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COMPARATIVE STUDY OF TWO INCISIONAL INFILTRATION TECHNIQUES IN DOGS UNDERGOING OVARIECTOMY

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Abstract

Incisional infiltration of local anaesthetic is a technique in support of multimodal analgesia. Usually, this technique is performed by injection of local anaesthetic directly into the incision site using a syringe. Comfort-in™ is a needle-free injection system that works by forcing the liquid medication at an elevated speed without piercing the skin; high pressure allows a wider diffusion of drugs. The aim of the study is to compare the analgesic effects of pre-operative incisional skin infiltration of lidocaine using the traditional technique and the Comfort-in™ in dogs undergoing ovariectomy.

The study was approved by University of Messina Review Board for Animal Care (No. 021/2018). Twenty-two ASA 1 cross-breed dogs were randomly divided into two groups: - S group (11 subjects): was infiltrated with 2% lidocaine (4 $mgkg^{-1}$) using an insulin syringe; - C group (11 subjects): was infiltrated with 2% lidocaine (4 $mgkg^{-1}$) using the Comfort-in™ Technology. The dogs were premedicated with medetomidine (5 $mcgkg^{-1}$) and tramadol (4 $mgkg^{-1}$) intramuscularly. General anaesthesia was induced with tiletamine and zolazepam (5 $mgkg^{-1}$) intravenously and maintained with isoflurane in oxygen diluted. After, the infiltration of the midline incision was performed depositing lidocaine in equal quantity in six injections that were made on each side of the incision, from the caudal to the cranial ends. During the procedure, heart rate, respiratory rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, peripheral capillary oxygen saturation and end-tidal carbon dioxide were recorded after premedication, after incisional block, at skin incision, at muscular incision, at removal of an ovary and at suture. The evaluation of intra-operative analgesia was performed with a cumulative pain scale. The surgery was completed in all the animals, and there were no anaesthetic or surgical complications.

The results of this study showed that the analgesia obtained at the skin incision was more satisfactory in C group than in S group, confirming that a better analgesia is provided from the better diffusion of drug and, also, from the absence of the needle trauma. Advantages of the Comfort-in™ use for incisional lidocaine block consist of lower quantity of local anaesthetics requirement, elimination of needle stick injuries and needle disposal, time saving and decrease of costs. Further studies are needed in order to demonstrate whether this technique can be a reliable alternative method to perform incisional infiltration.

Abstract

L'infiltrazione lungo la linea d'incisione di anestetico locale è una tecnica a supporto dell'analgesia multimodale. Di solito, questa tecnica viene eseguita mediante iniezione di anestetico locale direttamente nel sito di incisione utilizzando una siringa. Comfort-in ™ è un sistema di iniezione senza ago che funziona forzando il farmaco liquido a una velocità elevata senza perforare la pelle; l'alta pressione consente una più ampia diffusione dei farmaci. Lo scopo dello studio è confrontare l'analgesia cutanea ottenuta con lidocaina, utilizzando la tecnica tradizionale e il Comfort-in ™ in cani sottoposti a ovariectomia.

Lo studio è stato approvato dal Review Board for Animal Care dell'Università di Messina (n. 021/2018). Ventidue cani ASA 1 sono stati divisi casualmente in due gruppi: - S gruppo (11 soggetti): è stato infiltrato con lidocaina al 2% (4 mgkg-1) utilizzando una siringa da insulina; - C gruppo (11 soggetti): è stato infiltrato con lidocaina al 2% (4 mgkg-1) utilizzando la tecnologia Comfort-in™. I cani sono stati premedicati con medetomidina (5 mcgkg-1) e tramadolo (4 mgkg-1) per via intramuscolare. L'anestesia generale è stata indotta con tiletamina e zolazepam (5 mgkg-1) per via endovenosa e mantenuta con isoflurano in ossigeno. Successivamente, è stata eseguita l'infiltrazione della linea dell'incisione depositando lidocaina in uguale quantità in sei iniezioni che sono state effettuate su ciascun lato dell'incisione, dall'estremità caudale a quella craniale. Durante la procedura, frequenza cardiaca, frequenza respiratoria, pressione sanguigna sistolica, diastolica e media, saturazione di ossigeno capillare periferico e anidride carbonica di fine espirazione sono state registrate: dopo la premedicazione, dopo l'infiltrazione, all'incisione cutanea, all'incisione muscolare, alla rimozione di un'ovaia e alla sutura. La valutazione dell'analgesia intraoperatoria è stata eseguita con una

scala cumulativa del dolore. L'intervento è stato completato in tutti gli animali e non ci sono state complicazioni anestetiche o chirurgiche.

I risultati di questo studio hanno mostrato che l'analgesia ottenuta all'incisione cutanea era più soddisfacente nel C gruppo rispetto al S gruppo, confermando che una migliore analgesia è fornita dalla migliore diffusione del farmaco e, anche, dall'assenza del trauma dell'ago. I vantaggi dell'uso Comfort-in ™ per il blocco lungo la linea d'incisione con lidocaina consistono in una minore quantità di anestetici locali richiesti, l'eliminazione delle ferite da puntura dell'ago e lo smaltimento dello stesso, il risparmio di tempo e la riduzione dei costi. Sono necessari ulteriori studi per dimostrare se questa tecnica può essere un metodo alternativo affidabile per eseguire l'infiltrazione della linea d'incisione cutanea.

Introduction

Nowadays, ensuring proper pain relief is considered an essential matter for correct patient management, and hence safeguarding animal welfare. With the development of multiple veterinary fields, such as surgery, ophthalmology, oncology, dermatology, physiotherapy, dentistry and internal medicine, to mention but a few, the need to provide targeted analgesia is fundamental.

For this reason, it is essential to put every effort into finding anaesthetic and analgesic protocols that can be adapted to various needs and situations.

Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" by the International Association for the Study of Pain in 1979 (Bonica, 1979).

In 2016, Williams and Craig proposed another definition of pain that is "a distressing experience associated with actual or potential tissue damage, with sensory, emotional, cognitive and social components". It outlines the subjectiveness of every single pain experience, differentiating it from the objectives of physiological processes, although biological mechanisms guide that experience (Williams et al., 2016).

An unpleasant emotional experience can be the trigger of homeostatic responses, similar to those caused by a noxious stimulus, such as reflex withdrawal, behavioural, autonomic nervous system, neuroendocrine, and immune system responses. (Muir et al. 2001).

