LEBANESE UNIVERSITY

FACULTY OF AGRONOMY

EFFECT OF TARGETED PULSED ELECTROMAGNETIC FIELDS ON INFLAMMATION AND PAIN IN CATS UNDERGOING OVARIECTOMY

Presented by

Monica SAYEGH

Thesis in partial fulfillment of the requirements for the Diploma of

VETERINARY MEDICINE DOCTOR

Master equivalent

Option: small animals

Under the direction of Dr Rana CHAAYA

December 2023

EFFECT OF TARGETED PULSED ELECTROMAGNETIC FIELDS ON INFLAMMATION AND PAIN IN CATS UNDERGOING OVARIECTOMY

Presented by

Monica SAYEGH

Approved by:

Dr Walid DARWICHE

Chairperson of the Committee

Signature:

Dr Rabih EL RAMMOUZ

Member of the committee

Signature:

Dr Rana CHAAYA

Advisor

Signature:

December 2023

Acknowledgements

I sincerely appreciate all of the help and support I got during the course of my studies, which has resulted in the completion of this thesis.

First, I would like to express my appreciation to the dean of the Faculty of Agronomy and Veterinary Medicine of the Lebanese University, Pr Nadine Nassif for creating an enriching and supportive academic environment.

I extend my gratitude to Dr Mona Abboud, head of the Veterinary Medicine department, for her dedication towards improving veterinary education.

I am also indebted to my thesis jury members, Dr Walid Darwiche, Dr Rabih El Rammouz and Dr Rana Chaaya, for their valuable feedback and insightful remarks.

I am very grateful for Dr Rana Chaaya, my thesis advisor and Lebanese University professor, for her continuous guidance and mentoring, and for passing on her valuable knowledge in research methodology and techniques.

Grateful appreciation to Fear Free and IVAPM Foundation for their generous support and funding, which played a crucial role in the success of this research.

I send my gratitude to the veterinary faculty of VetAgro sup and staff who have been cooperative and helpful throughout the data collection process. A specific thanks to Pr Stéphane Junot, Pr Vanessa Louzier and Pr Thierry Buronfosse, for all the necessary help they provided.

A particular thank you to my family for their unwavering belief in me, and for their constant moral and financial support. Their encouragement and love have been my driving force.

I would like to extend my heartfelt appreciation to my close friends, who have never failed to support and motivate me over my years of study. Their friendship has been my constant source of inspiration.

Last but certainly not least, I would like to express my deepest gratitude to Dr Christelle Mansour for her direction and effort throughout the whole process, and for the opportunity she gave me. This thesis would not have been possible without her.

Abstract

Anti-inflammatory and analgesic therapies are crucial pre-operative practices that alleviate inflammation and pain in animals during and after surgery. Medications such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids and alpha-2 agonists, are commonly administered to prevent these surgical reactions. However, these drugs can cause many side effects in animals, prompting the exploration of non-pharmacological anti-inflammatory devices (NPAIDs), like targeted Pulsed Electromagnetic Field therapy (tPEMF). This study aims to evaluate tPEMF as an adjunctive therapy in feline ovariectomy surgery, potentially offering new insights into its effectiveness in managing pain and inflammation in anesthetized cats.

Thirty healthy female cats were equally divided into two groups; Control (n=15) and tPEMF (n=15) groups. Intraoperative hemodynamic parameters such as Heart Rate (HR), Mean Arterial Pressure (MAP), and Respiratory Rate (RR) were recorded for pain measurement, at different timepoints including, the steady state (SS), cutaneous incision (I), first ovarian manipulation (MO1), first ovarian traction (TO1), second ovarian manipulation (MO2), second ovarian traction (TO2), muscular suture (MS) and cutaneous suture (CS). The interleukin-1 beta (IL-1β) concentrations were assessed as well, before and after the surgery, as an indicator of inflammation.

The outcomes showed significant variations in both groups HR and MAP during surgical procedures, indicating nociception; however, there was no significant difference between the two groups, suggesting that tPEMF's ability to reduce intraoperative pain was limited. On the other hand, the tPEMF group showed significantly lower MAP during TO2 compared to the control group (79 mmHg *vs* 97 mmHg respectively), which shows a potential beneficial effect on blood pressure regulation. At the cutaneous incision, RR in the tPEMF group seemed to stabilize compared to the steady state, unlike the control group ($\Delta RR = 0.4$ vs 3.5 respectively), suggesting a potential early intraoperative nociception decrease. However, additional study is required to validate this effect. Although there was no significant difference in IL-1β concentrations across the groups, the tPEMF group's preoperative and postoperative levels continuously trended lower, compared to the control group's levels, implying a potential anti-inflammatory effect.

Keywords: tPEMF – Ovariectomy – Hemodynamic Parameters – IL-1β

Résumé

Les traitements anti-inflammatoires et analgésiques sont des pratiques préopératoires indispensables qui diminuent l'inflammation et soulagent la douleur chez les animaux durant une intervention chirurgicale. Les anti-inflammatoires non stéroïdiens (AINS), les opioïdes et les agonistes alpha-2 sont généralement administrés pour prévenir ces réactions chirurgicales. Cependant, ces médicaments peuvent entraîner de nombreux effets secondaires chez les animaux, ce qui incite à utiliser les dispositifs anti-inflammatoires non pharmacologiques (AINP) tels que la thérapie par Champ Electromagnétique Pulsé ciblé (CEMP). Cette étude vise à évaluer la CEMP en tant que thérapie d'appoint dans l'ovariectomie féline, offrant potentiellement de nouvelles perspectives sur son efficacité à gérer la douleur et l'inflammation.

Trente chattes ont été divisées en deux groupes : le groupe témoin (n=15) et le groupe CEMP (n=15). Les paramètres hémodynamiques peropératoires tels que la fréquence cardiaque (FC), la pression artérielle moyenne (PAM) et la fréquence respiratoire (FR) ont été enregistrés pour mesurer la douleur à différents moments, notamment à l'état stable (ES), à l'incision cutanée (I), à la première manipulation ovarienne (MO1), à la première traction ovarienne (TO1), à la deuxième manipulation ovarienne (MO2), à la deuxième traction ovarienne (TO2), à la suture musculaire (SM) et à la suture cutanée (SC). Les concentrations d'interleukine-1 bêta (IL-1β) ont également été évaluées, avant et après la chirurgie, en tant qu'indicateur de l'inflammation.

Les résultats montrent des variations significatives de la FC et de la PAM dans les deux groupes pendant la chirurgie, indiquant une nociception ; cependant, il n'y avait pas de différence significative entre les deux groupes, ce qui suggère que la capacité de la CEMP à réduire la douleur peropératoire était limitée. D'autre part, le groupe CEMP a montré une PAM significativement plus basse pendant la deuxième traction ovarienne (TO2) par rapport au groupe témoin (respectivement 79 mmHg vs 97 mmHg), ce qui montre un potentiel effet bénéfique sur la régulation de la tension artérielle. Lors de l'incision cutanée, la FR dans le groupe CEMP est stable par rapport à l'état stable, contrairement au groupe témoin (ΔRR = 0,4 et 3,5 respectivement), ce qui suggère une diminution potentielle de la nociception peropératoire précoce. Cependant, des études supplémentaires sont nécessaires pour valider cet effet. Malgré l'absence de différence significative dans les concentrations d'IL-1β entre les groupes, les niveaux préopératoires et postopératoires du groupe CEMP ont baissé par rapport au groupe témoin, ce qui implique un effet potentiel anti-inflammatoire.

Mots-clés: CEMP – ovariectomie – paramètres hémodynamiques – IL-1β.

Table of contents

List of Figures

List of Tables

List of Appendices

List of Abbreviations

ANOVA: Analysis of Variance

ASA: American Society of Anesthesiology

BP: Blood Pressure

Bpm: Beats Per Minute

Ca2+: Calcium

CaM: Calmodulin

cGMP: cyclic-Guanosine Monophosphate

cNOS: constitutive nitric oxide synthase

CNS: Central nervous system

COX: Cyclooxygenases

CS: Cutaneous suture

ΔHR: Delta- Mean Heart Rate

ΔMAP: Delta- Mean Arterial Pressure

ECG: Electrocardiogram

ETCO2: End-tidal carbon dioxide

FDA: Food and Drug Administration

GMP: Guanosine monophosphate

HR: Heart Rate

I: Incision

IL-1α: Interleukin-1 alpha

IL-1β: Interleukin-1 beta

IM: Intramuscular

ISFM: International Society of Feline Medicine

IV: Intravenous

MAP: Mean Arterial Pressure

μg: microgram

MO1: First ovary manipulation

MO2: Second ovary manipulation

MS: Muscular suture

mmHg: Millimeter of Mercury

nm: nanometer

NO: Nitric Oxide

NPAIDs: Non-Pharmacological Anti-inflammatory Drugs

NSAIDs: Non-Steroidal Anti-inflammatory Drugs

p: probability

pg: picogram

rcf: relative centrifugal force

rpm: revolutions per minute

SC: Subcutaneous

SD: Standard deviation

SPO2: Oxygen Saturation

SPSS: Statistical Package for the Social Sciences

SS: Steady State

TO1: First ovary traction

TO2: Second ovary traction

tPEMF: targeted Pulsed Electromagnetic Fields

WSAVA: World Small Animal Veterinary Association

INTRODUCTION

Veterinarians have been able to effectively execute increasingly complicated and invasive surgeries due to the advancements in the veterinary surgery field, in an effort to increase the lifespan and improve the quality of life of pets (Lamont, 2002). The most common responses to surgical procedures are pain and inflammation.

The inflammatory response is an inherent and physiological answer of the body to the surgical intervention and can be both beneficial and harmful (Rossaint & Zarbock, 2018). It is a dynamic and complex process that is characterized by a variety of events like the immune system being activated and the recruitment of immune cells to sites of injury and infection (Choileain & Redmond, 2006). Cytokines, such as interleukin-1 beta, and other sensitizing chemical mediators are also released, which is the main cause of surgical pain (Bosmans *et al*., 2009). The main goals of surgical inflammation are to start the healing process, prevent tissue damage, and defend the body against possible infections (Gaynor *et al*., 2018). However, if surgical inflammation is not properly controlled, it can result in a prolonged healing time, more pain and discomfort, as well as a higher risk of complications including infections and scarring (Hsing & Wang, 2015). In order to alleviate inflammation and pain in animals during and after surgery, anti-inflammatory and analgesic therapies are needed.

Based on the International Society of Feline Medicine (ISFM) guidelines on the management of pain in cats, acute visceral pain that is associated with elective procedures, such as feline ovariectomy, is effectively treated with NSAIDs, opioids and alpha-2 agonists (Steagall *et al*., 2022). However, these drugs can cause many side effects in animals, such as hypotension or toxicity with NSAIDs, as well as bradycardia or respiratory depression with opioids (Wright, 2002). Consequently, alternative therapies such as non-invasive non-pharmacological anti-inflammatory devices (NPAIDs) are being developed.

