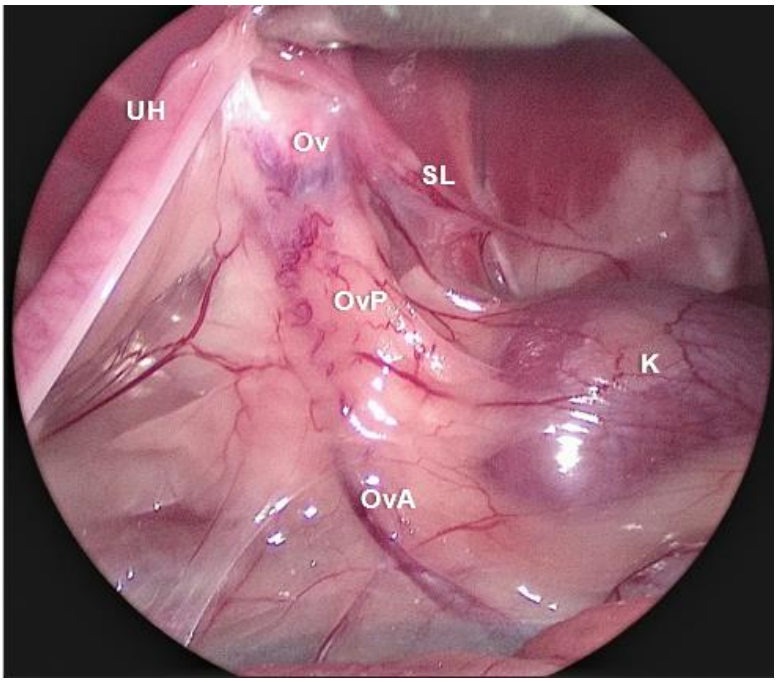


Figure 2 - Anatomy of the ovarian region, highlighting the ovarian pedicle. UH = Uterine Horn, Ov = Ovary, SL = Suspensory Ligament, K = Kidney, OvA = Ovarian Artery, OvP = Ovarian Pedicle. Photograph by Fausto Brandão.

Because the ovary is a highly vascularized organ, it is also highly innervated. Since the blood vessels have smooth muscle tissue, the nerve fibres penetrate their walls and reach the ovary through the ovarian pedicle. These fibres also innervate the fibromuscular tissue of the ovarian stroma, although there is no scientific evidence that there is innervation of the superficial layers of the ovarian cortex unless it exists a blood vessel in the area. [9; 11]

The ovarian innervation comes from the sympathetic autonomous nervous system, specifically from the lumbar sympathetic trunk (mainly from the fourth, fifth and sixth lumbar

ganglia) that send axons to the renal and aortic plexus. From these plexus, nervous fibres arise and enter the ovaries in proximity to the blood vessels [9; 11].

1.2 – Pain management during ovariectomy surgical procedure

Ovariectomy is a procedure considered to cause moderate pain, indicating that good analgesia must be provided in the perioperative period [12]. Perioperative pain is usually prevented by analgesics, such as fentanyl, methadone, buprenorphine, butorphanol or tramadol, and non-steroidal anti-inflammatory drugs (NSAIDs) like carprofen, firocoxib, meloxicam, ketoprofen or robenacoxib. The multimodal pharmacological approach provides a more adequate analgesia, as it targets different noxious pathways and nociceptive receptors. Also, it potentiates the effect of the analgesic/sedative/hypnotic drugs, which allows for a reduction in the drugs' doses with an increased effect, and a decreased anaesthetic risk. [13]

The Universal Declaration of Animal Rights proclaimed in 1978 at the United Nations Educational, Scientific and Cultural Organization (UNESCO) headquarters states that "all animals are entitled to respect" and thus should not be submitted to unnecessary pain. [14] Therefore, the anaesthetic drugs used in the premedication should provide a suitable degree of sedation and, usually adequate analgesia for the entire routine surgical procedure, such as ovariectomy. Although, there should be analgesic rescue medication prepared for administration when deemed necessary throughout the procedure. The combination of different anaesthetic drugs with sedative and analgesic effects, such as α_2 agonists, opioids and ketamine, are among the routinely used anaesthetic premedication for elective surgery. [13]

1.2.1 – α_2-Adrenergic agonists

α_2-adrenergic agonists are the class of sedatives most commonly used in veterinary medicine. They act on the α_2-adrenoreceptors which are divided into three pharmacological subtypes: α-2A, α-2B and α-2C. The α-2A and α-2C subtypes are mainly located in the central nervous system (CNS), namely in the dorsal horn of the spinal cord and in the brain stem (in the *locus ceruleus*); and the α-2B subtype is mostly found in the vascular smooth muscle. The stimulation of the α-2A and the α-2C subtypes is associated with analgesia, sedation and sympatholytic effects, and although these are the desirable effects of α_2-agonists, the sympatholytic effects cause bradycardia. The stimulation of the α-2B subtype is linked to vasopressor effects, namely an increase in the systemic vascular resistance index (SVRI), which clinically translates to an increase in arterial blood pressure. However, this hypertension tends to lower to normotension following about one hour of administration. [15; 16; 17]

Since medetomidine is a highly selective α_2-agonist, it has fewer side effects than other drugs of the class, such as xylazine, hence why it was used in the anaesthetic protocol of this study. However, dexmedetomidine, is more selective and more potent than medetomidine, because it is the pharmacologically active isomer of medetomidine. [15; 18]

1.2.2 – Ketamine

Ketamine is a phencyclidine derivative, which is part of the dissociative anaesthetics [19; 20]. In addition, ketamine can be used as an analgesic, if given in sub-anaesthetic doses, which are yet to be properly established for veterinary patients [19; 21]. It provides analgesia mostly by N-methyl-D-aspartate (NMDA) receptor antagonism, but also by δ and ƙ opioid receptor agonism, although it is an antagonist for the μ opioid receptors (MOR) [19; 20; 21; 22]. Ketamine prevents the excitatory neurotransmitters glycine and glutamate from binding to the NMDA receptors located in the dorsal horn of the spinal cord, inhibiting the transmission and modulation of nociceptive stimuli [19; 20].

1.2.3 – Opioids

Opioids are a class of drugs that interact with opioid receptors, which are located in the brain, spinal cord, peripheral neurons and the digestive tract. There are three different types of opioid receptors: μ (MOR), δ (DOR) and ƙ (KOR). When opioid agonists interact with the MOR, they mainly provide antinociception. However, this interaction is the main cause of the adverse effects of opioids, such as sedation and mild respiratory depression, which is not a clinically relevant side effect in healthy animals. [23; 24; 25; 26] Agonism of the DOR and KOR also provides analgesia but it can be linked to seizures and dysphoria, respectively [27]. The antagonism of opioid receptors is achieved by naloxone which is a competitive opioid receptor antagonist that can reverse all of the opioids' adverse effects [28]. Also, when agonist-antagonist drugs such as butorphanol are administered, they can partially antagonize full opioid agonists, significantly reducing their analgesic effect. [29]

Morphine is the most traditional μ-agonist that other opioids are compared to in terms of analgesic potency and opioid receptor binding [30]. In dogs, methadone is considered to have a similar analgesic potency to morphine (about 1.75 times more potent) but with fewer side effects. On the other hand, tramadol has 6000 times less affinity for MOR than morphine, making it a weak μ-agonist [31; 32].