Mechanism of pain

Pain can be classified by means of various criteria, including:

1) type:

- nociceptive (acute),
- inflammatory,
- neuropathic;

2) duration:

- acute,
- intermittent,
- chronic (it is present longer than the actual tissue damage and the expected recovery phase);

3) nature:

- adaptive (physiological): nociceptive pain and inflammatory pain (this is pain to protect and/or spare an injured body part);
- maladaptive (pathological, pain without purpose): neuropathic pain, such as pain due to damage to nerve tissue as a result of pressure from a herniated disc or ingrowth of a malignant tumour.

4) location:

- somatic: pain in skin, muscles and joints,
- visceral: pain in organs,
- neurogenic: pain emanating from nerve tissue.

Pain sensation involves five different physiologic processes: transduction, transmission, modulation, projection and perception.

The **transduction** is the conversion of nociceptive stimuli into nerve impulses.

Nociceptors are the free nerve endings of afferent neurons, specialized in registering damaging stimuli, the nociceptive stimuli. These nerve cells are known as nociceptive neurons and are special because they can transport information from peripheral to central and vice versa due to their particular structure.

The cell bodies of nociceptive neurons are located in:

- trigeminal nerve ganglia: for nociceptive information from the head.
- dorsal ganglia adjacent to the spinal cord: for nociceptive information from the rest of the body.

The concentration of nociceptors differs according to the tissue. Skeletal muscles, skin, joints and mucous membranes contain more nociceptors compared to organs; this is why pain from skeletal muscles etc. can be well localized and is more intense, while pain from organs is more diffuse and less intense in nature. Less than 10% of the nociceptive nerve fibres in the dorsal horn carry impulses from organs. The skin has the most nociceptors. Furthermore, the nociceptors distribution pattern also appears to differ according to animal species.

There are 4 types of nociceptors:

1) Thermal nociceptors register heat or cold stimuli, e.g. heat from a thermocautery or freezing cold.
2) Chemical nociceptors register chemical stimuli emanating from foreign chemicals or released inflammatory mediators: in the case of tissue damage, from the tissue cells themselves or from accelerated inflammatory cells in the event of tissue damage.

3) Mechanical nociceptors register mechanical stimuli such as by: tissue stretching e.g. stomach and intestines; injection; incision; kick, punch, slap or bite.

4) Polymodal nociceptors: these nociceptors register mechanical, thermal and chemical stimuli.

Thermal, chemical and mechanical nociceptors are located on nerve cells surrounded by a myelin sheath, through which nerve impulses are transmitted quickly: Aδ nerve fibres. They are responsible for the early first pain felt after stimulation and activation of nociceptors.

This very first pain is referred as primary pain or first pain. Primary pain is easy to localize because these Aδ nociceptors are located in high concentrations in the skin, muscles, joints, etc.

The polymodal nociceptors belong to the type C fibres and approx. 80% of all nociceptors are of this type. They are simultaneously activated with one of the aforementioned types of Aδ nociceptors. However, these nerve fibres do not contain myelin, so impulse conduction is slower.

Pain resulting from this is less clearly localized, duller, more burning and nagging in nature and is known as secondary pain or second pain.

All four types of nociceptors are only activated when the relevant stimuli are strong enough: they must exceed a threshold value. When this happens, ion channels in the cell membrane open and influx of Na^+ and Ca^{2+} ions follows, causing depolarization of the nociceptive neuron.

After transduction, transmission takes place: the nerve impulses run from the nociceptor to the dorsal horn of the spinal cord.

Also, the Aβ-fibres, normally involved in non-nociceptive impulses such as pressure, touch and sensation, are depolarized in contrast to Aδ-fibres by the action of weak stimuli on their nerve endings. Aβ-fibres can play a role in pain perception in several ways:

- gate theory: due to the transduction and depolarization of Aβ-fibres, pain in the overlapping area is perceived as less severe. This is because with depolarization of the Aβ-fibres, inhibitory GABAergic interneurons in the dorsal horn of the spinal cord are activated. These reduce the release of excitatory neurotransmitters from the Aδ and C neurons on which the nociceptors reside. This reduces their nociceptive output so that ultimately fewer nociceptive impulses arrive in the cortex. This reduces pain perception.
- due to central sensitization.

Transmission, Modulation, Perception

Nerve cells involved in nociception are classified into 3 categories:

1) First order neurons: the nerve cells on which the nociceptors are located. Their cell bodies are located in the dorsal root ganglia adjacent to the spinal cord. In the head, these are located in trigeminal nerve ganglia.
2) Second order neurons: nerve cells that synapse with first order neurons in the dorsal horn of the spinal cord. They carry both nociceptive and non-nociceptive impulses.

There are several types of second order neurons:

- nociceptive-specific projection neurons: these conduct nociceptive impulses to the anterior and midbrain; they ascend on the ipsilateral and/or contralateral side in the white matter of the spinal cord. Their nociceptive input comes from Aδ and C fibres.
- interneurons or switching neurons: these can have an excitatory or inhibitory effect and are involved in local modulation (or

processing) of nociceptive information. They are located in the spinal cord and in the brain. In the brain, they are found in high numbers at important switching stations, such as the thalamus and the reticular formation.

- intersegmental neurons: these transmit impulses across multiple spinal cord segments and are often part of a reflex arc. In doing so, they stimulate efferent α-motor neurons that induce muscle contractions as part of the reflex, for example the withdrawal reflex.
- Wide Dynamic Range (WDR) neurons: these are projection neurons that conduct impulses from Aδ, C and Aβ fibres to the brain.
- second order neurons, involved in transmission of nociceptive information from the head, are located in the caudal sub nucleus of the trigeminal nerve in the medulla.

The transmission of nociceptive information from the spinal cord to the brain or from one brain region to another is called projection.

Modulation takes place in the dorsal horn of the spinal cord: processing of the nociceptive input via various processes; this also occurs in the brain.

Modulation takes place because certain substances in the body act on specific receptors, which subsequently affect the release of neurotransmitters in the synaptic cleft. The receptors are presynaptic on the first order neuron and/or postsynaptic on the second order neuron. Both inhibitory (e.g. GABA) and excitatory (e.g. glutamate) neurotransmitters can be active in these synapses. Examples:

- GABA is an inhibitory neurotransmitter that has an inhibitory effect through binding to pre- and post-synaptic $GABA_B$ and $GABA_{AB}$ receptors, causing fewer second order neurons to depolarize and less nociceptive impulses to the brain.

- The excitatory neurotransmitter glutamate activates the postsynaptic NMDA and AMPA receptors. As a result, more nociceptive impulses reach the brain via second-order neurons, i.e. an increased feeling of pain.
- The dorsal horn contains pre- and post-synaptic μ, δ and κ opioid receptors and α2-adrenoceptors. When these receptors are activated, depolarization of the second order neurons is inhibited, ultimately resulting in a reduced sensation of pain.