Among the NPAIDs is the targeted Pulsed Electromagnetic Field therapy (tPEMF). By the use of inductive coils, electrical and magnetic fields are being applied to tissues. This mechanism of action renders the PEMF therapy a non-invasively treatment of a number of disorders. The electromagnetic field produced by these devices have been shown to have an impact on a number of biological processes, and in the last ten years, basic scientific understanding of the underlying mechanisms of tPEMF treatment has advanced (Gaynor *et al*., 2018). tPEMF has been proven to be an efficient and safe post-operative treatment for pain in veterinary medicine. However, no research has been conducted to date to evaluate the effectiveness of tPEMF in reducing pain and inflammation during surgery.

The main objective of the present thesis is to assess the analgesic and anti-inflammatory effect of tPEMF device as an adjunctive therapy to premedication in feline ovariectomy, by recording

intraoperative nociceptive parameters, and analyzing pro inflammatory cytokines concentrations in two different groups.

Therefore, findings could provide a fresh insight into a relatively understudied NPAID device in a surgical context in anesthetized cats.

LITERATURE REVIEW

1. Surgical inflammation and inflammatory markers:

Surgery can cause inflammation for a variety of reasons. The main one is a process called surgical trauma. During the operation, the body tissues are manipulated, cut, and torn, damaging the cells and tissues around them. This damage triggers the development of a response by the endogenous immune system, which leads to inflammation (Rossaint & Zarbock, 2018). Since this inflammatory response is related to surgery and trauma, it could be considered a surgical inflammation (Arias *et al.*, 2009). It is geared to promote tissue remodeling, and to provide protection and foster a physiological environment that is favorable to healing and hostile to infections (Paruk & Chausse, 2019). This response causes blood vessels to dilate, enhancing the flow of blood to the afflicted area, resulting in higher concentrations of white blood cells and immune cells at the site of injury which aids in the removal of any foreign intruders, including bacteria and debris (Meintjes, 2012). In addition, the body releases inflammatory mediators, such as cytokines and other molecules like platelet activating factor, reactive oxygen species, nitric oxide, prostaglandins, nerve growth factors, substance P and histamine (Meintjes, 2012; Hsing & Wang, 2015). Cytokines are key chemical immune mediators that guide the inflammatory reaction to the injury and infection locations and alert the nociceptors in the damaged areas, causing prolonged sensitization and pain (Bosmans *et al.*, 2009). Therefore, there can be two types of cytokines: Pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines possess a major role in the onset of an effective defense against exogenous pathogens. In contrast, anti-inflammatory cytokines play a critical role in reducing the exacerbated inflammatory response and preserving homoeostasis for the healthy functioning of vital organs (Ng *et al.*, 2003). Activated macrophages and endothelial cells are mainly responsible for the release of Interleukin-1, pro-inflammatory cytokines, as part of the innate acute phase response that is defensive in nature (Lin *et al.*, 2000). Two types of Interleukin-1 can be found: Interleukin-1 alfa (IL-1α) and Interleukin-1 beta (IL-1β). After the occurrence of cellular disruption from tissue injury, during the intraoperative and early postoperative periods, IL-1β are quickly released and more readily detectable in the circulation. These characteristics of IL-1β make them appropriate inflammatory markers that can be evaluated for the assessment of inflammation (Lin *et al.*, 2000).

2. Intra-operative pain: definition, pathway and assessment:

In the 17th century, Descartes postulated that due to the automata nature of animals, they were incapable of feeling pain. Unfortunately, for a long time, up through a great part of the 20th century, science embraced this theory, and it wasn't until the latter half of that century that animal pain became

the subject of scientific investigation. Nowadays, the majority of veterinarians recognize that animals actually experience pain (Bosmans *et al.*, 2009).

Pain from surgical procedures is a perception generated in response to noxious stimuli, such as tissue trauma (Urman *et al.*, 2014), while nociception is the detection of such stimulus and sensation of pain by specialized sensory neurons, called nociceptors, and the transmission of that information to the brain (Muir & Woolf, 2001). The nociceptive pathway is a 3-neuron chain, and it is illustrated in figure 1. The 1st order neuron or primary afferent neuron is in charge of transmitting signals from the periphery to neurons in the dorsal horn of the spinal cord and transducing noxious stimuli. The 2nd order neuron, also known as the projection neuron, takes information from the first neurons and transmits it to neurons in higher brain centers (medulla, midbrain, pons, thalamus and hypothalamus). Third order supraspinal neurons combine signals from spinal neurons in these centers, and send them to subcortical and cortical regions, where pain is finally perceived (Lemke, 2004) (Figure 1).

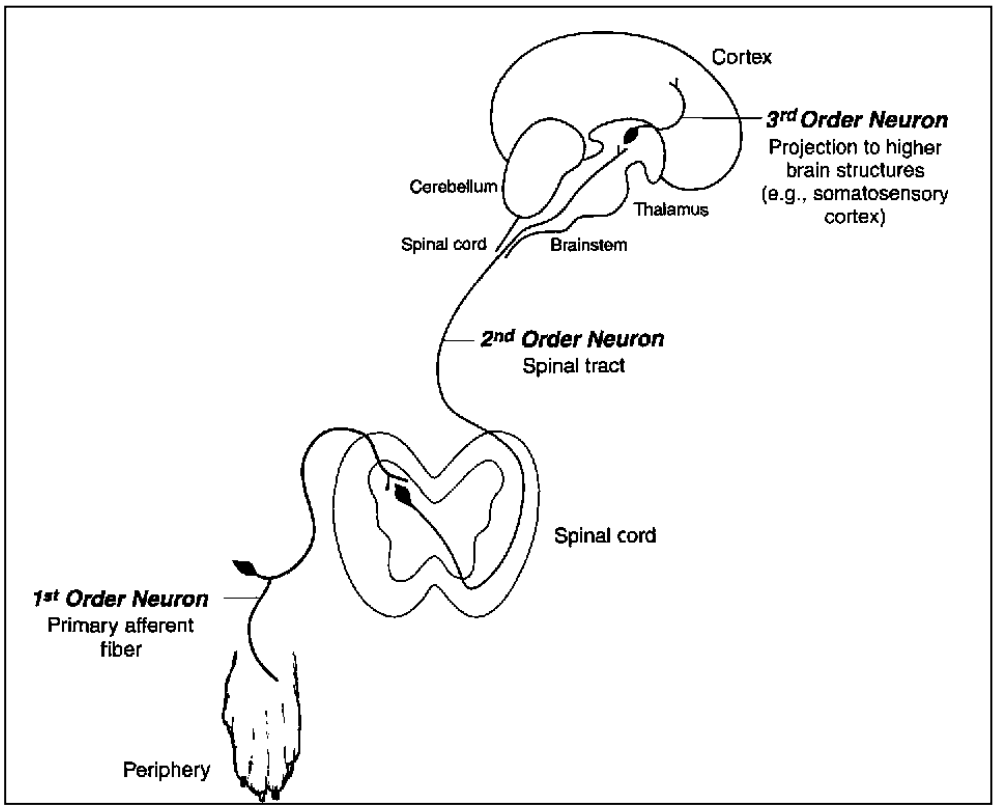

Figure 1: A simplified representation of nociceptive pathway as a three-neuron chain. A noxious stimulus in the periphery activates a primary afferent fiber that transmits the information to the dorsal horn of the spinal cord. Here, a second order projection neuron that ascends in a spinal tract to the level of the thalamus intervenes. Finally, a tertiary neuron transmits the modified noxious stimulus to higher brain centers, notably the cerebral cortex, for perception (Lamont *et al*., 2000).

Numerous physiological reactions, such as the fight or flight response, brought on by noxious stimuli, are inherently mediated by the sympathetic nervous system (Burton *et al.*, 2016). These include changes in blood pressure (BP), heart rate (HR) and respiratory rate (RR). This sympathetic response to noxious stimulation of tissues is caused by the close proximity of somatosensory and sympathetic nerves, and the direct coupling of sympathetic and sensory pathways in the dorsal root ganglion (Stewart & Panickar, 2013).

There is currently no reliable way to assess the severity of acute pain objectively. A number of devices that have been developed in humans and animals are now accessible in the market for intraoperative monitoring, however, they are only applicable in particular circumstances (Ledowski, 2019; Sylvain *et al.*, 2022). In veterinary medicine, vital signs, such as hemodynamic (HR and BP) and respiratory variables (RR), have long been explored as potential nociception indicators (Hernandez-Avalos *et al.*, 2019; Ruíz-López *et al.*, 2020). In a study done on 20 cats undergoing laparoscopic ovariectomy, these parameters were considered and recorded at surgical noxious events (incision, 1st ovary manipulation and traction, 2nd ovary manipulation and traction, muscular suture and cutaneous suture) (Conceição *et al.*, 2018). In another study done on 75 cats undergoing ovariohysterectomy and evaluating the intraoperative analgesia provided by incisional lidocaine and bupivacaine, BP, RR and HR were registered during the surgery (Vicente & Bergström, 2018). These cardiorespiratory parameters were also recorded in a study done on 60 cats undergoing orchiectomy in order to evaluate intra and postoperative analgesia after ropivacaine injection into the spermatic cord (Cicirelli *et al.*, 2022).

3. Preventive analgesia:

Pain management in veterinary medicine has evolved significantly over the years. Historically, due to the belief that animals were not subjected to pain in the same way as humans, pain management in animals was frequently neglected. Based on surveys of animal health technicians and veterinarians in Canada (Dohoo & Dohoo, 1996; 1998), Britain (Lascelles & Waterman, 1997; Lascelles *et al.*, 1999) and Australia (Watson *et al.*,1996), veterinarians were still hesitant in the late 1990s to give analgesics to animals following all surgical procedures. According to these surveys, only 13% to 26% of cats and dogs undergoing routine ovariohysterectomy or castration were given analgesics (Mathews, 2000).

Currently, veterinary surgeons and practitioners are openly supporting the concept of pain management in animals, which is steadily gaining traction (Wright, 2002). In fact, preventive analgesia

is now considered one of the main principles of pain management (Steagall *et al*., 2022). It refers to the practice of initiating analgesic treatment prior to the predicted noxious insult that leads to acute pain (Lamont, 2002). Indeed, untreated acute pain caused by illness, trauma, or surgery, has the potential to progress into a chronic pain state, which is much more challenging to manage. The lack of analgesic treatment for a painful animal may result in the onset of peripheral and/or central sensitization of nociceptive neurons (Adrian *et al.,* 2017; Simon *et al.,* 2017), which in turn can lower the nociceptive threshold to non-noxious and noxious stimuli (Steagall *et al.,* 2022) and can cause long-term emotional and psychological distress (Cohen & Raja, 2013). Therefore, preventive analgesia could prevent peripheral and central sensitization by anticipating and relieving pain and should be considered essential in every veterinary practice to optimize animal welfare (Kelly *et al*., 2001; Lamont, 2002; Wright, 2002; Steagall *et al*., 2022).