1.2.3.1 – Methadone

Methadone is a synthetic opioid and it primarily binds to the opioid receptors: μ, δ and ƙ [33; 34; 35]. In addition to being an opioid agonist, it is also an NMDA antagonist and an α_2-adrenergic agonist [36; 37].

Methadone is a racemic mixture of two enantiomers: R-methadone and S-methadone [33; 38]. Once in the plasma, methadone binds to about 65% of the α_1-acid glycoproteins, which is considered a high number [39]. The tissue affinity is high and consequently, the volume of distribution is large [40]. It undergoes hepatic metabolization by the phase I Cytochrome P450 (CYP450) enzymes (probably by CYP2B11), leading to inactive metabolites, which still require further investigation in canines. This means that this drug is eliminated by the liver and that after

this passage, methadone is no longer effective. Therefore, the parent compound is immediately available to exert its effect following absorption. [26; 33; 40; 41]

Following metabolization, methadone is excreted by the kidneys at 25.1 ± 9.8 mL/min*kg, with an elimination half-life of 1.8 ± 0.3 h in dogs, despite taking >20 h in humans [33; 36]. Because methadone is metabolized by the liver only minimal amounts of the parent compound are found in urine (± 3%) and bile (<1%) [34].

Since there are several highly irrigated organs (e.g. intestines, kidneys, lungs) that contain CYP enzymes and because methadone enters the liver at a high rate, it undergoes a fast clearance [34]. The CYP450 enzyme system is susceptible to breed and genetic variations, which lead to different clearance values in different studies and, consequently, interindividual variation in response to treatment [42; 43].

In veterinary medicine, methadone is routinely used as a strong analgesic, especially in neurologic, oncologic and chronic pain. But also, in the perioperative period as part of the pre-anaesthetic protocols when moderate to severe pain is expected. Due to its sedative effects, it integrates sedative protocols when pain is involved to provide both sedation and analgesia. [34; 35]

The clinically effective dose of methadone can vary between 0.1-0.3 mg/kg if given epidurally (EP) or intravenously (IV) and it provides analgesia for about eight hours (EP) or three to four hours (IV). If given intramuscularly (IM) the dose should be 0.1-0.5 mg/kg and repeated every three to four hours. It is important to state that accumulation can occur after prolonged use of methadone, which means that with time the dose should be lowered or the interval between administrations should be prolonged. [35] Although some authors claim that the doses can vary between 0.5 and 1 mg/kg administered every three to four hours [37], anecdotally the dose of 0.3 mg/kg is generally recommended to provide good analgesia and remove the dose-dependent adverse effects [44]. The intravenous lethal dose of methadone in 50% of dogs (IVLD50) is 29 mg/kg, which is one of the reasons why methadone is a safe analgesic choice for most patients [26].

Methadone has the general mild adverse effects of the group of opioids, although these adverse effects vary according to the animal being conscious or sedated/anaesthetized and are generally dose-related [31; 34; 37; 43]. In conscious dogs, methadone can cause mild respiratory depression (not clinically relevant), sedation, dysphoria/euphoria, salivation/drooling, slight cardiovascular changes (bradycardia), hypothermia, and less frequently panting, vomiting/regurgitation, constriction of the gastrointestinal sphincters and reduction of the gastrointestinal motility. These adverse effects occur especially if administered IV. However, IM injection of methadone alters its kinetics, diminishing the side effects. [26; 32; 35; 36; 43; 45; 46]

Sedation, although being considered a side effect, it is a desirable one if methadone is given in the perioperative period, or when analgesia is required in unsettled animals [26]. In anaesthetized dogs, the side effects of methadone are mostly related to changes in haemodynamics. It can cause a decrease in heart rate (HR) (usually related to direct action on vagal tone) and in the cardiac index (CI), and an increase in the SVRI. The median arterial blood

pressure (MBP) is not as affected as the SVRI because of the compensatory decrease in CI.[31; 43; 46; 47]

In anaesthetized patients or patients with underlying respiratory disease or head trauma, respiratory and cardiac function should be meticulously monitored, particularly when methadone is administered intravenously since the most common side effect is mild respiratory depression. In these patients, methadone can also cause apnoea, leading to the need for mechanical ventilation. Methadone also crosses the placenta and can affect neonates. [26; 35; 46]

Methadone can interact with several types of drugs. CNS depressants like anaesthetics, antihistamines, barbiturates, phenothiazines and tranquillisers can enhance opioid adverse effects (CNS and respiratory depression)[35]. Furthermore, if co-administered with CYP inhibitors (chloramphenicol and fluconazole, for example) or enhancers, methadone's plasma concentrations can be increased/prolonged or shortened/depleted, respectively [26; 38; 41; 48].

1.2.3.2 – Tramadol

Tramadol is an atypical opioid that causes activation of the MOR, inhibition of the norepinephrine/noradrenaline (NE) and serotonin/5-hydroxytryptamine (5-HT) reuptake on the synaptic cleft, and assistance in serotonin release [37; 49; 50; 51]. It is a very affordable and easy-to-find drug, which are crucial characteristics in low-resource settings. Moreover, because it is available in a number of formulations, it can be administered almost by any route, which provides great convenience. [52] Also, tramadol has a minor occurrence of adverse effects, compared to pure opioid agonists [50]. Due to its lack of traditional opioid side effects, it is recommended for long-term treatments of chronic pain, such as in the case of osteoarthritis or neoplasia, as an adjunctive agent [35; 50; 53].

Tramadol exists as a racemic mixture, which means that it consists of two enantiomers, (+)-tramadol and (-)-tramadol. The positive enantiomer is more potent than the racemic mixture, but the latter has less abuse potential and fewer adverse effects. [49; 50; 54]

Each mechanism of action is catalysed by a different enantiomer, which means that all of them are important to provide adequate analgesia [52]. (+)-Tramadol acts mainly on the 5-HT reuptake inhibition and partially on MOR activation. (-)-Tramadol acts mostly on the NE reuptake inhibition. (+)-M1 catalyses the MOR activation, but to a much lesser extent in dogs than in humans, because it is produced in much lower amounts [50].