The second order neurons that send nociceptive information to the brain are arranged in pathways in the spinal cord. These pathways are interesting because they transmit impulses - usually via the thalamus - to several brain regions, so that they partly determine the final effect of the nociceptive input.

Spinocerebral orbits

Interestingly, the effect of nociceptive impulses is partly determined by the trajectory through which they run to the brain. The shaded information regarding the various tasks are optional.

Spinothalamic Pathways: nociceptive impulses carried through this pathway are diverted in the ventral portion of the thalamus to the somatosensory cortex. Here, non-anesthetized animals become aware of the location, intensity, duration and type of stimulus (thermal, mechanical or chemical) of the nociceptive incident.

Spinoreticular pathways: run to the reticular nuclei in the brain stem to switch to neurons that supply the nociceptive information to the thalamus, among other things. From the thalamus, the impulses spread to the hypothalamus and cerebral cortex, among other things. The nociceptic impulses involved are partly responsible for the body becoming alert to

pain/nociception (increase of the sympathetic tone) and the autonomic reactions that occur as a result of pain/nociception.

Spinohypothalamic pathways: Nociceptive impulses reach the hypothalamus directly through this pathway. In the hypothalamus, the sympathetic nervous system becomes more active as a result of pain/nociception. This explains why patients under general anaesthesia respond with an increase in heart and respiratory rate, increase in blood pressure and peripheral vasoconstriction with strong surgical stimulus.

Spinolimbic pathways: These switch in the medial region of the thalamus to neurons that transmit nociceptive impulses to the limbic system.

The limbic system is involved in:

- behaviour, emotion and state of mind (cingulate gyrus, locus coeruleus): (chronic) pain can lead to fear, behavioural abnormalities (hiding) and dejection in awake patients;
- conditioned fear and anxiety (amygdala): pain can cause restless behaviour;
- memory (hippocampus): this remembers pain;
- sympathetic autonomic activity (hypothalamus, locus coeruleus);
- alertness, attentiveness, behaviour (locus coeruleus).

3) Third order neurons: These are the neurons that sympathize second order neurons in different regions of the brain. This mainly occurs in the thalamus, but also in the reticular formation, pons, medulla, mesencephalon, amygdala and hypothalamus.

(Duke-Novakovski et al., 2016; Fox, 2013; Greenspan, 1997; Mathews et al., 2014; Muir et al., 2001; Self, 2019; Sorkin et al., 1999; Yam et al., 2018)

Evaluation of pain

Differences in pain tolerance have been experimentally proven in humans and animals, and these play a significant role in pain management; indeed, it is important to be aware that some individuals have a lower threshold for pain and others have a particularly stoic nature, that may be sex-related (Guesgen et al., 2011; Mogil et al., 2010), age-related (Guesgen et al., 2011, Yezierski, 2012) or breed-related (Bowden et al., 2018; Mehrkam et al., 2014).

In 2006 the American College of Veterinary Anaesthesiologists (ACVA) has published a position paper stating that "animal pain and suffering are clinically important conditions that adversely affect an animal's quality of life, either in the short or long term". It also stated that "an important part of determining whether an animal is in pain is the ability to recognize departures from the normal behaviour and appearance of that animal". Some examples of behavioural changes to be considered for the evaluation of the presence or absence of pain in companion animals are:

- changes in personality or attitude (i.e. anxiety, depression, reclusive, irritable, aggressive);
- changes in vocalization;
- changes in posture or ambulation (i.e. curled position, hunched position, praying position in dogs and sphinx position in cats, reduced weight bearing and licking, biting, scratching, shaking or even self-mutilation of the painful area);
- changes in activity level (i.e. reluctance to move, reluctance to lie down and changing position);
- changes in facial expression and appearance (i.e. lack of hair coat grooming);

- changes in bowel movements or urination (i.e. diarrhoea with soiling of the perineum, dysuria and tenesmus);
- changes in appetite (reduced food and water consumption, leading to weight loss and dehydration) (Anonymous, 1998).

Moreover, there are physiological changes that can be used as indicators of pain:

- hyperventilation or tachypnoea (or breath holding in some species);
- tachycardia (mild, moderate, or severe);
- pupil dilation;
- hypertension;
- hyperthermia;
- increased serum cortisol and catecholamine (epinephrine).

These signs are caused by an increase in sympathetic tone after the hypothalamus has received nociceptive input.

Painful stimuli during light stages of general anaesthesia can create physiologic manifestations such as tachycardia, hypertension and hyperventilation with all the clinical consequences: an increase in myocardial work and thus in myocardial oxygen consumption, a decrease in the diastolic phase, an increase in oxygen demand, decrease in tidal volume.

Despite their value as indicators of pain, the sympathetic nervous system can be activated by various conditions such as fear (i.e. “white coat syndrome”) and disease (i.e. septic peritonitis). Moreover, very deep general anaesthesia can strongly depress all physiologic responses to surgical stimulation (Gaynor et al., 2002).

The detection of pain is the most challenging problem in veterinary anaesthesia. In human medicine, self-reporting is the gold standard for the evaluation of pain; in veterinary medicine, the recognition of pain is based on

the interpretation of the animal's behaviour and physiologic changes by the veterinary surgeon in case of acute pain and by the owner in case of chronic pain (Reid et al., 2018; Murrell et al., 2008).

Before analgesic drugs can be administered in order to reduce the nociceptor event, the signs of pain must be assessed quantitatively using pain scales.

In the past, the scales used to assess pain in animals and human have been based on assessments of its intensity alone, the so-called unidimensional scales:

- Simple Descriptive Scale (SDS)
- Numerical Rating Scale (NRS)
- Visual Analogue Scale (VAS) (Downie et al., 1978)

These scales have been shown to be unreliable in patients who cannot communicate (patients suffering from dementia, children and animals).

This limitation led to the development of multidimensional scales. In 1971, Melzack and Torgerson developed a 'language of pain', which considered other qualities of pain in addition to its intensity, based on the interpretation of behaviours such as facial expressions, postures, activities and so on. This language formed the basis of the McGill Pain Questionnaire created by Melzack in 1975, which was designed to provide quantitative evaluations of pain that could be treated statistically (Murrell et al., 2008).

It is only lately that the utility of such methods has been recognised by veterinary doctors. The most important multidimensional scales used in companion animals are:

- University of Melbourne Pain Scale (UMPS)
- Colorado State University Feline and Canine Acute Pain Scales
- Glasgow Composite Measure Pain Scale (GCMPS)
- Botucatu Multidimensional Composite Pain Scale.