A. Common analgesia practices in ovariectomy:

Ovariectomy, a typical elective surgical procedure, is known to induce acute pain in cats. A questionnaire to British veterinary practices found that after a routine ovariectomy, 99.9% of responders reported that cats were in pain (Capner *et al*., 1997; Slingsby & Waterman-Pearson, 1998). It can be categorized as a mild to moderately painful surgery if the animal is young and healthy, and moderately painful if the animal is obese, old, or if the procedure is more extensive (Mathews, 2000). This may be due to noxious stimuli such as pedicular manipulation and ovarian traction (Höglund *et al*., 2011; Höglund *et al*., 2014) or stretching of the heavily innervated peritoneum in an effort to locate the ovaries (Roizen, 1988).

A number of analgesics have been shown to be effective in reducing pain during ovariectomy, including alpha-2 agonists (eg, medetomidine), opioids (eg, morphine), NSAIDs and local anesthetic techniques (eg, incisional and intraperitoneal analgesia using bupivacaine) (Stanway *et al*., 1996; Lamont, 2002; Wright, 2002; Steagall *et al*., 2022).

i. Alpha-2 agonists:

Alpha-2 adrenergic agonists are potent dose-dependent sedatives frequently used in premedication. They are also known to provide muscle relaxation and some analgesia (Steagall *et al*., 2022). Analgesia can be produced by the stimulation of alpha-2 adrenergic receptors in the peripheral and central nervous systems (CNS), organs and vascular tissues (Kelly *et al*., 2001). After the administration of alpha-2

agonists, the presynaptic alpha-2 receptors in the CNS are stimulated which activates the inhibitory neurons and reduces the sympathetic output through a negative feedback mechanism resulting in a decrease in the secretion of norepinephrine (Norman & Nappe, 2023). The use of alfa2 adrenergic agonists should be reserved for cats with stable cardiovascular function, since they can lead to undesirable profound cardiovascular depression causing vasoconstriction, bradycardia, arrhythmias, and decreased stroke volume (Lamont, 2002; Wright, 2002). Alpha-2 adrenergic receptor antagonists (such as atipamezole) should always be on hand for drug reversal and may be necessary during extended recoveries or when cardiovascular collapse is noticed (Steagall *et al*., 2022). Other side effects such as vomiting, hyperglycemia and urination can also be observed. Drugs with higher specificity for the alpha-2 adrenergic receptors (medetomidine and dexmedetomidine) are preferred over other less selective alpha-2 adrenergic drugs (such as xylazine) thanks to their more predictable sedative effect and lower incidence of side effects (Steagall *et al*., 2022).

ii. Opioids:

Opioids are considered 'core medicines' and are included in the WSAVA list of essential drugs for cats and dogs (Steagall *et al*., 2022). In cats undergoing sterilization, one study found that opioid agonists were generally administered preoperatively while NSAIDs were prescribed postoperatively (Lascelles *et al*., 1999). Opioids are potent analgesic drugs and could have some sedative properties when administered with alpha-2 agonists or acepromazine thanks to their synergic activity. Morphine, buprenorphine, and fentanyl are some of the commonly used opioids in cats (Stanway *et al*., 1996; Robertson & Taylor, 2003). Pure opioid agonists, such as morphine, could result in potential side effects such as bradycardia, respiratory depression, urinary retention, gastrointestinal stasis, histamine release, vomiting, nausea as well as dysphoria, excitement and hyperthermia especially in cats (Fertziger *et al*., 1973; Lascelles *et al*., 1999; Lamont, 2002; Wright, 2002).

A synergistic effect was found to occur between opioids and alfa-2 agonists which could permit the administration of a lower dose of each drug resulting in less undesirable effects. As such, several studies (Omote *et al*., 1991; Goyagi & Nishikawa, 1995) indicated that the potency of the alpha-2 agonists analgesia could be enhanced by the concomitant administration of opioids. Additionally, it has been demonstrated that alpha-2 agonists could reduce the undesirable psychological and physiological effects of opioid withdrawal (Kelly *et al*., 2001).

iii. NSAIDs:

It has been demonstrated that NSAIDs, such as meloxicam, are extremely efficient postoperative analgesics following ovariectomy and minor soft tissue surgeries (Slingsby & Waterman-Pearson, 2000; Steagall *et al.*, 2022). They are extensively used in cats for their anti-inflammatory, analgesic and antipyretic effects particularly for post-operative pain control (Steagall *et al.*, 2022). They are cyclo-oxygenase (COX) enzyme inhibitors which could reduce inflammatory prostaglandin production and peripheral sensitization. Due to the fact that cats have reduced ability to metabolize drugs via hepatic glucuronidation, plasma drug concentrations are frequently prolonged, causing this species to be susceptible to toxicity from NSAIDs when given at high doses (Wright, 2002; Steagall *et al.*, 2022). Their administration is contra-indicated in case of gastrointestinal disease, NSAID intolerance, unmanaged renal disease, hepatic disease, coagulopathies, concurrent NSAID or corticosteroid administration, hypovolemia and hypotension (Steagall *et al.*, 2022).

iv. Locoregional anesthesia:

Locoregional anesthesia is a relatively safe technique which has the unique ability to completely block peripheral nociceptive input and prevent the development of central sensitization (Lemke, 2004). Excellent analgesia is provided by preventing transduction and transmission of nociceptive signals from the periphery to spinal and supraspinal centers (Steagall *et al.*, 2022). They are useful adjuncts to other perioperative regimens and as a part of the multimodal analgesia (Mathews, 2000). When these techniques are integrated into the plan, anesthetic maintenance and recovery are usually smooth. Other benefits of locoregional anesthesia include lower costs and reductions in injectable and volatile anesthetic requirements which could consequently improve the cardiopulmonary function. In addition, locoregional anesthesia has a long-acting effect which could extend into the postoperative period reducing the requirements for other drugs (Steagall *et al.*, 2022).

B. Targeted pulsed electromagnetic field therapy (tPEMF™):

i. Rationale and background:

Apart from the pharmacologic agents, non-invasive non-pharmacological anti-inflammatory devices (NPAIDs) have been designed and put to use in clinical settings to treat inflammatory conditions as well as pain in both humans and animals (Rohde *et al.*, 2010; Gaynor *et al.*, 2018).

In the last two decades, clinicians and scientists have developed compelling research demonstrating the basic science and clinical evidence of targeted Pulsed electromagnetic field (tPEMF™) therapy as a NPAID. This device was demonstrated to cure non-invasively and non-thermally a variety of illnesses by sending electric and magnetic stimulations to tissues through inductive coils (Strauch *et al.*, 2009). tPEMF treatment has received FDA clearance thanks to rigorous and peer-reviewed results obtained from human and preclinical studies which enabled this device to be considered safe and effective for many situations including orthopedic injuries, degenerative disorders, neurological issues, internal inflammation, wound care, post-surgical pain and swelling (Rohde *et al.*, 2010; Pilla, 2013; Gaynor *et al.*, 2018).

Many researches back up the efficacy of tPEMF to treat pain and inflammation in humans. Kubat *et al.* studies revealed that PEMF therapy caused gene expression alterations and was associated with resolution of inflammation in human cells (Kubat *et al.*, 2015). According to other studies, tPEMF therapy showed significant clinical effects reflecting a considerable drop in the concentrations of IL-1β at the wound site in patients undergoing breast reduction surgery (Rohde *et al.*, 2010), as well as in pain scores (Rawe *et al.*, 2012) and in narcotic pain medication use (Rohde *et al.*, 2015).

In veterinary medicine, tPEMF devices are increasingly being implemented as a clinical treatment modality (Gaynor *et al.*, 2018). They have been used to evaluate their efficacy in alleviating post-operative pain. As such, tPEMF was applied for several weeks in dogs treated for spinal decompression surgery. Compared to controls, it resulted in significantly less pain at the site of the surgical incision, reduced levels of inflammatory biomarkers, and improved proprioceptive function (Zidan *et al.*, 2018). Also, in dogs with osteoarthritis, previcox (5mg/kg) and tPEMF were compared for 20 days. Previcox treatment appeared to be unsatisfactory after 4 days whereas dogs treated with tPEMF were good during 12 months (Pinna *et al.*, 2013).

Overall, it may be said that to date, tPEMF technology is found to be an attractive NPAID device implemented during the postoperative recovery to reduce symptomatology, promote healing and facilitate administration of lower doses of drugs, underscoring the trend towards multimodal treatment in both humans and animals.

ii. Mechanism of action:

The mechanism of action is illustrated in figure 2.

The primary mechanism of action of tPEMF is complex and continue to be an area of research focus. However, there has been considerable progress in identifying some of the pathways that are relevant to the PEMF technologies. It consists of specifically targeting normal endogenous anti-inflammatory and repair responses by delivering electromagnetic pulses, which are therapeutic waveforms, to the damaged tissues. The response to the tPEMF treatment is ineffective outside of the context of injured tissues (Gaynor *et al*., 2018).

Because the induced currents are immediately present when the coil applicator is transmitting into the afflicted area, tPEMF effects are instantaneous and are not constrained by pharmacokinetics. In fact, the magnetic field created by these electromagnetic pulses penetrates the body's tissues and cells, causing increased intracellular calcium ion (Ca2+) signaling (Strauch *et al*., 2009; Brighton *et al*., 2001; Pilla *et al*., 2011). Increased levels of free calcium ions (Ca2+) in the cytoplasm enhance the binding of calcium (Ca2+) to calmodulin (CaM). Short-bursts of nitric oxide (NO) are produced as a result of constitutive nitric oxide synthase (cNOS) being activated by Ca2+/CaM binding. Cyclic guanosine monophosphate (cGMP) synthesis is then increased by NO's ability to bind to soluble guanylyl cyclase. The upregulation of NO and cGMP are known to activate endogenous anti-inflammatory responses, enhance blood flow and increase production of growth factors that promote neovascularization, tissue regeneration, and tissue remodeling (Gaynor *et al*., 2018) (see Figure 2).