As explained before, the MOR are the most predominant type of opioid receptors. They are responsible for the majority of the analgesic effects of these drugs, but they are also responsible for the majority of their side effects. Norepinephrine is a neurotransmitter released by the sympathetic nervous system that acts on the α_2-adrenoreceptors and it produces vasoconstriction. Serotonin functions both as a neurotransmitter and as a local hormone in the peripheral vascular system and it acts on the 5-HT receptors. [42; 55]

The mechanisms of action discussed take part in the antinociceptive effects of the descending inhibitory pathways in the CNS by binding to the opioid receptors on the superficial

layer of the dorsal horn of the spinal cord, which block the rostral transmission of the nociceptive impulses [49; 51; 56]. Furthermore, tramadol prevents the propagation of impulses on the nerve fibre terminations [51] by increasing the neurotransmitter NE in the synaptic cleft leading to a decrease or inhibition of the pain perception [52].

Thus, tramadol can also cause vasoconstriction and consequent hypertension, due to the action of norepinephrine and serotonin [42; 55]. It can also cause a reduction in the rectal temperature, and respiratory and heart rate (HR)[51].

Tramadol has been reported to have 15% protein binding in dogs [57; 58], while in humans it is 20%[49] and it is known for having a high volume of distribution which is in accordance with its high tissue affinity [53; 54; 59]. The distribution of tramadol leads it to the liver, where metabolization occurs. There, mainly in the microsomes and mitochondria, it undergoes demethylation, oxidation and hepatic conjugation mediated by the CYP450 different enzymes, producing more than 30 metabolites. Dogs also have CYPs in the intestinal mucosa, which enhance the generation of metabolites. [52; 60; 61; 62]

Tramadol is metabolized mainly into inactive metabolites in the dog's liver, and once it is metabolized it has a short half-life, conferring this drug an uncertain clinical effectiveness [50; 63]. Additionally, the analgesic effect of tramadol comes mostly from the NE reuptake inhibition [50]. When the CYP2D15 interacts with tramadol through demethylation, it produces the metabolite O-desmethyltramadol (M1), which in humans and a variety of species is the main active metabolite [49; 59; 61; 64]. However, although dogs produce M1, it reveals to be a minor metabolite compared to the other tramadol metabolites [48; 53; 54; 64; 65]. Further N-demethylation of M1 by CYP2C21 originates N,O-didesmethyltramadol (M5) which has a higher affinity than tramadol for the MOR, but, due to its high polarity, it does not penetrate the blood-brain barrier as easily as M1 [61; 64; 66].

The second tramadol metabolite, N-desmethyltramadol (M2), is the next metabolite to be formed by CYP2B11 and CYP3A12 mainly, although CYP2C21 and CYP2D15 also participate. Both M2 and M5 are inactive in dogs, even though they are produced in higher amounts than M1. [61; 64]

Following metabolization, the excretion of tramadol and its metabolites occurs via the kidneys [48; 54]. The elimination of M1 takes only one to two hours, following either oral or IV administration [48; 54]. This also contributes to the low utility of this metabolite in producing analgesia [26; 49; 53; 54].

All enzymes previously mentioned are encoded by genes which are subject to polymorphisms that may compromise the metabolism of certain drugs. Nonetheless, a polymorphism is not inevitably indicative of a change in the pharmacokinetics of a drug. [67] Humans can be ultra-metabolizers (UM), extensive-metabolizers (EM) or poor-metabolizers (PM), which explains the ample variation in their pharmacokinetic response toward tramadol [49; 68]. Dogs may endure a similar situation as some breeds have been associated with mutations leading to poor antinociceptive effects. However, additional clinical trials are necessary to confirm and

generalize these findings. [67; 69; 70] Thus, tramadol should not be used as a single analgesic agent in dogs, especially if it is given orally [37; 63]

Although there are mean values established for tramadol, it is important to state that there is considerable inter-individual and inter-species variability, due to the different absorption rates and metabolization [42; 48; 52]. When administered in low doses (1, 2 and 4 mg/kg), tramadol barely has any side effects noted [53]. However, if given at higher doses it may cause sedation, respiratory depression, dysphoria, constipation, vomiting, anorexia, mydriasis, and serotonin syndrome. Overdoses with tramadol can cause seizures that may end up in death, although they respond well to intravenous diazepam. [26; 37] The serotonin syndrome may present a large number of possible life-threatening clinical signs: tachycardia, bradycardia, arrhythmia, hypertension, tachypnoea, sedation, lethargy, hyperexcitability, temporary blindness, nystagmus, confusion, agitation, excitement, vocalizations, aggression, coma, hyperreflexia, muscle spasms and hyperthermia, trembling, rigidity, convulsions, recumbency, weakness, hyperesthesia, hypersalivation, nausea, vomiting, diarrhoea, abdominal pain, fever or hyperthermia with diaphoresis [60; 71].

In humans, following a single dose of 100 mg of oral tramadol the bioavailability is 68%[49; 72], while in Beagle dogs following an oral dose of 11.2 ± 2.0 mg/kg, it is 65 ± 38 %[54]. Giorgi et al (2010)[64] reported a bioavailability of 92 ± 9 % after IM injection of 4 mg/kg, also in Beagle dogs. Additionally, the bioavailability of tramadol in Beagle dogs after intranasal (IN) or rectal administration of a 4 mg/kg dose is 32.6 ± 20.6 %[73] and 10 ± 4 %[59], respectively. The huge standard deviation of the tramadol bioavailability is a strong indicator of interindividual variability.

In 2017, Simon & Steagall [37] claimed that for dogs, tramadol should be administered in a range of 4-6 mg/kg each six to eight hours. However, in 2019, Kukanich [26] wrote that to reach the targeted drug concentrations needed to treat subacute to chronic pain states, tramadol needed to be administered at doses up to 15 mg/kg each six to eight hours, which triples the daily dose stated two years prior.

After intravenous administration in dogs, the tramadol peak plasma concentration (C_{max}) will have its maximum effect shortly after administration [59]. Also, following a 4 mg/kg IM administration of tramadol the C_{max} obtained was 2.5 ± 0.4 µg/mL with the corresponding time to reach C_{max} (T_{max}) of 20.4 ± 3 min in Beagle dogs [64]. When given orally at a dose of 11.2 ± 2 mg/kg, the tramadol C_{max} was 1.4 ± 0.7 µg/mL with the corresponding T_{max} of 62.4 ± 30.6 min [54]. After rectal administration of 4 mg/kg, the tramadol C_{max} was 0.1 ± 0.1 µg/mL and the corresponding T_{max} was 33.6 min [59].