Beyond ethical considerations, pain can cause catabolism, damage respiration, delay wound healing, extend hospitalization times and increase morbidity and mortality (Cambridge et al., 2000).

In the past years, many studies have been performed to obtain information related to attitudes, opinions and knowledge of both veterinary doctors and owners of companion animals, regarding pain and the use of analgesic drugs in small animal veterinary practice (Bell et al., 2014; Simon et al., 2018; Steagall et al., 2017).

It seems there is a positive trend regarding the relevance to provide pain management for companion animals by the veterinary surgeons, but the use of perioperative analgesic drugs is still not optimal (Murrell et al., 2008).

More often, owners considered analgesic drugs to be consistently necessary for surgical procedures compared to the medical conditions (Simon et al., 2018; Steagall et al., 2017); indeed, difficulties with owner compliance is one of the main obstacles to proper treatment of chronic pain, together with problems with pain assessment and the cost of drugs (Bell et al., 2014).

Simon et al. conclude by reporting that the veterinary doctors need to improve the comprehension of owners' perceptions and knowledge regarding anaesthesia, surgery and pain in order to lead to better client education (Simon et al., 2018).

Management of pain

At the beginning of the 1900s, the concept of pre-emptive analgesia was introduced for the first time by Crile, who observed that if pain transmission was blocked before a nociceptive event, postoperative mortality was reduced as well as the intensity and duration of postoperative pain.

Pre-emptive analgesia is antinociception therapy, the goal of which is the prevention of both peripheral and central sensitization, in order to avoid the postoperative amplification of pain sensation.

Treatment of pain can be performed peripherally, blocking inputs along sensory axons, or at central nervous system sites using analgesic drugs, individually or in combination, administrated in the form of a single shot or as continuous rate infusion (Kelly et al., 2001).

1) Opioids

Opioids are the oldest and most frequently used drugs for pain relief in small animals. These drugs are very effective and mostly safe, with the advantage of reversibility, through the administration of an opioid antagonist, naloxone. They work by binding to opioid receptors located in the central and peripheral nervous system; binding causes a conformational change that increases the affinity of opioids for guanine nucleotide binding proteins: G-coupled proteins. During the perioperative period, opioids are given to provide analgesia and sedation as part of premedication (neuroleptoanalgesia) and as part of a multimodal analgesic protocol (Simon et al., 2016).

The side effects of opioids include: depression of tidal volume and gas exchange, diminution of breathing rate, respiratory arrest when very high doses are administered, decrease of chest wall compliance and increase of upper

airway resistance, their action on the discharges of respiratory bulbospinal, vagal, propriobulbar neurons and phrenic nerve activity (Lalley, 2003).

μ-opioid receptor agonists have been typically administered by intravenous, intramuscular, subcutaneous routes or intravenously by constant rate infusions.

The transdermal patch, such as buprenorphine (Andaluz et al., 2009; Moll et al., 2011) and fentanyl (Egger et al., 1998; Kukanich et al., 2012; Reed et al., 2011), or the transdermal solution of fentanyl (Linton et al., 2012), are recent alternative pharmaceutical forms of opioid administration; their goal is to extend their effect and duration and to mitigate the disadvantages of both oral and parenteral opioid administration.

Opioid drugs are also administered to improve loco-regional anaesthesia, both alone and concomitant with local anaesthetics. Epidural or spinal administration of morphine, a highly hydrophilic drug, produces analgesia for 30-40 minutes; this long duration of effect is due to the delayed systemic absorption and longer maintenance in the spinal cord. Epidural or spinal administered opioids give somatic and visceral pain relief, selectively blocking nociceptive impulses without the depression of the sympathetic nervous system and interference with sensory and motor function.

Intra-articular administration of morphine has become very common after joint surgery; moreover, following joint inflammation, μ-opioid receptors increase significantly in both articular and peri-articular tissues (Simon et al., 2016).

2) α2- agonists

Alpha2-adrenoreceptor agonists are largely used in small animal practice due to their strong sedative and analgesic effects and having the advantage of

the ability to reverse their action, in the case of the occurrence of side effects occur. The administration of the α2-antagonist, atipamezole, reverses side effects, but also the sedation and analgesia provided by the α2-agonist. This class of drugs has significant effects on:

- cardiovascular system: bradycardia, hypertension, hypotension, reduction in cardiac output and stroke volume.
- respiratory system: reduction in respiratory rate and tidal volume, which may result in respiratory acidosis and hypoxemia.

These effects can be observed even when very small doses of these drugs administered, so their use should be confined to patients capable of tolerating both cardiovascular and respiratory imbalance (Berry, 2015).

In premedication, administration with opioids can work synergistically, increasing analgesia and sedation and reducing the doses of each drug used. During the post-operative period, small doses can provide sedation and analgesia effects, especially in patients experiencing dysphoria.

Peripheral nerve blocks can be performed using α2-agonists as adjuvant to local anaesthetics, prolonging their duration. Intra-articular administration of α2-agonists also can be advantageous, as shown in studies concerning humans undergoing arthroscopy and rats with arthritic pain (Murrell et al., 2005).

Dexmedetomidine can be used as continuous rate infusion, alone or in combination with other drugs, such as lidocaine and fentanyl, to increase analgesia during the perioperative period. (Gutierrez-Blanco et al., 2015; Valtolina et al., 2009) or to reduce the halogenated agent's minimum alveolar concentration during anaesthesia (Acevedo-Arcique et al., 2014; Moran-Muñoz et al., 2014).

3) Local anaesthetics

Local anaesthetics are inexpensive, simple to use and uncommonly associated with major adverse events. The administration of local anaesthetic in combination with general anaesthetic is commonly used in many balanced anaesthesia protocols, making it possible to work on patients undergoing major surgery with less use of analgesic drugs (such as opioids) and general anaesthetic (Brower et al., 2003; Campagnol et al., 2012).

Local anaesthetics such as lidocaine, bupivacaine, levo-bupivacaine, ropivacaine exert their analgesic effect by preventing the generation and transmission of nerve impulses by blocking the influx of sodium across the cell membrane of nerve axons and inhibiting action potentials conduction (Campoy et al., 2013). Adverse events related to local anaesthetic administration are generally due to unintentional intravenous administration or overdose. Clinical signs of their toxicity are ataxia, nystagmus, and tremors, which may advance to convulsions, unconsciousness and respiratory arrest; other signs of toxicity, regarding the cardiovascular system, are bradycardia and sinus arrest.