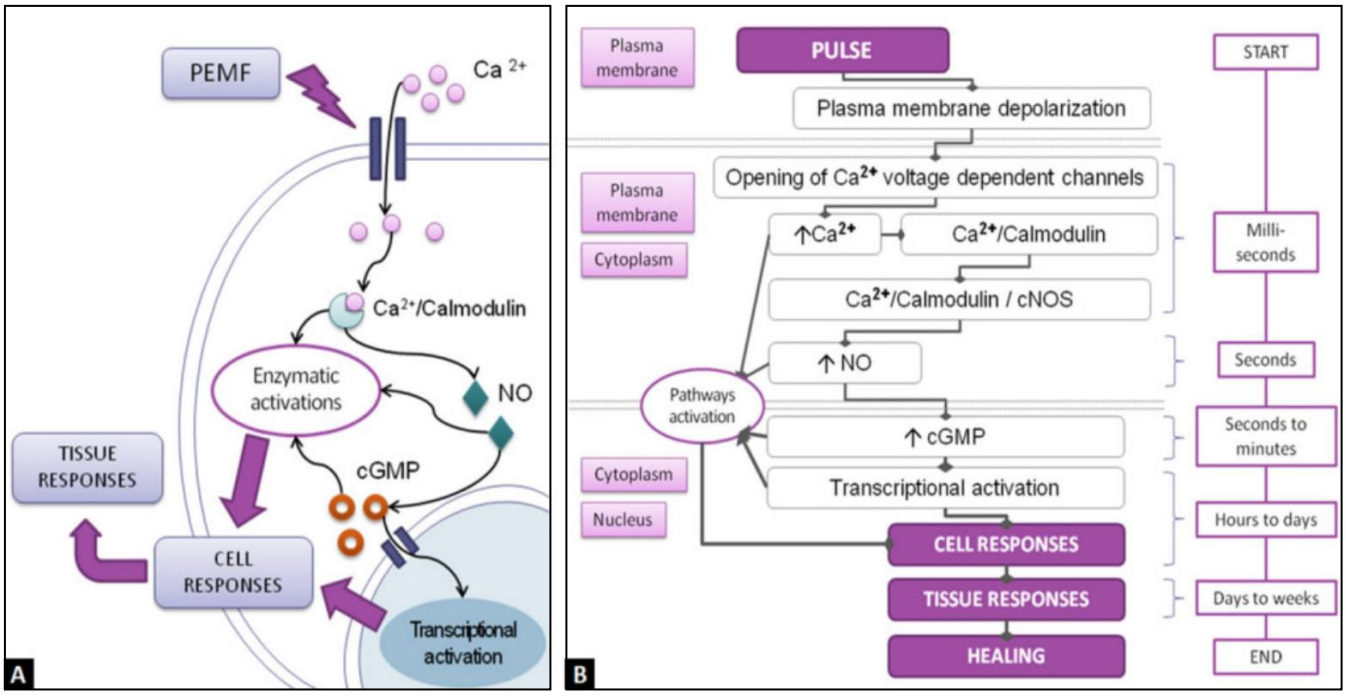

Figure 2: Mechanism of action of tPEMF. (A) General graphical representation of the model; (B) Schematic detail of the sequence of events starting from the first PEMF pulse with the alignment of the temporal flow diagram of the individual events and the involvement of the various cellular compartments (Luigi & Tiziano, 2020).

MATERIALS AND METHODS

Data collection was held between May and December 2022 at the Small Animal Hospital of VetAgro Sup (Veterinary Campus of Lyon, France) with an informed consent of the owners.

1. Animals:

Forty female cats admitted for a routine ovariectomy were recruited in this study. Four cats were excluded due to recording errors while six cats received ketamine in addition to propofol for induction and were thus withdrawn from the study. Therefore, thirty patients were analyzed and included in the study (Appendix 1). The demographic data of each group are represented in table 1.

		Age (months)	Weight (kg)	Procedure length (min)
Control (N=15)	Mean ± SD	9.2 ± 6.7	2.8 ± 0.53	151.4 ± 31.24
tPEMF (N=15)		9.1 ± 5.9	2.8 ± 0.5	154.4 ± 33.95

Table 1: Average age and weight of cats, and procedure length of each group
SD: Standard Deviation, tPEMF: targeted Pulsed Electromagnetic Field

All cats were examined prior to premedication and only healthy cats (ASA physical status of I or II) were included. The cats were blindly and equally allocated among two groups: Control group (cats receiving morphine and medetomidine in premedication) and tPEMF group (cats receiving morphine and medetomidine in premedication in addition to the targeted Pulsed Electromagnetic Fields therapy).

2. Anesthetic Protocol:

The anesthetic procedure adapted in both groups is illustrated in figure 3.

Following a fasting period of approximately 8 hours, cats of both groups received a combination of medetomidine (0.02 mg/kg) (*Dorbene vet, Zoetis, France*) and morphine (0.1 mg/kg) (*morphine chlorhydrate, Aguettant, France*) intramuscularly (*IM*) 30 minutes before the surgery. All cats were then put in a plastic cage placed on the tPEMF pad during 15 minutes and in a quiet environment. A plastic cage devoid of metal was chosen to avoid any effect on the strength of the electromagnetic field treatment. A person (MM/JP), who was not involved in the evaluation of the hemodynamic parameters during the surgery, was charged to randomly activate or not the tPEMF pad, in order to ensure a blind procedure.

After the 15 minute-wait, an intravenous catheter was placed in the cephalic vein and anesthesia was induced with diazepam (0.3 mg/kg) (*Diazépam TVM, France*) and a slow injection of propofol (2 mg/kg) (*Propomitor, Osalia, France*) to effect (see Figure 3).

To intubate the cats, lidocaine spray was used to facilitate the intubation and maintenance of anesthesia was provided by inhalation of isoflurane (*Isoflo, Axience, France*) in 100% oxygen. Intravenous fluids (*Ringer lactate*) were administered at a rate of 3 mL/kg/h. During the surgery, rescue analgesia consisting in an *IV* bolus of fentanyl (1 µg/kg) (*Fentadon, Dechra, France*) was administered in case of a hemodynamic reaction (defined as an increase by ≥ 20% of the heart rate and/or mean blood pressure and/or respiratory rate).

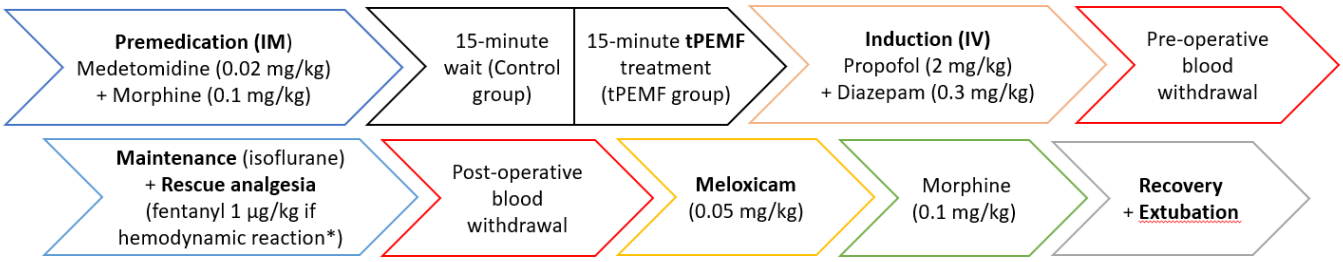

Figure 3: Anesthetic procedure of Control and tPEMF groups.
*Hemodynamic reaction: increase by ≥ 20% of heart rate and/or mean blood pressure and respiratory rate

3. Anesthesia monitoring:

After stabilization, the mean arterial pressure (MAP), heart rate (HR), respiratory rate (RR), electrocardiogram (ECG), oxygen saturation of hemoglobin (SpO2), capnography (ETCO2) and oesophageal temperature were continuously monitored (*Advisor® Vital Signs Monitor V9203®, Surgivet, USA*). These data were collected every 5 min during the procedure as well as at the times of interest. The arterial pressure measurement was repeated twice for further accuracy. The depth of anesthesia was assessed clinically using the palpebral reflex, eyeball rotation and jaw tone. In case of a light anesthetic depth, a bolus of propofol (0.5 mg/kg) was administered with an adjustment of the concentration of inhalant anaesthetic in order to maintain the animal in a surgical plane of anesthesia.

At the end of the surgery, isoflurane flow was discontinued. A *SC* combination of morphine (0.1 mg/kg) and meloxicam (0.05 mg/kg) (*Meloxym®, Osalia, France*) was administered. Once the laryngeal reflex was detected, the animal was extubated.

4. Description of tPEMF device:

The tPEMF device used in this study is represented in figure 4. It is a multi-coil pad (18 inches x 36 inches) that delivers FDA cleared tPEMF therapy for in-clinic use. Its effects can last for about 2 hours after a 15 minute-full body treatment. The coil of the tPEMF is the component that delivers the therapeutic electromagnetic field. It generates a twice-per-second, 2-millisecond burst of a 27.12 megahertz radio wave signal with an amplitude of 4 microtesla. That field extends 4-5 inches on both sides from the plane of the coil. The tPEMF strength is about 100 times less than that from a cell phone, though, its signal characteristics enable it to enhance the normal anti-inflammatory and repair responses in challenged human and animal tissue. In fact, the signal is specifically targeted to enhance the binding of calcium (Ca2+) to calmodulin (CaM). This, in turn, accelerates the nitric oxide (NO) cascade, which regulates inflammation and healing. The tPEMF waveform acts as a first messenger in tissue growth, repair, and maintenance. Therefore, this pulse-modulated field is non-thermal and non-invasive, yet is sufficient in strength to have therapeutic benefit (see Figure 4).

Figure 4: Assisi Loop Lounge® Medium tPEMF System device

5. Surgical procedure of ovariectomy:

Cats were positioned in dorsal recumbency, where they were shaved, cleaned and sterilized on the surgical site, to minimize the risk of infection. And then they were clipped, prepped, and draped from pubis to xiphoid area. Using a scalpel blade, a cutaneous incision was made at the cranial third of the abdomen, just below the umbilicus, on the abdominal midline. After all abdominal layers were incised, the ovaries were located through palpation or visualization of the fallopian tubes. A spay hook was used to sweep the abdomen caudal to the kidney in order to snare the uterine horn. After the ovary, suspensory

ligament, and tip of the uterus were identified and visualized, the first ovary was exteriorized. Hemostatic forceps were applied to the proper ligament (located between ovary and uterine horn) to maintain traction, and the suspensory ligament (fibrous band connecting ovaries to kidneys) was palpated, stretched, and then torn using the left index finger. The ovarian pedicle was clamped with two hemostatic forceps (modified 3 clamp technique) and a third forceps at the level of the proper ligament, after a fenestration was made in the broad ligament (caudal to mesovarium). Dorsal to the most dorsal forceps was placed an encircling ligature, which was later taken away when the ligature was being tightened. A transfixing-encircling absorbable suture followed, which was placed between the encircling ligature and the second forceps. Transection of the pedicle took place between the second forceps and the ovary. The second ovary was treated using the same approach. After checking for any hemorrhage, all abdominal layers were sutured and the cutaneous incision was closed.

6. Study design:

After induction and intubation, the pre-operative blood withdrawal is done, and the blood is collected in heparin tubes and left for a few hours at room temperature.

Before starting the surgery, the cats were positioned in dorsal recumbency and the surgical site was clipped, scrubbed and draped.

MAP, HR and RR were recorded during the whole procedure with a particular focus at 8 specific times of interest: SS (Steady State, defined as the baseline phase between the preparation of the surgical site and the cutaneous incision), I (Cutaneous incision), MO1 (first ovarian manipulation, defined as the manipulation and stretching of the abdominal content to locate the first ovary), TO1 (first ovarian traction, defined as the traction and tearing of the suspensory ligament and the ligation and transection of the pedicle), MO2 (second ovarian manipulation), TO2 (second ovarian traction), MS (muscular sutures) and CS (cutaneous sutures) (Appendix 2).