The C_{max} can be altered due to multiple factors inherent to each patient. In any route of administration, the bioavailability and the rate of absorption will have an impact on C_{max}. And if given orally, the presence of food, gastrointestinal transit time and the time of dissolution of the drug form, will also impact the C_{max}. [54]

Naloxone is the gold standard opioid antagonist, as previously mentioned. But because tramadol is an atypical opioid, it is only partially reverted by it. Other antagonists are needed to

revert the other mechanisms of action. Yohimbine, for example, is an α_2-adrenoreceptor antagonist that together with naloxone can almost completely abolish tramadol effects. [48; 56; 74] On the other hand, ondansetron, a potent antiemetic, is a 5-HT receptor antagonist and therefore it can also partially reverse tramadol [75].

1.2.4 – Inhalant Anaesthetics

Inhalant anaesthetics or volatile anaesthetics are the typical choices for the maintenance of general anaesthesia in veterinary patients. They are administered with oxygen or a mixture of oxygen and medical air and are absorbed by the lungs. [76; 77] Because they are made of small lipophilic molecules, they easily penetrate the blood-brain barrier and have multiple mechanisms of action. It is not yet precisely known each target and receptor for this type of anaesthetics, but it is known that they interact with different membrane receptors and ion channels that involve neurotransmitters such as acetylcholine, glutamate, serotonin, gamma-Aminobutyric acid (GABA) and glycine. Volatile anaesthetics cause unconsciousness and suppression of reflexes, which are their desirable effects. However, they have a few dose-dependent adverse effects. They cause cardiovascular depression, reduce the cardiac output and cause peripheral vasodilation leading to hypotension. Also, volatile anaesthetics have sympatholytic and parasympatholytic properties, cause respiratory depression, may reduce the hepatic and renal blood flow, and inhibit insulin secretion. [78; 79; 80]

Isoflurane is a commonly used volatile anaesthetic that, besides interacting with the previously mentioned receptors, has been shown to be an antagonist of the NMDA receptors. Isoflurane can cause greater respiratory depression than other inhalant anaesthetics and some reflex tachycardia, which compensates for hypotension. [78; 81]

2 – OBJECTIVE

The aim of this study is to evaluate the effect of tramadol administration during elective ovariectomy procedures in female dogs under ketamine, medetomidine, methadone and isoflurane general anaesthesia.

3 – MATERIALS AND METHODS

3.1 – Animals

The inclusion criteria for this study were healthy female dogs from all breeds, American Society of Anaesthesiologists (ASA) physical status I-II, aged between 6 months to 8 years old, scheduled for routine ovariectomy at the Portimão Veterinary Hospital. According to the Portuguese legislation Decree-Law nº 113/2013, which transposes to the legal order Directive Nº. 2010/63/EU of the European Parliament, and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes, this routine clinical study is excluded from the scope of the mentioned Decree-Law ((lines b) and f) of the point 7)), meaning that ethics approval from legal entities was not required for this routine clinical study. Pet guardians were personally informed about the details of the study design, and a declaration of consent was signed before the surgery for each animal included in the study.

Animals were submitted to a thorough physical exam. Blood samples were withdrawn from the cephalic vein for haemogram and biochemistry analysis, according to the routine veterinary hospital practice applied to all animals scheduled for anaesthetic procedures.

3.2 - Anaesthetic protocol

Animals were randomly assigned into two distinct groups: the control group and the tramadol group. Randomisation was performed by alternately allocating each dog to one of the study groups, considering when the surgical procedure was scheduled.

On surgery day, animals had a minimum food fasting period of eight hours. Water was allowed *ad libitum* until the pre-operative period. A 20G-24G intravenous catheter (Introcan Winged IV Catheter, B. Braun) was introduced in the left cephalic vein of each animal and connected to a fluid delivery line with an initial NaCl 0.9% (B. Braun, Germany) 2 mL/kg/hr delivery rate using an infusion pump (Cygnus Vet Infusion, Servive).

All animals in both groups were premedicated with intramuscular 0.2 mg/kg methadone (Insistor, Richter Pharma, Austria), 0.025 mg/kg medetomidine (Sedator, Dechra, Netherlands) and 2 mg/kg ketamine (Ketamidor, Richter Pharma, Austria) in the *longissimus dorsi* muscle. Induction of anaesthesia was performed with propofol 1% (Lipuro, Barcelona, Spain) when needed, adjusting the minimum dose to allow for tracheal intubation, followed by trichotomy and asepsis of the surgical area using diluted chlorhexidine gluconate (Clorhexidina, Zoopan, 1%) and 70% alcohol (Aga, Álcool etílico sanitário, 70%). Animals in the tramadol group also received an intravenous 4 mg/kg bolus of tramadol (Tramadol Labesfal, Labesfal, Portugal) one minute prior to skin incision. Rescue medication with intravenous methadone 0.1 to 0.2 mg/kg would be administered if systolic blood pressure, mean blood pressure and heart rate values increased 20% or higher when compared to the previous measurement, followed by exclusion of the patient from data analysis.

The ovariectomy procedures were performed by the same surgeon using a midline approach technique and, for additional postoperative analgesia, a 2 mg/kg robenacoxib (Onsior, Elanco, USA) was administered subcutaneously to all animals when suturing the abdominal wall.

3.3 – Anaesthesia monitoring and data collection

During the study period, heart rate (HR), expired carbon dioxide ($EtCO_2$), peripheral oxygen saturation (SpO_2), non-invasive systolic (SBP), diastolic (DBP) and mean (MBP) arterial blood pressures, body temperature (TºC) using an oesophageal thermometer, respiratory rate (RR) and the isoflurane selected vaporised concentrations (Iso%) were recorded every minute. Arterial blood pressure was measured on the left hind limb at the metatarsal region using a petMAP monitor (petMAP graphic II, Ramsey Medical inc., Cardio Command, USA). The other variables were collected from the haemodynamic monitor Mindray uMEC12 (Shenzhen Mindray Bio-Medical Electronics Co., LTD, China). Animals were mechanically ventilated with a mixture of air and oxygen using a Mindray WATO EX-35 (Shenzhen Mindray Bio-Medical Electronics Co., LTD, China) mechanical ventilator and a Mindray V60 Anaesthetic Vaporizer (Shenzhen Mindray Bio-Medical Electronics Co., LTD, China). Ventilatory parameters were adjusted for each animal to maintain SpO_2 values above 95% and $EtCO_2$ values between 40-50 mmHg.

Baseline values (Bas) were recorded for all variables in animals under general anaesthesia, one minute before skin incision and, in the tramadol group, before its administration. The response to surgical noxious stimuli was analysed at skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.

3.4 – Statistical analysis

Data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk normality test and Levene's test, respectively. Repeated measures analysis of variance (ANOVA) with a Greenhouse-Geisser correction and pairwise comparisons with a Bonferroni adjustment were used to compare data within groups. The Mann-Whitney test was used to compare data between groups.

Data are presented as median (interquartile range), and statistical significance is considered when $p<0.05$. Statistical analysis was performed using the SPSS version 28 for windows.

4 – RESULTS

A total of 17 animals were included in the study and no animal required rescue medication.