The versatility of these drugs allows them to be utilized in many ways:

- Topical: application to skin or mucosa in order to desensitize areas (lidocaine spray, lidocaine patch);
- Infiltrative: injection near specific nerves or placement in wounds (soaker catheters);
- Systemic: administration intravenously and as continuous rate infusion, in order to decrease inhaled anaesthetic requirements and to provide anti-inflammatory, antiarrhythmic and analgesic effects;
- Neuraxial: application into the intrathecal or epidural space (Berry, 2015).

4) NSAID (non-steroidal anti-inflammatory drug)

The anti-inflammatory and analgesic effects of the NSAIDs are mediated by the inhibition of COX-1 and COX-2. These enzymes are responsible for the production of inflammatory mediators of interest in nociception: prostaglandins, thromoboxane A2 and prostacyclins. NSAIDs are useful in both acute and chronic pain management, particularly osteoarthritis (Bradbrook et al., 2018).

5) Ketamine

Ketamine is a dissociative anaesthetic, an antagonist of NMDA and a sympathomimetic. At low doses it has analgesic effect, while at high doses it also has anaesthetic effects. This drug provides less analgesia than pure μ-opioid receptor agonists and is frequently administered with an opioid for multimodal analgesia (White et al., 2017).

In one study from 2000, Slingsby&Waterman-Pearson reported that the use of ketamine in dogs undergoing ovariohysterectomy as pre-emptive analgesia has required less rescue analgesia than the control group receiving no ketamine; however, the control group did not require much more rescue analgesia than the group treated with ketamine post-operatively, suggesting that administration of ketamine at induction confers certain advantages over administration of ketamine at extubation.

Ketamine can also be used in continuous rate infusion, with an important sparing effect on the minimum alveolar concentration of isoflurane (Gianotti et al., 2014). In their study from 2016, Kaka et al. reported that the minimum serum concentration of ketamine to produce analgesic effect in dogs is between 100-200 ng/mL during infusion.

6) Tramadol

Tramadol is an antinociceptive drug that provides analgesia through various mechanisms: activation of μ-opioid receptors, facilitation of serotonin release and inhibition of norepinephrine reuptake. In dogs experiencing mild to moderate pain, the administration of this drug gives proper analgesia. In cats undergoing ovariohysterectomy, the administration of tramadol combined with an NSAID gives sufficient analgesia. Side effects of this drug are bradycardia, nausea and constipation or diarrhoea (Berry, 2015).

7) Gabapentin

Gabapentin is an anticonvulsant that has analgesic properties. The analgesic effect is achieved through reduced release of neurotransmitters, such as substance P and glutamate, and the interaction with γ-aminobutyric acid receptors located in the spinal cord. During the perioperative period, the administration of gabapentin in combination with opioids can increase sedation and analgesia and reduce post-operative analgesic medications (Berry, 2015; Crociolli et al., 2015; Wagner et al., 2010).

Loco-regional analgesia

Loco-regional anaesthetic techniques, either neuraxial or peripheral, are becoming more and more popular for perioperative pain control in companion animals.

The influence of regional anaesthesia on the stress response has been extensively studied. In a study from 2016, Romano et al. have found that analgesia provided by loco-regional techniques performed pre-operatively, avoid the stress biomarkers responses to surgery (glycaemic and cortisol), give better quality of recovery, and reduced postoperative pain scores compared to systemic analgesia in dogs undergoing stifle surgery, so loco-regional anaesthesia techniques were found to be major alternatives to the intravenous administration of analgesic drugs.

There are many studies, both in human and in animals, that largely demonstrate the benefits of using loco-regional analgesia instead of systemic analgesia, or a combination of thereof, in order to reduce systemic opioid administration and then, to avoid their side effects such as bradycardia, hypotension, hypoventilation, ileus, nausea, vomiting and dysphoria (Lalley, 2003; Romano et al., 2016).

Complications of loco-regional anaesthetic techniques can be categorized as:

- systemic toxicities, due to intravascular injection of local anaesthetic, such as muscle twitches, tremors, seizures, tachycardia, hypotension, arrhythmias, cardiovascular collapse, and even death.
- nerve injuries, due to one or more of the following causes: needle/mechanical trauma, neuronal ischemia, neurotoxicity caused by local anaesthetics, drug error, infection, allergic reaction.

These can lead to short-term complications such as temporary dysesthesia, localized tenderness, and hematoma formation or even permanent signs, like neuropraxia, with persistent numbness in the affected area (Campoy et al., 2013).

There are several loco-regional techniques:

- Topical or surface anaesthesia
- Local or infiltration anaesthesia
- Regional or nerve (plexus) block anaesthesia
- Neuraxial anaesthesia
- Intravenous regional anaesthesia (IVRA)

Further papers investigating the use of various methods of neuro-localization, such as electroneurostimulation and ultrasound, are published frequently.

Needle-free injection

Jet-injection technology was developed in the 1930s and designed for mass vaccination programs. Needle-free jet-injections, exploiting the mechanical compression of a fluid through a micro-hole, generate a high-pressure flow which penetrates the skin and spreads under the skin.

Most of the devices available on the market inject a fluid at a speed ranging from 80 to 100 m/s through a hole which on average has a diameter of 150 μm.

The devices used for vaccinations were of three types: spring, battery or gas.

The use for immunological purposes was abandoned over time because there was no disposable instrumentation at that time, fearing contagion and spread of pathogens from one patient to another, and because it was feared that the amount of vaccine to be injected with this system was not sufficient or not spread adequately, compromising the patient immunization process. (Hingson et al., 1963; Mitragotri, 2005).

In 1971 Bennett et al. began to study the characteristics of jet-injection diffusion in rats. The injected liquid follows the physical law of fluids, spreading in the tissues according to the resistance encountered, though without being distributed systemically. In rats, infiltration was into the skin, sub cutis and muscle; bone tissue was not penetrated, but the periosteum was soaked.

A study of Schramm et al. from 2002 analysed the diffusion characteristics based on the speed set by the instrument and the hole through which the drug was injected, succeeding in identification of the ideal characteristics for speed and diameter of the hole for a favourable diffusion.

More recently the jet-injector has been used for the infiltration of local anaesthetic prior to performing needle aspiration of thyroid nodules: the comparison with the use of EMLA (lidocaine/prilocaine patch) has shown comparable efficacy but greater handling for the jet-injector, as the patch needs to be applied at least one hour before the procedure, while the jet-injection device guaranteed immediate effectiveness, ease of handling and practicality in performing the procedure (Gursoy et al., 2007).