In order to assess the dynamic variation of MAP, HR and RR between the different times of interest within each group, ΔHR, ΔMAP and ΔRR were calculated for every 2 consecutive times-points as follows:

$\Delta HR = [(HR2-HR1)/(HR2+HR1)/2]*100$, where HR1 is the value of HR at the first time of interest and HR2 is the value of HR at the consecutive time of interest.

$\Delta MAP = [(MAP2-MAP1)/(MAP2+MAP1)/2] * 100$, where MAP1 is the value of MAP at the first time of interest and MAP2 is the value of MAP at the consecutive time of interest.

$\Delta RR = [(RR2-RR1)/(RR2+RR1)/2] * 100$, where RR1 is the value of RR at the first time of interest and RR2 is the value of RR at the consecutive time of interest.

After the end of the surgery, and before extubation, the post-operative blood withdrawal is effected and the blood is collected in heparin tubes and left for a few hours at room temperature.

The tubes are then centrifugated for 5 minutes at the speed of 3700 rpm/rcf, in order to separate the plasma from the blood. The plasma is collected in conical tubes and stored at -20°C until analyzed.

7. Enzyme-Linked Immunosorbent Assays (ELISA):

IL-1 beta was analyzed using a sensitive solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) for feline IL-1β (*Invitrogen, Feline IL-1 beta ELISA Kit*). The immunoassay allowed specific IL-1β quantification in serum / plasma up to 4000 pg/mL. The sensitivity of the method was reported to be 0.05 pg/mL. The assay was performed according to the manufacturer's recommendations after appropriate dilution of samples. IL-1β sample concentrations were determined following readings at 595 nm (for high concentrations) or 420 nm (for low concentrations), depending on the level of IL-1β.

8. Statistical Analysis:

Statistical analysis was performed using IBM SPSS. Normality of distribution was assessed using Kolmogorov-Smirnov test. Parametric data were expressed as mean ± standard deviation (SD).

A one-way repeated measures ANOVA was conducted for each group to compare MAP, HR and RR between the different times of interest within each group. Wilks' Lambda value and its p value were reported to check the effect of time on the parameters. A pairwise comparison was used to compare each parameter at each time of interest between the groups.

Friedman test was conducted to compare ΔHR, ΔMAP, ΔRR and the mean ranks within each group. Mann-whitney U test was used to compare ΔHR, ΔMAP, ΔRR between the groups.

Friedman test was used to compare the inhaled concentration of isoflurane between the different times of interest within each group.

Regarding the evaluation of the IL-1β concentration, Wilcoxon Signed Ranks test was used to compare the preoperative and postoperative IL-1β concentrations within each group whereas Mann-Whitney U test was used to compare the preoperative and postoperative IL-1β concentration between groups.

RESULTS

Thirty cats were recruited and were divided equally into 2 groups (see Appendix 1). Within each group, HR, MAP and RR were compared at each time-point to their measurements at Steady-State (SS). These parameters were also compared between groups at each time of interest. ΔHR, ΔMAP and ΔRR were compared within and between groups.

1. Evaluation of the Respiratory Rate:

In the control group, a significant increase in RR was noted only at the time of cutaneous sutures (CS) (33 bpm at CS *vs* 23 bpm at SS, $p \leq 0.05$) (Figure 5).

In tPEMF group, RR appeared to increase at the time of muscular sutures (MS) as well as cutaneous sutures compared to SS (29 bpm and 34 bpm at MS and CS respectively *vs* 25 bpm at SS, $p \leq 0.05$) (see Figure 5).

No significant difference was found in RR between groups nor in its dynamic variation (ΔRR) within each group. However, it is relevant to mention that, unlike Control group, RR didn't show any change at incision (I) compared to SS in tPEMF group (ΔRR = 3.5 and 0.4 in the Control and tPEMF groups respectively).

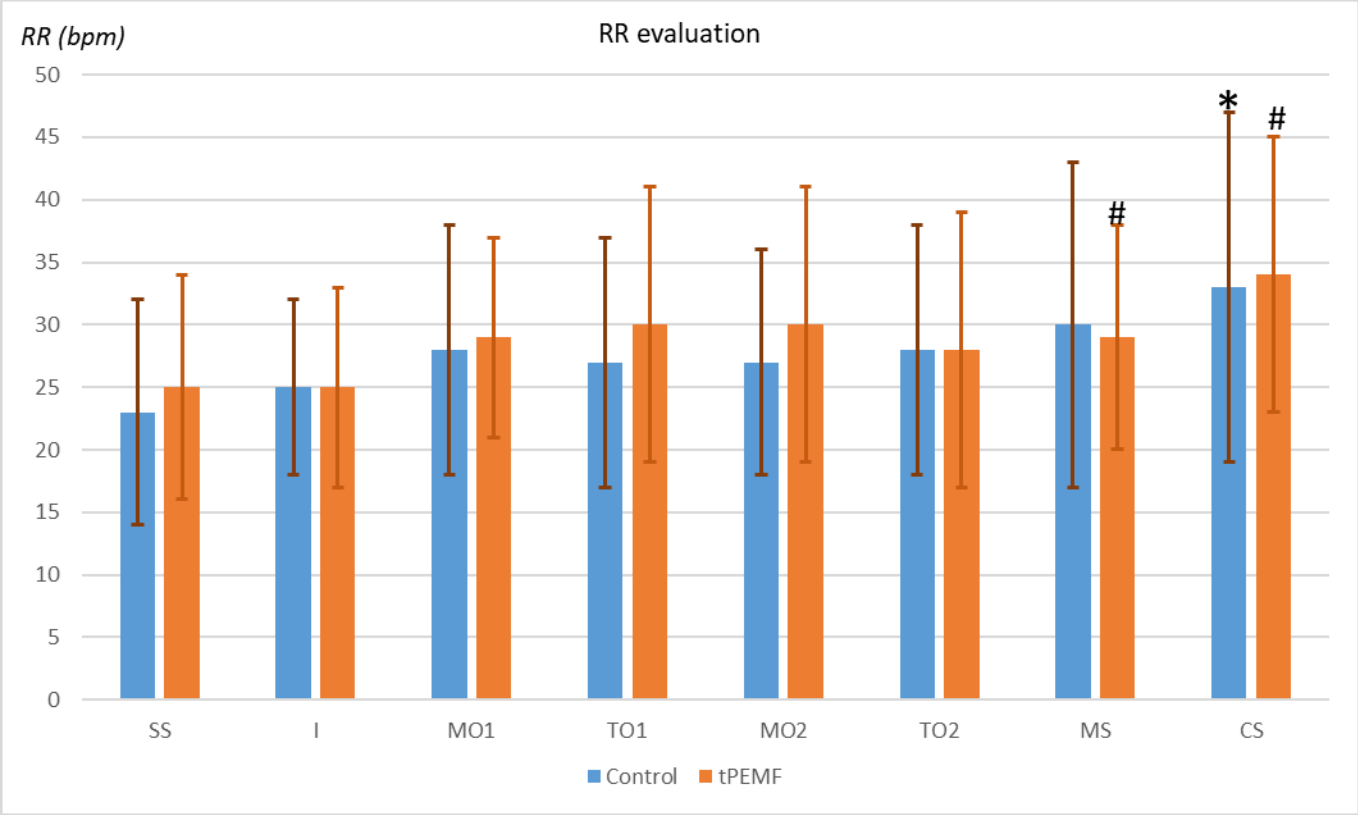

Figure 5: Evaluation of RR at each time of interest compared with SS.
RR: Respiratory Rate, SS: Steady State, I: Incision, MO1: Manipulation of 1st Ovary, TO1: Traction of 1st Ovary, MO2: Manipulation of 2nd Ovary, TO2: Traction of 2nd Ovary, MS: Muscular Suture, CS: Cutaneous Suture.
*indicates a significant variation ($p<0.05$) of RR at each time point compared to SS in Control group.
#indicates a significant variation ($p<0.05$) of RR at each time point compared to SS in tPEMF group.

2. Evaluation of the Heart Rate:

In both groups, HR appeared to vary significantly ($p\leq0.05$) at MO1, TO1, MO2, TO2, MS and CS compared to SS (Control group : MO1: 153 bpm, TO1: 160 bpm, MO2: 161 bpm, TO2: 162 bpm, MS: 158 bpm, CS: 152 bpm *vs* SS: 115 bpm and tPEMF group : MO1: 146 bpm, TO1: 148 bpm, MO2: 157 bpm, TO2: 151 bpm, MS: 147 bpm, CS: 152 bpm *vs* SS: 122 bpm) (Figure 6). Also, its dynamic variation (ΔHR) appeared to vary significantly between the different time-points within each group ($p=0.0001$).

No significant difference in HR and ΔHR was detected between groups.