Eight female dogs were included in the control group, age 1.5 (1) years and weighing 14.7 (8.2) kg. The duration of the study period was 9 (6) min, during which HR was 59 (29) bpm, SBP was 156 (28) mmHg, DBP was 92 (25) mmHg, MBP was 116 (23) mmHg, RR was 12 (0) rpm, $EtCO_2$ was 47 (5) mmHg, SpO_2 was 97 (6) % and TºC was 37.7 (1.2) ºC.

Nine female dogs were included in the tramadol group, age 2 (4) years and weighing 8 (22.9) kg. The duration of the study period was 16 (5) min, during which HR was 77 (20) bpm, SBP was 147 (37) mmHg, DBP was 94 (18) mmHg, MBP was 108 (24) mmHg, RR was 12 (0) rpm, $EtCO_2$ was 44 (11) mmHg, SpO_2 was 96 (4) % and TºC was 37.3 (0.6) ºC.

No statistically significant differences were observed between groups in the demographic variables and duration of the study period.

Data recorded at Bas, T0, T1, T2, T3 and T4 for control and tramadol groups are presented in tables 1 and 2, respectively.

Table 1 - Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MBP), respiratory rate (RR), expired carbon dioxide pressure (EtCO2), peripheral oxygen saturation (SpO2), body temperature (TºC) and isoflurane concentration (Iso%) from animals in the control group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle. Data are median (interquartile range).

	HR bpm	**SBP mmHg**	**DBP mmHg**	**MBP mmHg**	**RR rpm**	**$EtCO_2$ mmHg**	**SpO_2 %**	**TºC ºC**	**Iso% %**
Bas	48 (21)	143 (39)	89 (17)	116 (24)	12 (0)	44 (8)	99 (3)	38 (1)	1.5 (0)
T0	55 (17)	156 (21)	90 (24)	115 (21)	12 (0)	47 (8)	97 (5)	38 (1)	1.5 (0)
T1	57 (17)*	140 (25)	90 (32)	107 (19)	12 (0)	48 (8)	96 (5)	38 (1)	1.5 (0)
T2	66 (11)*	156 (22)	92 (18)	117 (20)	12 (0)	46 (10)	94 (6)	38 (1)	1.5 (0)
T3	77 (33)	160 (8)	108 (26)	122 (25)	12 (0)	48 (1)	98 (5)	37 (1)	1.5 (0)
T4	80 (27)	166 (23)	94 (17)	120 (6)	12 (0)	47 (4)	97 (4)	37 (1)	1.5 (0)

No statistically significant differences were observed within the group for all variables. *$p<0.05$ when compared to the tramadol group.

Table 2 - Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MBP), respiratory rate (RR), expired carbon dioxide pressure (EtCO2), peripheral oxygen saturation (SpO2), body temperature (TºC) and isoflurane concentration (Iso%) from animals in the tramadol group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle. Data are median (interquartile range).

	HR bpm	SBP mmHg	DBP mmHg	MBP mmHg	RR rpm	$EtCO_2$ mmHg	SpO_2 %	TºC ºC	Iso% %
Bas	66 (22)	151 (32)	98 (13)	117 (19)	12 (0)	44 (11)	97 (4)	38 (0)	1.5 (0)
T0	59 (22)	145 (37)	95 (16)	108 (31)	12 (0)	44 (13)	97 (4)	38 (0)	1.5 (0)
T1	79 (24)*	127 (29)	94 (24)	103 (29)	12 (0)	45 (11)	96 (4)	37 (1)	1.5 (0)
T2	84 (24)*	142 (29)	96 (17)	110 (16)	12 (0)	44 (12)	95 (6)	37 (0)	1.5 (0)
T3	80 (14)	149 (32)	93 (20)	108 (23)	12 (0)	44 (7)	96 (2)	37 (0)	1.5 (1)
T4	90 (10)	155 (36)	92 (17)	119 (20)	12 (0)	44 (7)	96 (3)	37 (0)	1.5 (1)

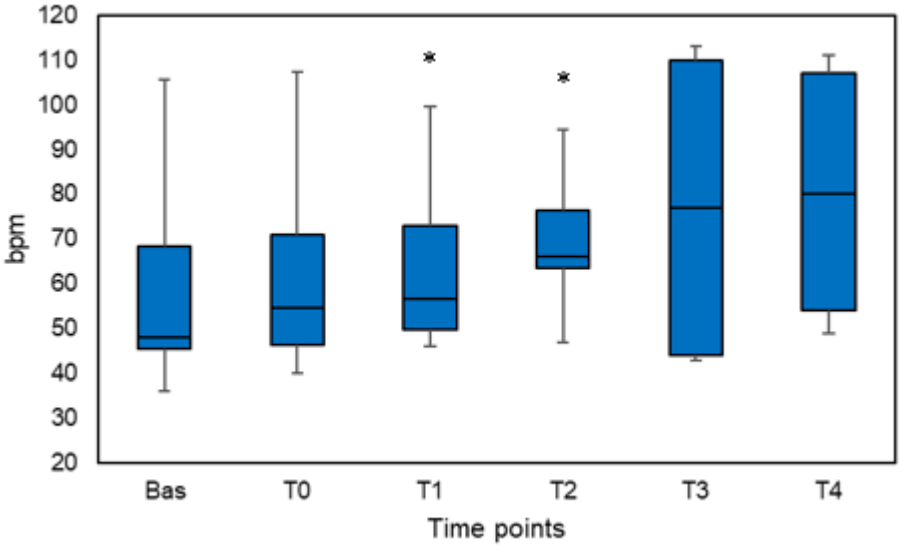
No statistically significant differences were observed within the group for all variables. *p<0.05 when compared to the control group.

The time elapsed between Bas and each of the recorded times (T0, T1, T2, T3 and T4), for both the control and tramadol groups, is presented in table 3.

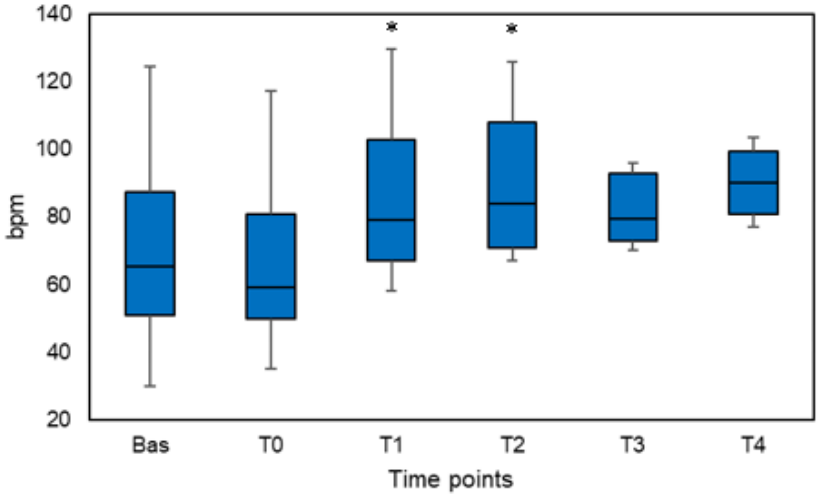
Table 3 - Time elapsed between baseline (Bas) and skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle in the control and tramadol groups. Data are median (interquartile range).