Nowadays in human medicine, the widespread use of needle free injection system has been employed in:

- the subcutaneous injection of insulin in type I diabetic patients (with considerable advantage avoiding the daily use of needles and marked pain reduction) (Engwerda et al., 2011, 2013);
- local anaesthetic injection in dental surgery (Munshi et al., 2001);
- subcutaneous injection of drugs used for aesthetic medicine (Barolet et al., 2018; Ravi et al., 2015);
- reproduction (Wilson et al., 2020).

In 2005, a study was performed testing the effectiveness of a needle-free jet injector device (ALGRX 3268), injecting lidocaine powder prior to venepuncture in children. Indeed, by waiting for 3 minutes after administration, there was a reduction in pain during the procedure compared to children who did not receive treatment (Migdal et al., 2005).

In veterinary medicine, needle free injection systems have been recently proposed as a valid alternative method for vaccination and drug administrations in swine (Temple et al., 2017; Chase et al., 2008). Moreover, the technology has been studied for immunization procedures in sheep, cattle and dogs (van Drunen Littel-van den Hurk et al.,2006; Goubier et al., 2008).

Objective

The aim of the study was to compare the analgesic effect between pre-operative incisional skin infiltration with lidocaine using a traditional syringe and the needle-free injection system(Comfort-in™) in dogs undergoing ovariectomy. Our hypothesis was that infiltration using the Comfort-in™ system might offer better analgesia than with a traditional syringe, and the Comfort-in™ technology may be a valid alternative method for incisional block.

Materials and methods

The study has been conducted over the period between January 2019 and September 2019, in the Veterinary Teaching Hospital of the University of Messina, Italy. This study was approved by the University of Messina Review Board for Animal Care (No. 021/2018), in accordance with Italian law (D.M. 116192), European law (O.J. of E.C. L 358/1 12/ 18/1986), and informed consent was obtained from all owners.

Study design

Prospective, randomized, double-blind clinical trial.

Animals

Twenty-two adult female cross-breed dogs, median age 4.4 years (1 – 11) and median weight 9.3 kg (5 - 15) undergoing ovariectomy have been included in the study.

The enrolled population had been assessed according to the American Society of Anesthesiologists physical status classification I, considering history, general physical examination and haematology analyses. Patients were selected for their docile temperament.

Study procedure

The surgical procedure was ovariectomy, removal of the ovaries, in order to reduce risk of mammary neoplasia and pyometra.

All the dogs received a standard anaesthesia protocol:

- premedication was performed by intramuscular injection of dexmedetomidine 5 $mcgkg^{-1}$; (Dexdomitor 500 $mcgml^{-1}$, Orion, Italy) and tramadol 4 $mgkg^{-1}$ (Altadol 50 $mgml^{-1}$, Formenti, Italy) mixed in the same syringe;
- after ten minutes, a catheter was aseptically placed in a cephalic vein, and all dogs received tiletamine/zolazepam 5 $mgkg^{-1}$ intravenously (Virbac, Carros, France), as induction;
- after intubation, using a cuffed endotracheal tube attached to a non-rebreathing system (Mapleson A in parallel of Lack), anaesthesia was maintained with isoflurane (IsoFlo, ESTEVE, United Kingdom) in diluted oxygen.

Lactated Ringer's solution (Ringer's lactate solution, NovaSelect, Italy) was administered intravenously at 5 $mLkg^{-1}h^{-1}$ throughout the period of anaesthesia and in the early post-anaesthesia recovery.

Hair over the planned surgical field, ranging from 4 cm cranial to xiphoid to 4 cm caudal to the cranial pubic brim (in order to allow the incision to be extended readily if needed) was clipped; and prior to the incisional block, the skin was aseptically prepared for injection.

Dogs were randomly allocated to two techniques:

- **S group** (11 subjects) was infiltrated with lidocaine 2% 4 $mgkg^{-1}$ (Zoetis, Spain) using an insulin syringe (capacity 1 ml - 27Gx1/2'' - 0.4 x 13 mm, Benefis S.r.l., Italy).
- **C group** (11 subjects) was infiltrated with lidocaine 2% 4 $mgkg^{-1}$ (Zoetis, Spain) using the Comfort-in™ Technology (GamasTECH, Italy).

Selection of the technique was made by the same anaesthetist performing infiltration. Intraoperative assessments have been recorded by another anaesthetist unaware of the technique used.

The injected volume of lidocaine was not diluted in sterile saline solution, and care had to be taken so as not to exceed the maximum recommended dose for lidocaine in dogs.

The planned incisional site was on the ventral midline, encompassing the cranial third of the distance between umbilicus to pubis; usually it was a line 3-4 cm long, depending on the size of the patient.

Then infiltration blocks were performed as described in the literature:

Local anaesthetic was deposited in equal quantities in six injections that were made on each side of the incision, from the caudal to the cranial ends, in order to achieve instillation of local anaesthetic into the skin and subcutaneous tissue (Campoy et al., 2013).

In S group, the injections were performed in a traditional manner; prior to each injection, aspiration of the syringe was performed to ensure intravenous injection does not occur.

In C group, the injections were performed using the Comfort-in™ technology.

The **Comfort-In™** is a spring-loaded Needle-free Liquid Jet Injection device (Figure 1). It is available in three variants distinguished by injection pressure and consequently by the depth of penetration into the tissues. These three variants are advertised by the manufacturer as follows:

- **soft** is intended for use in dentistry, aesthetic medicine and paediatrics, being characterized by reduced pressure.
- **medium** is intended for areas of the body where moderate pressure is required such as hands and feet.
- **normal** is intended for areas of the body where greater pressure is required such as the abdomen and thighs.

The penetration range varies from 2.1 mm to 6.1 mm depending on the variants used.

The device is equipped with sterile disposable syringes with a 0.15 mm micro-hole, which are able to contain solutions up to 0.5 ml. These micro syringes are called nozzles.

Before each injection, the device spring must be loaded and the nozzle must be filled with fluid. The procedure is divided into the following steps:

1) the nozzle is filled with the liquid for injection;
2) without the nozzle being inserted in its seat, the Comfort-in™ is loaded with the appropriate tool supplied (pressure box/pressure lever). This has the function of blocking the device and allows, the introduction of a metal punch in the seat of the nozzle, to manually apply a force on the lever connected to the metal punch which is thus pushed against the spring which is compressed;
3) the dose to be injected is preselected on the device;
4) the nozzle is screwed into the Comfort-in™;
5) the piston inside the nozzle is advanced by rotating the seat clockwise and any air bubbles are released which could cause problems during the injection;
6) the device is positioned perpendicularly to the injection site.

To carry out the injection, the device is gripped and, once the nozzle containing the fluid is attached to the tissue and a preload is performed, it is activated by pushing the button located at the opposite end.