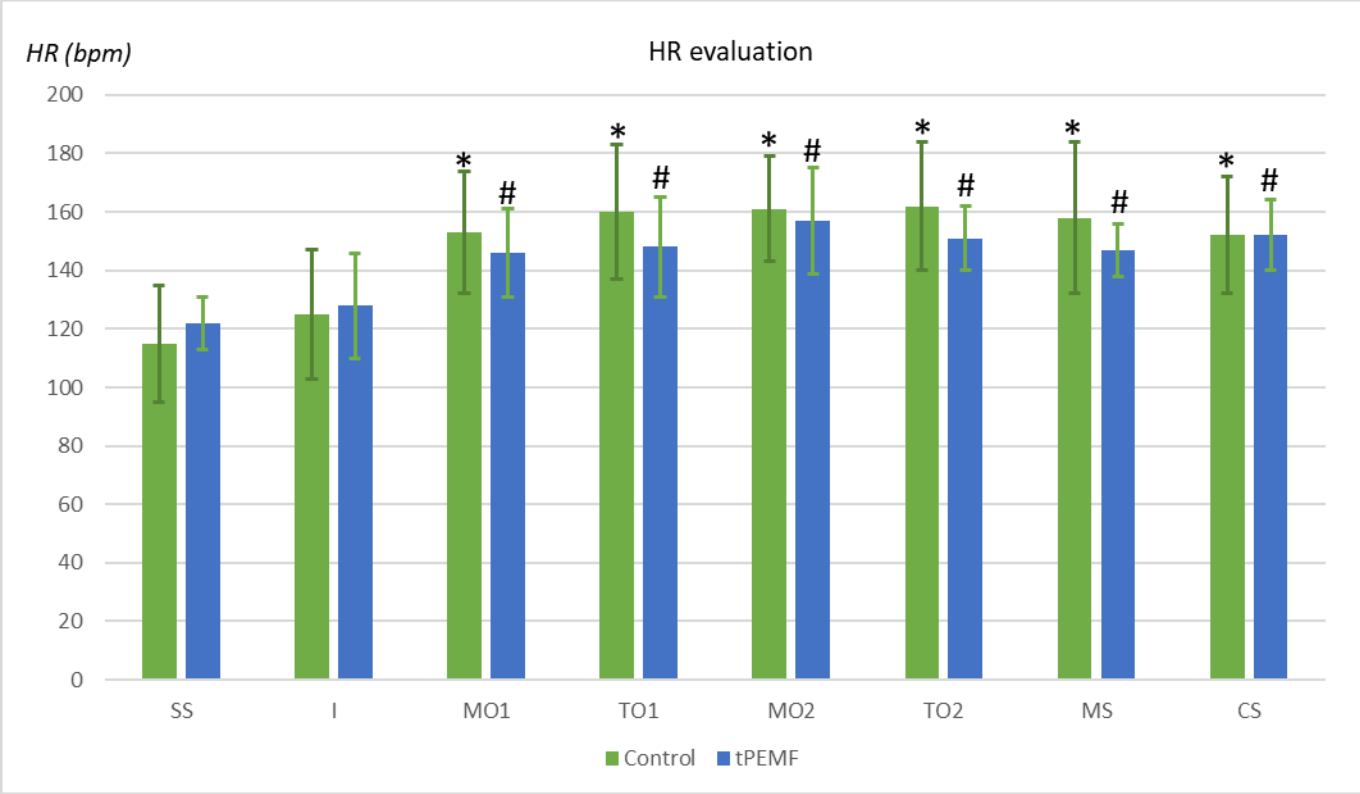

Figure 6: Evaluation of HR at each time of interest compared with SS.
HR: Heart Rate, SS: Steady State, I: Incision, MO1: Manipulation of 1st Ovary, TO1: Traction of 1st Ovary, MO2: Manipulation of 2nd Ovary, TO2: Traction of 2nd Ovary, MS: Muscular Suture, CS: Cutaneous Suture.
*indicates a significant variation ($p<0.05$) of HR at each time point compared to SS in Control group.
#indicates a significant variation ($p<0.05$) of HR at each time point compared to SS in tPEMF group.

3. Evaluation of the Mean Arterial Pressure:

Similar to HR, MAP appeared to significantly increase in both groups ($p\leq0.05$) at MO1, TO1, MO2, TO2 compared to SS (Control group: MO1: 80 mmHg, TO1: 94 mmHg, MO2: 86 mmHg, TO2: 97 mmHg *vs* SS: 60 mmHg and tPEMF group: MO1: 76 mmHg, TO1: 83 mmHg, MO2: 81 mmHg, TO2: 79 mmHg *vs* SS: 60 mmHg) (Figure 7).

When comparing both groups, a significant difference in MAP at TO2 was detected (97 mmHg for the Control group *vs* 79 mmHg for the tPEMF) (see Figure 7).

Regarding the dynamic variation of MAP, ΔMAP appeared to vary significantly between the different time-points within each group ($p=0.0001$) but no significance was noted between groups. However, it is worth noting that MAP appeared to be stable to a certain extent between MO1 and TO2 in the tPEMF group when compared to the Control group (Control group: ΔMAP = 3 (at TO1), -1.3 (at MO2) and 2 (at TO2) *vs* tPEMF group: ΔMAP = 1.8 (at TO1), -0.6 (at MO2) and -0.5 (at TO2))

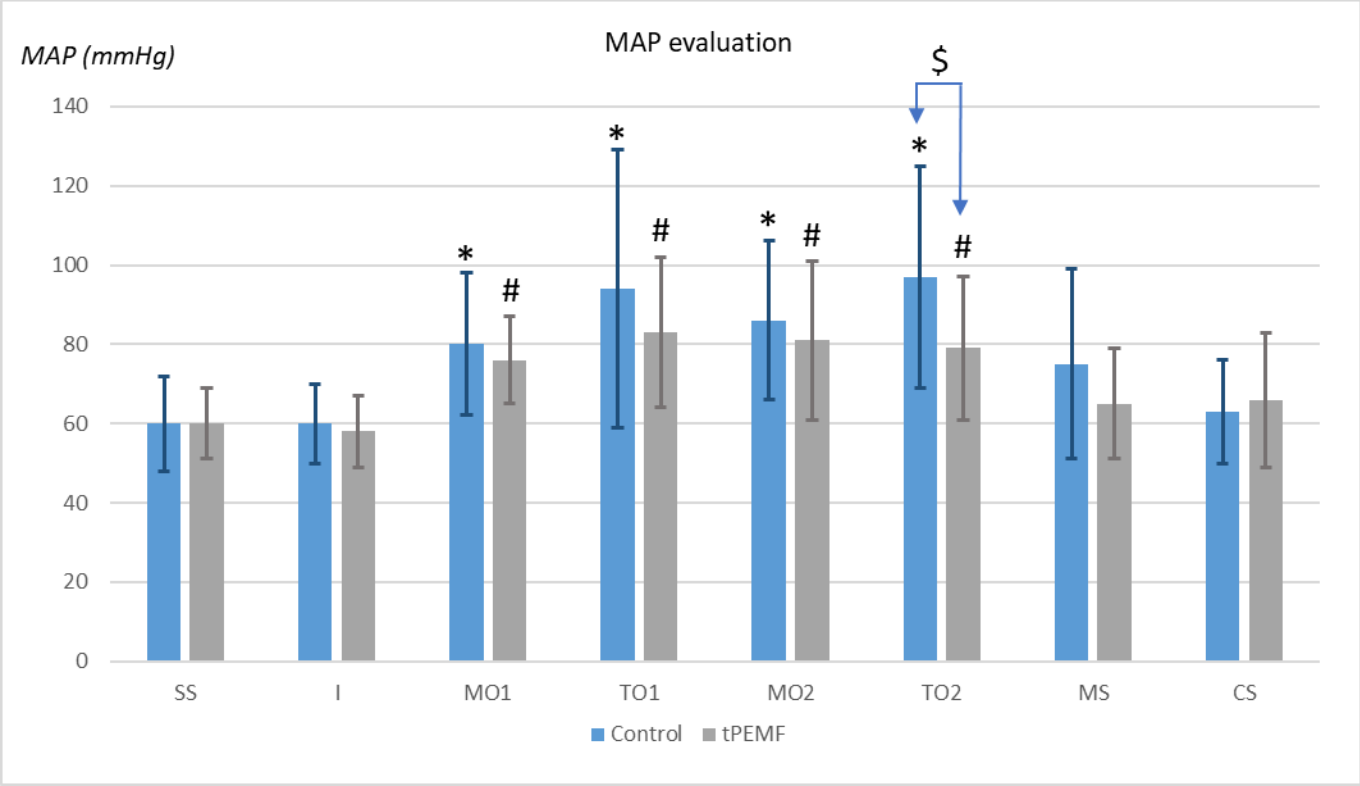

Figure 7: Evaluation of MAP at each time of interest compared with SS.
MAP: Mean Arterial Pressure, SS: Steady State, I: Incision, MO1: Manipulation of 1st Ovary, TO1: Traction of 1st Ovary, MO2: Manipulation of 2nd Ovary, TO2: Traction of 2nd Ovary, MS: Muscular Suture, CS: Cutaneous Suture.
* indicates a significant variation ($p<0.05$) of MAP at each time point compared to SS in Control group.
# indicates a significant variation ($p<0.05$) of MAP at each time point compared to SS in tPEMF group.
$ indicates a significant variation ($p<0.05$) of MAP at TO2 between the two groups.

4. Isoflurane concentration

No significant variation of isoflurane concentration was shown between the time-points within each group nor between groups at any time-point. The overall mean isoflurane concentration was 1.324 ± 0.318 in Control group and 1.334 ± 0.335 in tPEMF group.

5. Propofol and fentanyl requirements

No significant difference was detected in propofol and fentanyl administration between the time-points of each group nor between groups at any time-point.

6. Evaluation of the inflammation:

The preoperative and postoperative concentration of IL-1β in each group were as follow:

- The Control group presented a preoperative and postoperative IL-1β concentration of 1115.63 ± 2875.23 pg/ml and 1106.36 ± 2841.81 pg/ml respectively.
- The tPEMF group revealed a preoperative and postoperative IL-1β concentration of 528.28 ± 1535.66 pg/ml and 537.74 ± 1567.44 pg/ml respectively (table 2).

		Preoperative IL1-β concentration	Postoperative IL1-β concentration
Control	Mean ± SD (pg/ml)	1115.63 ± 2875.23	1106.36 ± 2841.81
tPEMF		528.28 ± 1535.66	537.74 ± 1567.44

Table 2: Average preoperative and postoperative concentration of IL-1β in each group
SD: Standard Deviation, tPEMF: targeted Pulsed Electromagnetic Field

No significant difference was noted between the preoperative and postoperative IL-1β concentration within each group (p=0.7). In addition, the comparison of the preoperative IL-1β concentration on one hand and the postoperative IL-1β concentration on the other hand between groups was shown to be unsignificant (p=0.2 and p=0.4 respectively).

DISCUSSION

The main objective of this study was to evaluate if the tPEMF device could reduce the intraoperative hemodynamic alterations and drug administration in healthy cats undergoing ovariectomy. Another goal was to evaluate if this technology could help decrease the postoperative level of surgical inflammation in these cats. In both groups, the main results are a significant variation of HR and MAP from the time of the 1st ovarian manipulation until the cutaneous sutures. Also, MAP value appeared to be significantly lower when treated with tPEMF therapy at the 2nd ovarian traction compared to control group. Regarding the intraoperative drug administration, propofol and fentanyl appeared to be similarly administered in both groups. As for the IL-1β concentration, no significant differences were noted in the preoperative and postoperative IL-1β concentrations within and between groups.

Advancement in the field of non-invasive non-pharmacological anti-inflammatory therapy has significance for both human and veterinary medicine, particularly in the areas of pain management, reduction of inflammation, bone healing, and wound healing (Gaynor *et al.*, 2018). These technologies include the tPEMF therapy which delivers electromagnetic stimulations to tissues and accelerates the nitric oxide (NO) cascade leading to the regulation of the inflammation and healing process. Incidentally, physicians who discovered NO's physiological properties were awarded the Nobel Prize in Medicine in 1998. In veterinary medicine, tPEMF was demonstrated to be a successful and safe post-operative pain treatment (Pinna *et al*., 2013; Zidan *et al.*, 2018). As such, it is suggested that tPEMF can be used as a standalone or adjunct therapy for pain and inflammation regimens which can help sparing postoperative opioids and other pain medications (Strauch *et al.*, 2009; Rohde *et al.*, 2015). However, apart from the postoperative period, no studies have been conducted to date in veterinary medicine to assess the intraoperative efficiency of tPEMF to prevent pain and inflammation. Therefore, to our knowledge, the present study is the first to evaluate the performance of tPEMF in cats during general anesthesia.