	T0 min	T1 min	T2 min	T3 min	T4 min
Control	1 (0)	3 (0)	3 (1)	9 (1)	9 (1)
Tramadol	1 (0)	7 (0)	8 (1)	16 (6)	17 (6)

Graphics 1 and 2 show the HR data during the study periods in the control group and the tramadol group, respectively.

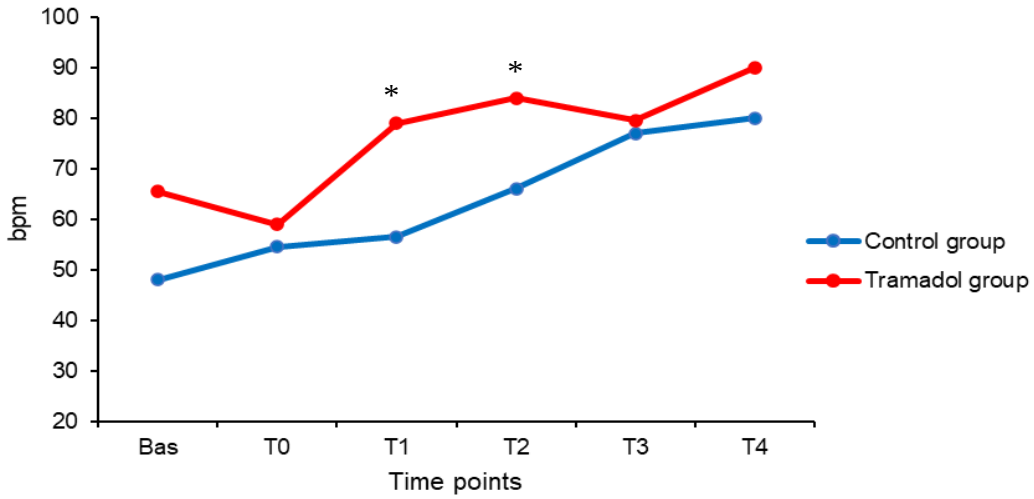


Graphic 1 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of heart rate in animals in the control group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle. *p<0.05 when compared to the tramadol group.



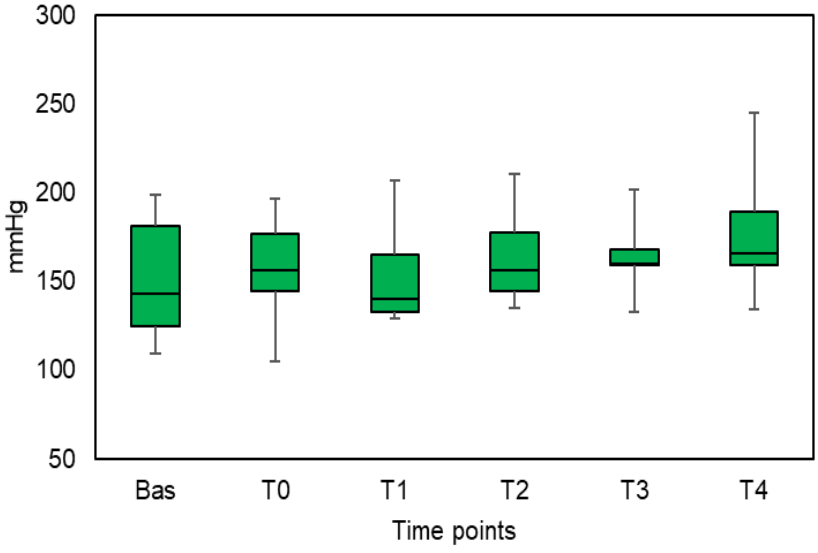
Graphic 2 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of heart rate in animals in the tramadol group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle. *p<0.05 when compared to the control group.

Graphic 3 shows the trend in HR for both control and tramadol groups during the study period.

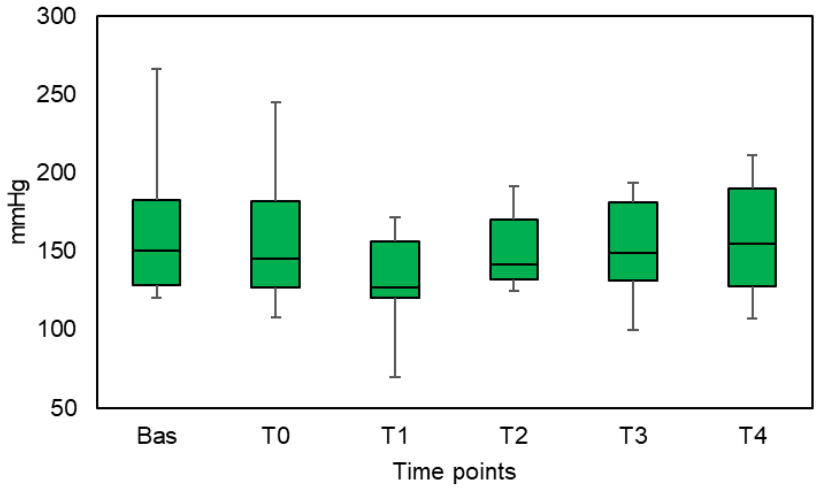


Graphic 3 - Median values trend of heart rate in animals in the control and tramadol groups at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle. *p<0.05 when compared to the control group.

Graphics 4 and 5 show the SBP data during the study periods in the control group and the tramadol group, respectively.

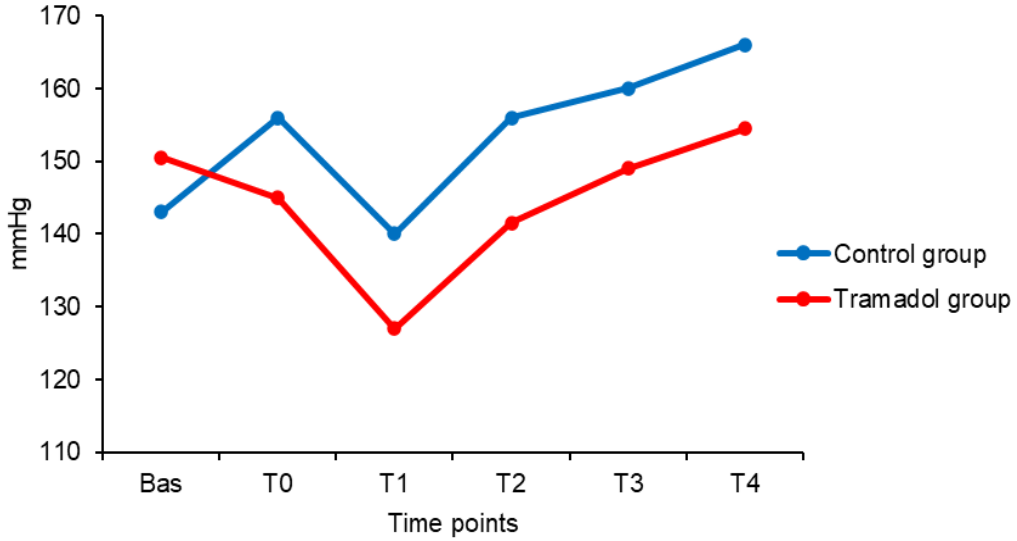


Graphic 4 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of systolic blood pressure in animals in the control group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.