The injection speed in this device can be modulated according to the actual amount of drug to be injected in the nozzles and can vary from 80 to 100 m/s.

In this study the soft variant of Comfort-In™, which has an injection pressure (pmax) of 282.39 atm and a penetration depth of approx. 2 mm, has been used.

Five minutes after the injection of local anaesthetic, incision of skin, laparotomy and ovariectomy had been performed by one highly skilled surgeon working at the Veterinary Teaching Hospital of the University of Messina. After closure of the laparotomy incision and suture of the skin, isoflurane was stopped.

A bolus of 2μgkg^{-1} Fentanyl (Fentadon 50 mcg ml^{-1} Dechra, Italy) was intraoperatively administered as rescue analgesia.

Meloxicam 0.2 mgkg^{-1} (Metacam, Boehringer Ingelheim, Italy) was administered subcutaneously at the end of anaesthesia.

Figure 1: Comfort-in™.

Monitoring

Heart rate (HR), respiratory rate (RR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), were recorded with the animal awake (baseline values), after premedication, after incisional block, at skin incision, at muscular incision, at removal of an ovary and at suture.

After intubation, peripheral capillary oxygen saturation (SpO_2) and end-tidal carbon dioxide ($EtCO_2$, mmHg) were also recorded at the same points.

These parameters were recorded using a multiparameter monitor (Leonardo model, AMI Italia S.r.l., Italy), while blood pressure was monitored with an oscillometric non-invasive blood pressure method with a cuff placed around the antebrachium.

When systolic and median pressures fell below 80 and 60 mmHg respectively, the Lactated Ringer's solution infusion rate has been increased from 5 to 10 $mLkg^{-1}h^{-1}$, and a bolus of 10 $mLkg^{-1}h^{-1}$ in administered over a ten minutes period; if this was insufficient, a bolus of 2-4 $mLkg^{-1}h^{-1}$ was administered over an additional ten minutes period; if all this was still not sufficient, cardiovascular support drugs were administered.

Evaluation of intra-operative analgesia was performed by means of a cumulative pain scale by assigning a score, from 0 to 4, based on percentage changes in heart rate, respiratory rate and systolic blood pressure, compared to the values recorded after incisional block.

For the score assignment, the following scheme was used:

$0 \leq 0\%$

$1 \geq 0\%$ but $\leq 10\%$

$2 \geq \leq 10\%$ but $\leq 20\%$

$3 \geq 20\%$ but $\leq 30\%$

4 ≥ 30% but ≤ 40%

The sum of the scores gave us the total score. When the total score was ≥ 10, rescue analgesia was administered because it was indicative of severe pain. (Costa et al., 2019).

Statistical analysis

Statistical analyses were performed using SPSS 15.0 (IBM Company, Italy). Shapiro-Wilk normality test have been performed to verify the normal distribution of data.

Data was expressed as the median and range values.

Changes with time were evaluated by means of the Wilcoxon Test, while differences between groups were compared using the Mann-Whitney U. A value of $p < 0.05$ was considered statistically significant.

Results

All the dogs were included in the study and all recovered from anaesthesia and surgery uneventfully.

The Shapiro-Wilk normality test shows that the data was not normally distributed.

Heart rate was reduced compared to baseline in the C group at all monitoring time points, while in the S group it showed no statistically significant variation from baseline. Between the two groups there were significant differences in heart rate during surgery, since in the S group the values of this parameter were higher than those for the C group.

The respiratory rate was reduced or remained unchanged from baseline in both groups. After incisional block, a significant difference ($P = 0.020$) in this parameter was recorded between the two groups.

Systolic blood pressure in C group changed significantly with time ($P = 0.003$), showing a reduction leading up to the skin incision and an increase during the remainder of the surgery. Similarly, in the S group increases or reductions in this parameter were recorded compared to baseline.

It was not necessary to increase the infusion rate of Lactated Ringer's solution because hypotension did not occur to any patient.

Haemoglobin saturation was 98%-100% and end-tidal carbon dioxide was 35 mmHg-42 mmHg for the entire duration of anaesthesia in both groups.

Stimulus response scores during the skin incision were statistically lower in the C group ($P=0.030$) compared to the S group, while during the rest of the surgery the stimulus response scores in the two groups were similar; in some dogs belonging to both groups, after skin incision, fentanyl has been administered because the cut off of ≥ 10% was exceeded (Table 1).

Recorded Data	Group	B	AP	AI	SI	MI	RO	S
HR (beats/ min)	C	131 (100/140)	80 (38/138)	97 (52/136)	88 (65/104)	91 (82/106)	88 (48/104)	90 (78/110)
	S	138 (52/189)	104 (46/180)	106 (59/131)	103 (83/126)	117 (86/148)	110 (88/142)	104 (95/145)
RR (breaths/ min)	C	29 (20/50)	22 (12/36)	18 (12/31)	17 (9/32)	27 (10/50)	32 (20/60)	28 (21/50)
	S	28 (20/36)	14 (8/28)	12(8/25)	14 (9/31)	28 (11/60)	29 (23/40)	32 (23/46)
SBP (mmHg)	C	158 (119/207)	142 (99/191)	143 (113/204)	144 (114/216)	167 (116/212)	172 (138/205)	151 (125/184)
	S	170 (119/186)	136 (110/191)	138 (113/203)	144 (114/197)	161 (116/200)	172 (148/205)	156 (127/184)
SpO_2 (%)	C			100 (98/99)	99 (98/99)	100 (98/99)	98 (98/99)	99 (98/99)
	S			99 (98/99)	100 (98/99)	100 (98/99)	98 (98/99)	100 (98/99)
$EtCO_2$ (mmHg)	C			35 (35/37)	35 (32/37)	32 (30/37)	35 (35/37)	35 (34/37)
	S			37 (35/37)	40 (35/42)	35 (35/37)	32 (30/37)	32 (30/38)
Stimulus response scores: CPS 0/4	C				0 (0/1)	2 (0/4)	2 (0/4)	1 (0/4)
	S				1 (0/2)	2 (1/4)	3 (1/4)	3 (1/4)

Table 1:
HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; SpO_2, peripheral capillary oxygen saturation; $EtCO_2$, end-tidal carbon dioxide; CPS, Cumulative Pain Scale.
B, baseline; AP, after premedication; AI, after incisional block; SI, skin incision; MI muscular incision; RO, removal of an ovary; S suture.
Group C, Comfort-in™ Technology; Group S, insulin syringe.
Underlined values significant differences along the time line, whereas bold highlights significant differences between groups ($p < 0.05$).