In terms of cardiovascular reactions, both groups revealed significant variations in HR and MAP compared to SS at an early stage of the surgery. These alterations in HR and MAP could be considered as hemodynamic reactions due to nociception at the different surgical stimulations, especially during ovarian manipulation and traction (Burton *et al.*, 2016; Hernandez-Avalos *et al.*, 2019). In addition, as the dynamic variation of these parameters didn't differ between groups, one could assume a poor performance of the tPEMF to reduce intraoperative nociception and the consumption of rescue analgesia. This is in accordance with the study done by Shafford *et al.* (2002) which failed to demonstrate any benefits of tPEMF alone when compared to untreated controls. However, during the traction of the second ovary, tPEMF group showed a considerably lower MAP compared to Control group. Interestingly, Stewart *et al.* (2020) reported a similar trend of reduced MAP after tPEMF therapy

application on a group of hypertensive individuals when compared to control group. As such, the results of our study are attractive as no discernable differences were noted between the two groups in terms of isoflurane concentration, fentanyl or propofol administration, therefore, the lower blood pressure shown in tPEMF group at the second ovarian traction is not expected to be related to the effect of isoflurane level alteration or to intraoperative drugs' administration. Therefore, a potential effect of tPEMF on blood pressure cannot be ruled out at this phase of the surgery.

Regarding the respiratory pattern, although not significant and unlike the Control group, the respiratory rate appeared to be stable in tPEMF group at the cutaneous incision, which is considered as the first nociceptive surgical stimulation. This may point to a potential stabilizing effect of the tPEMF during the early phase of the surgery.

Lastly, this study didn't present any significance when analyzing the results of the preoperative and postoperative IL-1β concentrations within and between groups. However, despite the non significance and the large dispersion of the IL-1β concentrations, both preoperative and postoperative concentrations of IL-1β in tPEMF group appeared to be lower than in Control group. The interpretation of these results could be difficult due to the large IL-1β data dispersion which might be due to the limited number of cats recruited in this study. Therefore, these results could not be conclusive and further studies that evaluate the intraoperative anti-inflammatory effect of tPEMF are required.

We acknowledge several limitations regarding this study. A limited number of cats was included which could have lowered the statistical power of the study. Also, in order to facilitate the implementation of the protocol in our clinical context, the measurement of arterial pressure during the procedure was non-invasive and thus not as accurate as an invasive method. However, the blood pressure measurement was repeated twice to avoid aberrant values. Another limitation could be related to the competence level of the veterinary students performing the ovariectomy which could have contributed in the prolongation of the surgical procedure. Additionally, this study did not assess post-operative recovery due to the immediate administration of meloxicam and morphine after extubation which can certainly affect the quality of recovery.

CONCLUSION

In order to reduce inflammation and pain in animals both during and after surgery, anti-inflammatory and analgesic therapies are essential pre-operative measures. Medications such as opioids, alpha 2 agonists, and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used. But because these medications can have a number of adverse effects in animals, researchers are looking into non-pharmacological anti-inflammatory devices (NPAIDs), such as targeted pulsed electromagnetic field therapy (tPEMF).

The purpose of this study was to examine the possible intraoperative advantages of tPEMF therapy, and evaluate its effects on inflammation and hemodynamic stability as an adjunctive treatment to premedication in healthy female cats undergoing ovariectomy. A number of interesting results were found after recording and assessing intraoperative nociceptive parameters, and analyzing pro inflammatory cytokines concentrations in the control and tPEMF groups.

HR an MAP considerably varied during the surgical procedure in both control group and tPEMF group, indicating hemodynamic reactions presumably caused by surgical nociception. However, this variation of HR and MAP did not differ significantly between the two groups, reflecting limited efficacy in decreasing intraoperative nociception.

Yet during the second ovarian traction, an interesting observation was made: the tPEMF group had a significantly lower MAP than the control group. This finding highlights questions regarding the potential impact of tPEMF on blood pressure regulation and calls for additional research.

Regarding RR, tPEMF application proved to have a stabilizing effect during the first nociceptive surgical stimulus, the cutaneous incision, raising the possibility that tPEMF may help to reduce early intraoperative nociception, although more studies are required to validate this effect.

IL-1β concentrations used to assess postoperative inflammation did not show any significant variations between the groups. The preoperative and postoperative IL-1β concentrations in the tPEMF group, however, were consistently lower than those in the control group, suggesting a potential anti-inflammatory impact that needs additional research, ideally with a larger sample size.

Finally, in a veterinary clinical context, the use of tPEMF device was associated with a suspicious intraoperative analgesic and anti-inflammatory effect in anesthetized cats. Based on these results, this device needs further investigation on a larger number of animals and in different clinical contexts, with assessment of post-operative recovery, and invasive arterial pressure measurement for more accuracy.

REFERENCES

- Adrian, D., Papich, M., Baynes, R., Murrell, J. and Lascelles, B. D. X. (2017). *Chronic maladaptive pain in cats: A review of current and future drug treatment options. Vet J. 230:52-61.* doi:10.1016/j.tvjl.2017.08.006. Epub 2017 Aug 20. PMID: 28887012.
- Arias, J. I., Aller, M. A. and Arias, J. (2009). *Surgical inflammation: a pathophysiological rainbow. Journal of Translational Medicine, 7(1), 19.* doi:10.1186/1479-5876-7-19.
- Bosmans, T., Doom, M., Gasthuys, F., Simoens, P., Van Ham, L. and Polis, I. (2009). *Perioperative pain: physiology and pathophysiology. Vlaams Diergeneeskundig Tijdschrift, 78, 302-313.*
- Burton, A. R., Fazalbhoy, A. and Macefield, V. G. (2016). *Sympathetic Responses to Noxious Stimulation of Muscle and Skin. Frontiers in Neurology, 7.* doi:10.3389/fneur.2016.00109.
- Capner, C. A., Lascelles, B. D. X. and Waterman-Pearson, A. E. (1997) *Journal of Veterinary Anaesthesia 24, 42*
- Choileain, N. N. and Redmond, H. P. (2006). *Cell response to surgery. Arch Surg., 141(11), 1132-1140.*
- Cicirelli, V., Matteo, B., Di Bella, C., Lacalandra, G. and Aiudi, G. (2022). *The ultrasound-guided funicular block in cats undergoing orchiectomy: ropivacaine injection into the spermatic cord to improve intra and postoperative analgesia. BMC Veterinary Research. 18(1).* doi:10.1186/s12917-022-03279-4.
- Cohen, S. P. and Raja, S.N. (2013). *Prevention of chronic postsurgical pain: the ongoing search for the holy grail of anesthesiology. Anesthesiology. 118(2):241-3.* doi:10.1097/ALN.0b013e31827d4129. PMID: 23340346.
- Conceição, M. E. B. A. M. D., Uscategui, R. A. R., Bertolo, P. H. L., de Souza, D. C., Rolemberg, D. D. S., de Moraes, P. C., Teixeira, P. P. M. and Dias, L. G. G. G. (2018). *Assessment of postoperative inflammatory markers and pain in cats after laparoscopy and miniceliotomy ovariectomy. Vet Rec. 2018 Dec 1;183(21):656.* doi: 10.1136/vr.104776. Epub 2018 Sep 25. PMID: 30254131.
- Crockett, A. and Panickar, A. (2011). *Role of the sympathetic nervous system in pain. Anaesthesia & Intensive Care Medicine, 12(2), 50–54.* doi:10.1016/j.mpaic.2010.10.023.
- Dohoo S. E. and Dohoo I. R. (1996). *Factors influencing the postoperative use of analgesics in dogs and cats by Canadian veterinarians. Can Vet J 37552-556.*
- Dohoo S. E. and Dohoo I. R. (1998). *Attitudes and concerns of Canadian animal health technologists toward postoperative pain management in dogs and cats. Can Vet J 39491-496.*

- Fertziger A. P., Stein E. A. and Lunch J. J. (1973). *Suppression of morphine-induced mania in cats. Psychopharmacology 36:185-187*.
- Gaynor, J. S., Hagberg, S. and Gurfein, B. T. (2018). *Veterinary applications of pulsed electromagnetic field therapy. Research in Veterinary Science, 119, 1-8.* doi:10.1016/j.rvsc.2018.05.005.
- Goyagi, T. and Nishikawa, T. (1995). *The addition of epinephrine enhances postoperative analgesia by intrathecal morphine. Anesth Analg. 81(3):508-13.* doi:10.1097/00000539-199509000-00014. PMID: 7653813.
- Hernandez-Avalos, I., Mota-Rojas, D., Mora-Medina, P., Martínez-Burnes, J., Casas Alvarado, A., Verduzco-Mendoza, A., *et al*. (2019). *Review of different methods used for clinical recognition and assessment of pain in dogs and cats. Int J Vet Sci Med. 7:43–54.* doi:10.1080/23144599.2019.1680044.
- Höglund, V., Olsson, K., Hagman, R., Öhlund, M., Olsson, U. and Lagerstedt, S., (2011). *Comparison of haemodynamic changes during two surgical methods for neutering female dogs. Research in veterinary science, 91 (1): 159–163. 43.*
- Höglund, V., Hagman, R., Olsson, K. and Olsson, U. (2014). *Intraoperative changes in blood pressure, heart rate, plasma vasopressin, and urinary noradrenalin during elective ovariohysterectomy in dogs: repeatability at removal of the 1st and 2nd ovary. Vet Surg., 43: 852–859.*
- Hsing, C. H. and Wang, J. J. (2015). *Clinical implication of perioperative inflammatory cytokine alteration. Acta Anaesthesiologica Taiwanica, 53(1), 23–28.* doi:10.1016/j.aat.2015.03.002.
- Kelly, D. J., Ahmad, M. and Brull, S. J. (2001). *Preemptive analgesia I: physiological pathways and pharmacological modalities. Canadian Journal of Anesthesia/Journal Canadien D'anesthésie, 48(10), 1000–1010.* doi:10.1007/bf03016591.
- Lamont, L.A., Tranquilli, W.J., and Grimm, K.A. (2000). *Physiology of pain. The Veterinary clinics of North America. Small animal practice, 30 4, 703-28, v.*
- Lamont, L. A. (2002). *Feline perioperative pain management. Veterinary Clinics of North America: Small Animal Practice, 32(4), 747–763.* doi:10.1016/s0195-5616(02)00028-1
- Lascelles B. D. X. and Waterman A. (1997). *Analgesia in cats. In Practice 19:203-213.*
- Lascelles, B. D. X., Capner, C. A. and Waterman-Pearson, A. E. (1999). *Current British veterinary attitudes to perioperative analgesia for cats and small mammals. Vet Rec 145:601404.*

