

**EFFECTS OF BUTORPHANOL, MELOXICAM AND THEIR
COMBINATION ON POSTOPERATIVE PAIN, STRESS RESPONSE AND
WOUND HEALING IN DOGS FOLLOWING OVARIOHYSTERECTOMY**

A thesis submitted in fulfillment of the requirement for Doctor of Philosophy degree of the
University of Nairobi

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DECLARATION

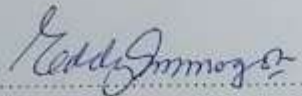
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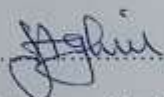
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DEDICATION

To my loving wife Millicent Malemba Mwangi, incredible son Liam Kiama Mwangi and caring mother Teresia Wambui Mwangi.

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LIST OF ABBREVIATIONS

ABP	Arterial blood pressure
ACVS	American College of Veterinary Surgeons
AMPA	μ -amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid
CMPS-SF	Glasgow composite measure pain scale – Short form
COX-2	Cyclooxygenase type 2
ECM	Extracellular matrix
EDTA	Ethylene-diamine-tetraacetic acid
EGF	Epidermal growth factor
FGF	Fibroblast growth factor
GABA	Gamma-aminobutyric acid
HADS	Hospital Anxiety and Depression Scale
HB	Hemoglobin concentration
HR	Heart rate
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NMDA	N-methyl-D-aspartate
NRS	Numerical rating scale
NSAIDs	Non-steroidal anti-inflammatory drugs
PAG	Periaqueductal gray matter
PCV	Packed cell volume
PDGF	Platelet-derived growth factor

PSS	Perceived Stress Scale
ROS	Reactive oxygen species
RR	Respiratory rate
RT	Rectal temperature
SDS	Simple descriptive scale
STAI	State Trait Anxiety Inventory
TEC	Total erythrocyte count
TGF)- β	Transforming growth factor
TLC	Total leucocytes count
TPC	Total platelet count
VAS	Visual analogue scale

ABSTRACT

Pain, defined as an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage, is associated with trauma, surgery and disease processes. If pain is not well managed in surgical patients, it can cause stress and impair wound healing in the affected patient. However, the interplay between pain, stress and wound healing in dogs is yet to be elucidated hence the need for this study.

This was a two-phased study, where in phase one, a systematic review was carried out to evaluate the type of analgesic drugs and protocols used to manage pain postoperatively in dogs following ovariohysterectomy. Phase two was a randomized controlled clinical study aimed at evaluating and comparing the effects of butorphanol, meloxicam and their combination on postoperative pain, stress response and wound healing in dogs after ovariohysterectomy.

In the systematic review, literature searches in Pub Med, Google Scholar and Science Direct were conducted for peer reviewed articles written in English and published between 1995-2015. The key search words were dogs, ovariohysterectomy, pain and analgesics. This was followed by a manual search of the references within the primary data sources. Inclusion and exclusion of trials into the study was performed independently by two reviewers. All randomized trials evaluating efficacy of analgesics after ovariohysterectomy in dogs were included. Data on the type of analgesic drugs used, the technique of their administration and the need for rescue analgesia were extracted from the papers.

In the clinical trial, forty-eight healthy client-owned dogs scheduled for ovariohysterectomy were randomly assigned to four treatment groups of twelve animals each. The treatment groups were designated as B, M, BM and C. All dogs in the study were sedated using acepromazine at 0.1mg/kg intramuscularly. Ten minutes later, induction was achieved by administering propofol at 5mg/kg intravenously. Anaesthesia was then maintained using isoflurane. Routine ovariohysterectomy was performed on each dog and test analgesics administered at the time of placement of the last skin suture. Dogs in group B received butorphanol at 0.2 mg/kg, group M received meloxicam at 0.2 mg/kg, group BM dogs received butorphanol-meloxicam combination at half the dosage of each drug (0.1 mg/kg butorphanol and 0.1 mg/kg meloxicam), and those in group C, acting as the control, received saline at 0.5ml/10kg body weight. All the test analgesics and placebo were administered subcutaneously.