Discussion

Incisional infiltration of local anaesthetic is defined as the direct injection of the drug into the surgical field. It is a local anaesthetic and analgesic technique supporting of multimodal analgesia and is popular due to its relative simplicity, safety and low cost (Campoy et al., 2013).

Several studies have shown the technique of incisional infiltration with local anaesthetics has been showed by different studies to be effective in managing intra-operative and post-operative pain and to reduces the use of analgesic drugs (Campagnol et al. 2012; Carpenter et al., 2004; Savvas et al., 2008).

No adverse events were reported in our study, indicating that a local anaesthetic infiltrative block is harmful to a patient, as instead suggested in another study that examining incisional line block may have seen higher complications because the authors choose to infiltrate the site of the incision (midline) where as we infiltrated the tissue surrounding the incision (Fitzpatrick et al., 2010).

The traditional technique of incisional infiltration consists of use of a syringe attached to a needle; however, over the past century, several types of innovative needle-free devices, such as the Comfort-in™, have been developed, resulting in consistently increasing use in human medicine and, eventually, in addition, in veterinary medicine.

In a recent review, regarding the needle free intradermal injection of local anaesthetic in human medicine, the benefits of this technique are summarised as followed:

- tiny amount of local anaesthetic used;
- immediate analgesia;

- broader diffusion;
- reduced bleeding (Barolet et al., 2018).

Similar advantages of needle-free injection over conventional needle-syringe administration, this time regarding swine immunity, have been described in another review as follows:

- lower vaccine volume;
- less pain and stress;
- higher antigen dispersion;
- consistent vaccine delivery (Chase et al., 2008).

In fact, based on these results, we supposed that since Comfort-in™ delivers the drug across the skin in the subcutaneous tissue at a high velocity, it may dispense the liquid drug over a wider area compared to injection of the drug using a needle attached to a syringe. This may enhance the efficiency with which the local anaesthetic is absorbed from the subcutaneous compartment into the circulation so that the effect can be advanced.

Moreover, similar results were found in two studies by Engwerda and co-workers that reported a more rapid onset of insulin action when the insulin is administered through a jet injector instead of a traditional insulin pen (Engwerda et al., 2011, 2013).

In our study, the analgesia obtained at the skin incision was more satisfactory in the C group than in S group. Indeed, some patients of the S group received a bolus of Fentanyl as rescue analgesia at skin incision, because the score obtained through the percentage changes in heart rate, respiratory rate and systolic blood pressure exceeded the established cut-off of 20%. However, after the skin incision, and especially during suturing, in some patients of belonging to both groups, the administration of fentanyl as rescue analgesia was necessary.

Nevertheless, the statistical significance of the difference between analgesia scores, recorded at the time of the skin incision, was weak.

As shown in the results, the improved analgesia obtained at skin incision in the group that had been infiltrated using Comfort-in™, was probably the result of better diffusion of drug, and also, of course due to the absence of the needle trauma. Moreover, improved diffusion of the drug may lead to a lower quantity of local anaesthetic required, thus reducing potential side effects.

At suturing, it was difficult to evaluate the analgesia obtained because the local anaesthetic was distributed in the dermal and hypodermal layers and was not distributed into the muscle layers. Consequently, the surgical stimulus on the muscle layers may have been masked by the analgesia obtained through the administration of lidocaine into the skin.

Furthermore, there are parameters that can alter needle-free injection, such as driving pressure, contact pressure, volume per spurt, nozzle aperture and the distance from the tip of the nozzle to the skin surface (Barolet et al., 2018; Ravi et al., 2015). That is why a limitation of this study is the lack of studies on the needle-free injection system that has been used, the Comfort-in™, that can help to prove our thesis. However, there are two test that have been performed by a Canadian laboratory in order to verify the safety and performance of the specific Comfort-in™ in human medicine. These tests are called depth penetration and dispersion test and dose accuracy test. During these tests, the soft variant of Comfort-in™ has been used, as in our study (Globe Laboratories, 2012).

Beyond these various advantages regarding the welfare patient, there are health care workers benefits like the elimination of needle stick injuries that are quite common and hazardous in veterinary medicine (Weese et al., 2008). Also, economic and environmental benefits, such as elimination of needle disposal, time saving and so decrease of cost (Chase et al., 2008). The lower

risk of needle injury is extremely important especially when anaesthetic drugs are handed.

All the advantages mentioned above may make the device suitable for injection of local anaesthetic in all the following veterinary services: surgery (i.e. castration, C-section, laparotomy, nodulectomy, mastectomy), diagnostic imaging (i.e. fine-needle aspiration), endoscopy (i.e. nasal), dentistry (i.e. extraction, epulis, tumours, gingival hyperplasia, fracture of the premolar), just to mention a few. Moreover, in awake animals, Comfort-in™ technology may find a valid clinical application, such as injection of vaccines and in mesotherapy, especially because it doesn't have the trauma of the needle.

The challenging of evaluating intra-operative pain is uniquely because despite the use of objective physiological that are easy to perform and analyse statistically, there is not a greater evidence that these measures are reliable indicators of pain (Cambridge et al., 2000; Conzemius et al., 1994). So, in order to maximize the potential for successful pain identification, the choice of protocol with tramadol was made. This decision was not made lightly, and the use of rescue analgesia (fentanyl) was very strict because of this.

We have decided to give rescue analgesia when cut-off score was ≥ 10, corresponding to an increase of heart rate, respiratory rate and systolic blood pressure between 20% and 30% or more, is recorded, following as suggested in one study (Costa et al., 2019). To the best of my knowledge, for the intraoperative pain evaluation, there is not a scientific accordance that established a cut-off point, exceeding which rescue analgesia is administrated. In two studies, cut-off point was subjectively established: Caniglia et al. (2012) have used a cut-off point > 10%, whereas for Portela et al., (2013) the cut-off point was > 25%.

An objective method for measuring intra-operative pain is electroencephalographic entropy determination, but we do not have the equipment (Mahidol et al., 2015).

Conclusions

On the basis of the results of this study, the analgesia of the midline incisional skin obtained with the infiltration of lidocaine using the Comfort-in™ technology was better than to the infiltration using the conventional syringe. To statistically confirm the tendency found by our results a larger and homogenous sample will be indispensable.

This new technique of injection may be used in veterinary medicine both for the administration of local anaesthetic and for other type of drugs.

It could be helpful in reducing doses of local anaesthetic and, thanks to the lack of the needle, drug inoculation can take place without trauma.

Further studies are needed in order to demonstrate whether this technique can be a reliable alternative method to perform incisional infiltration.

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