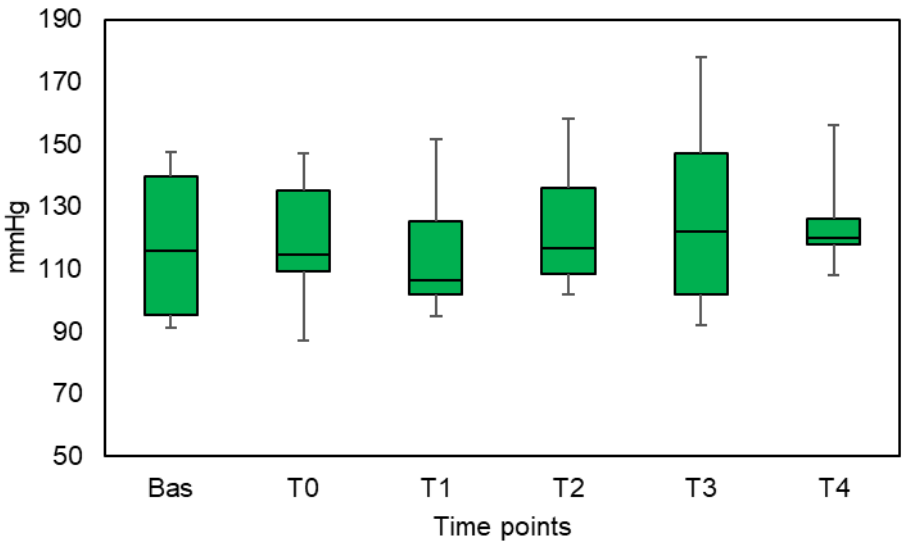
Graphic 5 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of systolic blood pressure in animals in the tramadol group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.

Graphic 6 shows the trend in SBP for both control and tramadol groups during the study period.

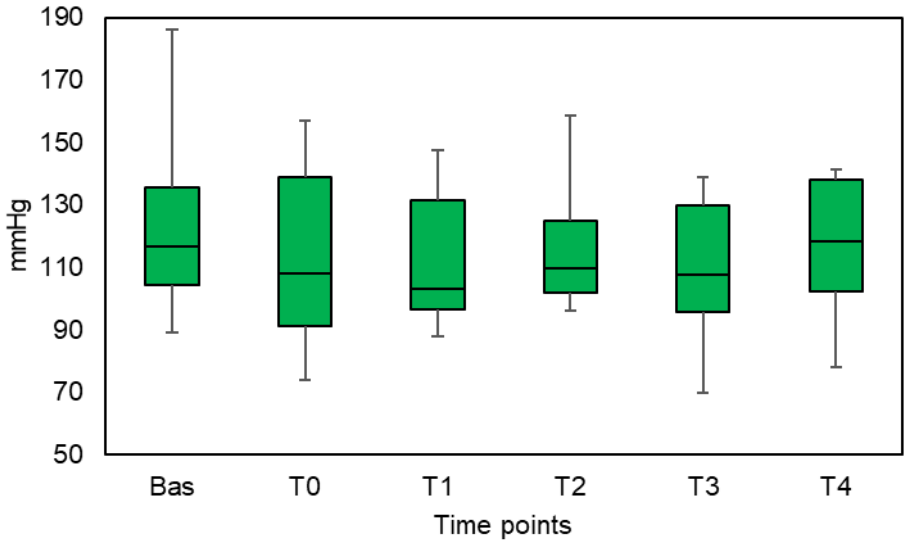


Graphic 6 - Median values trend of systolic blood pressure in animals in the control and tramadol groups at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.

Graphics 7 and 8 show the MBP data during the study periods in the control group and the tramadol group, respectively.

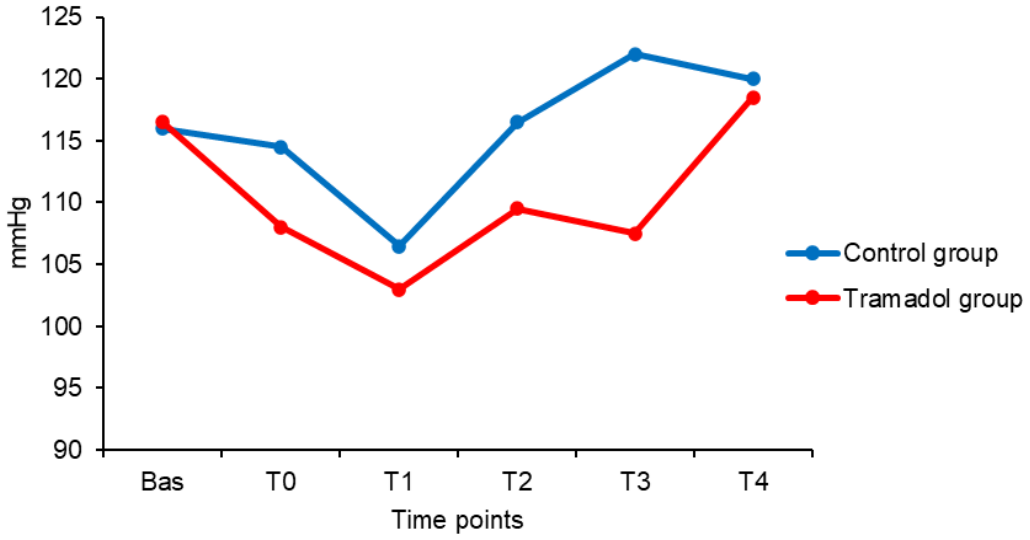


Graphic 7 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of mean blood pressure in animals in the control group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.



Graphic 8 - Median, 1st interquartile, 3rd interquartile, minimum, and maximum values of mean blood pressure in animals in the tramadol group at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.

Graphic 9 shows the trend in MBP for both control and tramadol groups during the study period.