Parameters for pain and stress response were monitored before sedation (baseline) then 1, 2, 4, 6, 12 and 24 hours postoperatively. Pain scores were assessed using the short form Glasgow composite measure pain scale. Sedation scores were assessed using Likert scale based on clinical signs of sedation. Arterial blood pressure, heart rate, respiratory rate and rectal temperature were also assessed. Stress response was assessed by measuring serum cortisol, glucose, neutrophil-lymphocyte ratio and hematological parameters. Wound healing was assessed on day 1, 2, 3 and 8 day postoperatively using clinical appearance of wounds (swelling, erythema, dehiscence, discharge) and histopathology of wound biopsies (attributes of collagen, epithelialization, neovascularization, fibroblasts, macrophages and neutrophils). In this study, parametric variables were analyzed using ANOVA and student t-test while non-parametric variables were analyzed

using Kruskal Wallis rank sum test and Mann Whitney test. Statistical significant was set at $p < 0.05$.

Thirty-one studies met the inclusion criteria in the systematic review phase of this study. Individual analgesic protocols were used in 83.9% of the studies compared to multimodal drug therapy, which was used in 16.1% of the studies. Opioids were used in 39.0% of studies, NSAIDs in 19.4%, combinations of NSAIDs and opioids in 19.4%, local analgesics in 6.5% and acupuncture in 3.2% of the studies. Drug administration was done using three approaches; pre-operative (64.5%), post-operative (22.6%) as well as combined pre and postoperative approach (12.9%). In 77.4% of the studies, administration of analgesics was done only once while in 12.9% it was done as a 72-hour postoperative course. Twenty four-hour and 48-hour courses of postoperative pain therapy were done in 6.5% and 3.2% of the studies, respectively. About 57% of the dogs in the control groups required rescue analgesia as compared to 21.6% in the single and 11.3% in multimodal drug therapy. The requirement for rescue analgesics was highest in dogs treated using acupuncture (43.8%) and lowest in dogs treated using NSAID-Opioid drug combinations (8.6%). Fewer dogs among those that received pain medication pre- and post-operatively required rescue analgesia as compared to those given analgesics only before or after surgery. More dogs (26.4%) among those given analgesics only once postoperatively required rescue analgesia as compared to those that received analgesics daily for 72 hours (4.4%).

In the clinical trial, dogs under meloxicam had statistically similar ($p = 0.68$) pain scores compared to those under butorphanol-meloxicam combination but significantly lower pain scores compared to dogs under butorphanol ($p = 0.01$) and those in the control group ($p = 0.01$). Sedation scores were

significantly ($p=0.01$) higher for dogs under butorphanol compared to those under meloxicam, the butorphanol-meloxicam combination and those in the control group. Dogs under butorphanol had significantly ($p=0.000$) lower mean blood pressure (92.0 ± 5.3 mmHg) when compared to those under meloxicam (100.9 ± 2.7 mmHg), butorphanol-meloxicam combination (105.2 ± 4.4 mmHg) and those in the control group (103.1 ± 3.8 mmHg). There were no significant differences in heart rate, respiratory rate and temperature between the four treatment groups.

Mean serum cortisol was statistically similar in the four treatment groups ($p=0.36$). Dogs under butorphanol-meloxicam combination had significantly lower mean blood glucose (4.7 ± 0.4 mmol/l) compared to that in dogs under butorphanol (5.6 ± 0.6 mmol/l, $p=0.008$) and those in the control group (5.6 ± 0.7 mmol/l, $p=0.01$). There were no significant differences in mean neutrophil-lymphocyte ratio, total leucocyte count, total platelet count, total erythrocyte count, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and the number of neutrophils, lymphocytes and monocytes in the four treatment groups.