- Ledowski, T. (2019). *Objective monitoring of nociception: a review of current commercial solutions. Br J Anaesth. 123:e312–21.* doi:10.1016/j.bja.2019.03.024.
- Lemke, K.A. (2004). *Understanding the pathophysiology of perioperative pain. Canadian Veterinarian Journal 45, 405-413.*
- Lin, E., Calvano, S. E. and Lowry, S. F. (2000). *Inflammatory cytokines and cell response in surgery. Surgery, 127(2), 117–126.* doi:10.1067/msy.2000.101584.
- Luigi, C. and Tiziano, P. (2020). *Mechanisms of Action And Effects of Pulsed Electromagnetic Fields (PEMF) in Medicine. J Med Res Surg 1(6): pp. 1-4.* doi:10.52916/jmrs204033.
- Mathews, K. A. (2000). *Pain Assessment and General Approach to Management. Veterinary Clinics of North America: Small Animal Practice, 30(4), 729–755.* doi:10.1016/s0195-5616(08)70004-4.
- Meintjes, R. A. (2012). *An overview of the physiology of pain for the veterinarian. The Veterinary Journal, 193(2), 344–348.* doi:10.1016/j.tvjl.2012.03.001.
- Moffett, J., Kubat, N. and Fray, L. (2015). *Effect of pulsed electromagnetic field treatment on programmed resolution of inflammation pathway markers in human cells in culture. Journal of Inflammation Research, 59.* doi:10.2147/jir.s78631.
- Muir III, W. W. and Woolf, C. J. (2001). *Mechanisms of pain and their therapeutic implications. Journal of the American Veterinary Medical Association, 219(10), 1346-1356.* doi:10.2460/javma.2001.219.1346.
- Ng, P. C., Li, K., Wong, R. P. O., Chui, K., Wong, E., Li, G. and Fok, T. F. (2003). *Proinflammatory and anti-inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed. 88(3):F209-13.* doi:10.1136/fn.88.3.f209. PMID: 12719394; PMCID: PMC1721542.
- Norman, K. and Nappe, T. M. (2023). *Alpha Receptor Agonist Toxicity. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.* PMID: 29763199.
- Omote, K., Kitahata, L. M., Collins, J. G., Nakatani, K. and Nakagawa, I. (1991). *Interaction between opiate subtype and alpha-2 adrenergic agonists in suppression of noxiously evoked activity of WDR neurons in the spinal dorsal horn. Anesthesiology. 74(4):737-43.* doi:10.1097/00000542-199104000-00018. PMID: 1672579.
- Paruk, F. and Chausse, J. M. (2019). *Monitoring the post surgery inflammatory host response. J Emerg Crit Care Med, 3:47.* doi: 10.21037/jeccm.2019.08.06.
- Pilla, A. A. (2013). *Nonthermal electromagnetic fields: from first messenger to therapeutic applications. Electromagnetic Biology and Medicine 32 (2), 123–136.*

- Pinna, S., Landucci, F., Tribuiani, A. M., Carli, F. and Venturini, A. (2013). *The effects of pulsed electromagnetic field in the treatment of osteoarthritis in dogs: clinical study. Pak Vet J, 33(1): 96-100.*
- Rawe, I. M., Lowenstein, A., Barcelo, C. R. and Genecov, D. G. (2012). *Control of Postoperative Pain with a Wearable Continuously Operating Pulsed Radiofrequency Energy Device: A Preliminary Study. Aesthetic Plastic Surgery, 36(2), 458–463.* doi:10.1007/s00266-011-9828-3.
- Robertson, S. A. and Taylor, P. M. (2004). *Pain management in cats past, present and future. Part 2. Treatment of pain--clinical pharmacology. J Feline Med Surg. 6(5):321-33.* doi:10.1016/j.jfms.2003.10.002. PMID: 15363764.
- Rohde, C., Chiang, A., Adipoju, O., Casper, D. and Pilla, A. A. (2010). *Effects of Pulsed Electromagnetic Fields on Interleukin-1β and Postoperative Pain: A Double-Blind, Placebo-Controlled, Pilot Study in Breast Reduction Patients. Plastic and Reconstructive Surgery, 125(6), 1620-1629.* doi:10.1097/prs.0b013e3181c9f6d3.
- Rohde, C. H., Taylor, E. M., Alonso, A., Ascherman, J. A., Hardy, K. L. and Pilla, A. A. (2015). *Pulsed Electromagnetic Fields Reduce Postoperative Interleukin-1β, Pain, and Inflammation. Plastic and Reconstructive Surgery, 135(5), 808e–817e.* doi:10.1097/prs.0000000000001152.
- Roizen F. (1988). *The compelling rationale for less preoperative testing. Can J Anaesth., 35 (3): 214- 218.*
- Roizen, M. F. (2012). *Effects of pulsed electromagnetic fields on interleukin-1 beta and postoperative pain: a double-blind, placebo-controlled, pilot study in breast reduction patients. Yearbook of Anesthesiology and Pain Management, 2012, 347.* doi:10.1016/j.yane.2012.02.021.
- Rossaint, J. and Zarbock, A. (2018). *Perioperative Inflammation and Its Modulation by Anesthetics. Anesthesia & Analgesia, 126(3), 1058–1067.* doi:10.1213/ane.0000000000002484.
- Ruíz-López, P., Domínguez, J. M. and Granados, M. D. M. (2020). *Intraoperative nociception-antinociception monitors: A review from the veterinary perspective. Vet Anaesth Analg. 47:152–9.* doi:10.1016/j.vaa.2019.09.006.
- Shafford, H. L., Hellyer, P. W., Crump, K. T., Wagner, A. E., Mama, K. R. and Gaynor, J. S. (2002). *Use of a pulsed electromagnetic field for treatment of post-operative pain in dogs: a pilot study. Vet Anaesth Analg. 29(1):43-48.* doi:10.1046/j.1467-2987.2001.00072.x. Epub 2016 Nov 15. PMID: 28404268.

- Simon, B. T., Scallan, E. M., Carroll, G. and Steagall, P. V. (2017). *The lack of analgesic use (oligoanalgesia) in small animal practice. J Small Anim Pract. 58(10):543-554.* doi:10.1111/jsap.12717. Epub 2017 Aug 1. PMID: 28763103.
- Slingsby, L. S. and Waterman-Pearson, A. E. (1998). *Comparison of pethidine, buprenorphine and ketoprofen for postoperative analgesia after ovariohysterectomy in the cat. Veterinary Record 143, 185189.*
- Slingsby, L. S. and Waterman-Pearson, A. E. (2000). *Postoperative analgesia in the cat after ovariohysterectomy by use of carprofen, ketoprofen, meloxicam or tolfenamic acid. Journal of Small Animal Practice, 41(10), 447–450.* doi:10.1111/j.1748-5827.2000.tb03139.
- Stanway, G. W., Taylor, P. M. and Brodbelt, D. C. (1996). *A comparison of preoperative morphine and buprenorphine in cats. Journal of Veterinary Anaesthesia 23, 78.*
- Steagall, P. V., Robertson, S., Simon, B., Warne, L. N., Shilo-Benjamini, Y. and Taylor, S. (2022). *2022 ISFM Consensus Guidelines on the Management of Acute Pain in Cats. Journal of Feline Medicine and Surgery, 24, 4–30.* doi:10.1177/1098612X211066268.
- Stewart, G. and Panickar, A. (2013). *Role of the sympathetic nervous system in pain*. *Anaesthesia & Intensive Care Medicine. 14(12): 524-527.* doi:10.1016/j.mpaic.2013.09.003.
- Stewart, G. M., Wheatley-Guy, C. M., Johnson, B. D., Shen, W. K. and Kim, C. H. (2020). *Impact of pulsed electromagnetic field therapy on vascular function and blood pressure in hypertensive individuals. J Clin Hypertens (Greenwich). 22(6):1083-1089.* doi:10.1111/jch.13877. Epub 2020 May 13. PMID: 32401418; PMCID: PMC8030044.
- Strauch, B., Herman, C., Dabb, R., Ignarro, L. J. and Pilla, A. A. (2009). *Evidence-based use of pulsed electromagnetic field therapy in clinical plastic surgery. Aesthet. Surg. J. 29, 135–143.*
- Sylvain, L., Jerneja, S. and Karine, P. (2022). *Respiratory response to finger clamping in dogs under general anesthesia: A descriptive pilot study. Front. Vet. Sci. 9:843956.* doi:10.3389/fvets.2022.843956.
- Urman, R., Vadivelu, N., Mitra, S., Kodumudi, V., Kaye, A. and Schermer, E. (2014). *Preventive analgesia for postoperative pain control: a broader concept. Local and Regional Anesthesia, 17.* doi:10.2147/lra.s62160.
- Vicente, D. and Bergström, A. (2018). *Evaluation of intraoperative analgesia provided by incisional lidocaine and bupivacaine in cats undergoing ovariohysterectomy. J Feline Med Surg. 20(10):922-927.* doi:10.1177/1098612X17735167. Epub 2017 Oct 11. PMID: 29019444.

- Watson A.D.J., Nicholson A., Church D.B., *et a1*. (1996). *Use of anti-inflammatory and analgesic drugs in dogs and cats. Aust Vet J 74203-210.*
- Wright, B. D. (2002). *Clinical pain management techniques for cats. Clinical Techniques in Small Animal Practice, 17(4), 151–157.* doi:10.1053/svms.2002.36609.
- Zidan, N., Fenn, J., Griffith, E., Early, P. J., Mariani, C. L., Muñana, K. R., Guevar, J. and Olby, N. J. (2018). *The Effect of Electromagnetic Fields on Post-Operative Pain and Locomotor Recovery in Dogs with Acute, Severe Thoracolumbar Intervertebral Disc Extrusion: A Randomized Placebo-Controlled, Prospective Clinical Trial. Journal of Neurotrauma, 35(15), 1726–1736.* doi:10.1089/neu.2017.5485.

Appendices

Appendix 1: Characteristics of cats undergoing ovariectomy

Control group (n=15)

Animal Breed	**Weight** (kg)	**Age** (months/years)
European	2	1.5 yo
European	1.8	4 mo
Maine Coon	3.8	2.5 yo
European	3.75	1 yo
European	2.35	1 yo
European	2.8	6 mo
European	2.54	6 mo
European	2.79	6 mo
European	2.17	6 mo
European	3.38	8 mo
European	3.17	6 mo
European	3.2	7 mo
European	3	5 mo
European	2.83	7 mo
European	2.8	6 mo

tPEMF group (n=15)

Animal Breed	**Weight** (kg)	**Age** (months/years)
European	2.85	1 yo
European	2.7	2 yo
European	2.57	5 mo
European	2.2	6 mo
European	2.76	5.5 mo
European	2.9	8 mo
European	2.62	6 mo
European	3.43	1 yo
European	2.8	8 mo
European	2.95	5 mo
European	2.8	1.5 yo
European	2.6	7 mo
European	4.15	15 yo
European	2.9	6 mo
European	3.3	8 mo

Appendix 2: Vital measurements at each time-point

		Iso (%)	**MAP** (mmHg)	**HR** (bpm)	**RR** (bpm)
SS	__h__				
	__h__				
	__h__				
I	__h__				
	__h__				
	__h__				
MO1	__h__				
	__h__				
	__h__				
TO1	__h__				
	__h__				
	__h__				
MO2	__h__				
	__h__				
	__h__				
TO2	__h__				
	__h__				
	__h__				
MS	__h__				
	__h__				
	__h__				
CS	__h__				
	__h__				
	__h__				