Graphic 9 - Median values trend of mean blood pressure in animals in the control and tramadol groups at baseline (Bas), skin incision (T0), traction (T1) and clamping (T2) of the right ovarian pedicle, and traction (T3) and clamping (T4) of the left ovarian pedicle.

5 – DISCUSSION

The intravenous administration of 4 mg/kg tramadol to female dogs under general anaesthesia with methadone, medetomidine, ketamine and isoflurane caused a 39.8% ($p<0.05$) and 31.9% ($p<0.05$) increase in HR at traction (T1) and clamping (T2) of the right ovarian pedicle, respectively, when compared to the control group. This tramadol effect in HR occurred, respectively, 7 (0) and 8 (1) minutes after tramadol administration (one minute before skin incision (T0)) and lasted a maximum of 15 minutes, the time when variables at traction of the left ovarian pedicle (T3) were recorded.

Tramadol is effective immediately after administration [50] (before metabolization), but it also has active metabolites [61]. When tramadol is administered intravenously, which was the case in this study, it can instantly interact with the MOR, α_2-adrenoreceptors and 5-HT receptors providing immediate analgesia [37; 51; 52; 64]. Following metabolization, its metabolites contribute to and potentiate the analgesic effect already in place, even though not as markedly in dogs as in other species [49; 60]. In graphics 3, 6 and 9 tramadol seems to affect the HR, SBP and MBP until T1, but from T2 to T4 it is not clear.

The increase in HR in the tramadol group (graphic 2), when compared to the control group (graphic 1) in our study is hardly related to a sympathetic reflex response to hypotension or noxious stimulation, because there were no statistically significant differences in the SBP nor MBP values within (graphics 4, 5, 7 and 8) or between groups (graphics 6 and 9). Plus, MBP and SBP values were always above 60-70 mmHg (graphics 7 and 8) and 80-90 mmHg (graphics 4 and 5), respectively, the minimum physiologic threshold for blood pressure values below which it is considered hypotension [82]. Also, the noxious stimuli were similar in both groups and the SBP trend was also similar between groups (graphic 6). SpO_2 and $EtCO_2$ values were maintained above 95% and between 40-50 mmHg, respectively, which indicates that hypoxemia and hypercapnia also did not occur [82] (tables 1 and 2). Thus, the increase in HR at T1 and T2 in the tramadol group, when compared to the control group, is not likely to be attributed to the reasons mentioned above.

However, because the increase in HR was followed by a decrease in BP (graphics 3 and 6), it can be considered that it was a response to the BP decrease and not an exaggerated response to noxious stimulation. Considering that when the HR increases as a response to a noxious stimulus, the BP always follows that trend, which did not happen in this study [82].

Ronagh et al., 2020[83] reported that the HR decrease was less accentuated when tramadol was co-administered with medetomidine, when compared to medetomidine alone, despite the fact that tramadol can cause bradycardia [51]. The increase in HR observed in the tramadol group when compared to the control group in our study is in accordance with the Ronagh et al., 2020[83] findings.

Although not statistically significant but with clinical relevance, in the tramadol group SBP decreased 3.7% and 15.6%, respectively 1 (0) minute (at T0) and 7 (0) minutes (at T1) after tramadol administration; MBP decreased 7.3% and 11.6% in the same time periods, also

respectively. In the control group, SBP increased 9.1% and decreased 2.1%, respectively 1 (0) minute (at T0) and 3 (0) minutes (at T1) after baseline; MBP decreased 1.3% and 8.2% in the same time periods, also respectively. It is important to notice that, despite the significant noxious stimulation associated with the presence of the surgical stimuli between baseline and clamping of the left ovarian pedicle (T4), the decrease in SBP and MBP in the tramadol group only returned to near baseline values at T3 and T4, respectively, 16 (6) minutes and 17 (6) minutes after tramadol administration, also respectively. These trends were not observed in the control group where SBP showed a 9.1%, 9.1%, 11.9% and 16.1% increase at T0, T2, T3 and T4, respectively, when compared to baseline values, and near baseline values at T1. MBP showed a 1.3% and an 8.2% decrease at T0 and T1, respectively, and a 0.4%, 5.2% and 3.4% increase at T2, T3 and T4, also respectively, when compared to baseline values.

Even though during the study period SBP and MBP values were above the normotensive range (80-160 mmHg and 70-120 mmHg, respectively) at T3 and T4 in the control group (graphics 6 and 9) animals cannot be considered hypertensive because they were physically examined prior to surgery, and their blood pressure values were considered normal [77; 82]. Hence it can be concluded that the blood pressure values are elevated due to the drugs administered, mainly medetomidine that causes vasoconstriction and, consequently, increases blood pressure [15; 17].

From Bas to T1 SBP and MBP values show a decreasing tendency in the tramadol group. This could be attributed to the speed of the tramadol's intravenous administration. However, if that was the case, it would be expected a more significant decrease at T0, which occurred one minute after tramadol administration. Yet, in graphic 6 it is shown that the decrease was more significant at T1, which makes it unlikely that the bigger decrease in blood pressure was due to the speed of administration of tramadol. Since the only difference between groups was the administration of tramadol in the tramadol group, the decrease in BP should be attributed to it. Tramadol inhibits the NE reuptake, increasing its amount in the synaptic cleft [52]. Since NE binds to α_2-adrenergic receptors it produces vasoconstriction and therefore can increase the BP, which is an adverse effect of tramadol administration [42].

Then, from T1 the noxious stimuli can be considered more intense than the previous stimuli (traction and clamping of both ovarian pedicles), which may have triggered a sympathetic response causing a steadily increase in blood pressure values [65] until T4 in both study groups in our study.

It has been shown that tramadol should not be used as a single analgesic agent in dogs [37; 63] because of its uncertain clinical effectiveness [50; 63] that comes from the way tramadol is metabolized in the liver and the metabolites it produces [61; 63]. Therefore, this study used a combination of methadone and tramadol to assess tramadol's clinical usefulness during general anaesthesia in animals submitted to elective ovariectomy.

6 – CONCLUSION

Tramadol is a safe drug when combined with methadone, medetomidine and ketamine, and it could be useful to reduce the bradycardia usually associated with the anaesthetic protocols that use α_2-agonists and pure μ-agonists.

When tramadol is used under general anaesthesia, it should be considered that an increase in heart rate without an increase in blood pressure or other physiologic parameters, may be due to tramadol and not to a sympathetic response to a noxious stimulus.

Tramadol should be carefully used in patients with heart disease because it may increase the cardiac workload, as a consequence of its potential positive chronotropic effect.

While it is unclear if tramadol can produce additional analgesia in dogs undergoing elective ovariectomy under general anaesthesia, it is clear that it had a beneficial effect on the intrasurgical wellness of the patients in this study.

Further studies should be performed to assess the norepinephrine role in the tramadol's analgesic effect.

7 – REFERENCES

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