Dogs treated using meloxicam had significantly lower scores for clinical appearance of the wounds compared to those under butorphanol ($p=0.03$) and those in the control group ($p=0.02$) but statistically similar scores to dogs under butorphanol-meloxicam combination ($p=0.39$). Dogs in the control group had the highest scores for wound swelling, erythema and dehiscence while those under meloxicam had the lowest scores. Histologically, wound biopsies from dogs under meloxicam and the butorphanol-meloxicam combination had better scores for collagen,

epithelialization, neovascularization, fibroblasts, macrophages and neutrophils compared to dogs under butorphanol and those in the control group.

The systematic review in this study demonstrated that opioids are the mainstream analgesics used to manage pain in dogs undergoing ovariohysterectomy and that one-time drug administration, preoperative and individual drug therapies are the commonly used techniques. Furthermore, NSAIDs were shown to be more effective in managing postoperative pain in dogs following ovariohysterectomy compared to opioids. Multimodal drug therapies, administration of analgesics before and after surgery, as well as a 72-hour course of pain therapy were practices that provided better outcomes in managing acute postoperative pain in dogs.

The clinical trial demonstrated that ovariohysterectomy causes moderate to severe pain lasting for up to 12 hours postoperatively. Meloxicam and butorphanol-meloxicam combination provide an equal level of analgesia without significant adverse effects in dogs following ovariohysterectomy. Butorphanol provides short term analgesia in early postoperative period but is associated with severe sedation and hypotension. Better stress management as indicated by lower cortisol, glucose and neutrophil-lymphocyte ratio was observed in dogs whose pain was treated than in those in the control. Butorphanol-meloxicam combination was the only protocol effective in minimizing stress response in dogs following ovariohysterectomy. Better response to wound healing was indicated by higher scores for wound collagen, epithelialization, neovascularization, fibroblasts and gradually diminishing levels of neutrophil and macrophage scores in pain treated dogs than in those in the control. The quality of wound healing was better in dogs treated with butorphanol-meloxicam combination than individual drugs.

It is therefore recommended that veterinarians be informed and encouraged to adopt the practice of administering analgesics both before and after surgery and for at least 72-hours postoperatively while managing pain in dogs after ovariohysterectomy. Further, it is recommended that both opioids and NSAIDs, be made part of routine pain management protocols for dogs undergoing ovariohysterectomy with opioids being administered preoperatively and NSAIDs being administered postoperatively.

Butorphanol-meloxicam drug combination as administered in this study is recommended for use in management of acute postoperative pain and stress in dogs undergoing ovariohysterectomy. This study further recommends that a more focused study, using a large number of animal, be conducted in order to quantify the relationship between pain, stress and wound healing in dogs.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 General background

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey and Bogduk, 1994; Short, 1995; Muir, 1998). Pain occurs when nociceptors are stimulated by thermal, mechanical, or chemical stimuli and impulses sent to the central nervous system for interpretation and modulation (ACVA, 2006). In veterinary surgical patients, the main sources of pain include trauma, surgical procedures and anaesthesia induced by muscle ischemia. Consequently, all animals undergoing surgical procedures require pain relief post-operatively to overcome the associated deleterious physiological effects as well as take care of humane and ethical concerns (Hansen, 2005). The harmful physiological effects of unmanaged pain include: increased post-operative stress, immunosuppression, increased arterial blood pressure, delayed wound healing, negative protein balance, decreased food intake and development of maladaptive behaviors including self-mutilation (Gwendolyn and Carrol, 1996; Gaynor, 1999).

Pain can be controlled by interventions targeted at different points in the pain transmission pathway. Techniques that are used to control pain are based on limitation of nociceptor stimulation, interruption of peripheral neural transmission, inhibition of nociceptive transmission at the level of the spinal cord, modulation of brain pathways or combined use of any of these techniques (ACVS, 1996; Muir, 1998; Mogoia and Mbithi, 2004).

Current trends in pain management in animals emphasize a more comprehensive approach by managing pain peri-operatively using a wide range of therapies including several categories of drugs administered through local, regional or systemic techniques. This allows for blocking of pain at several different places along the nociceptive pathways (Epstein, 2011). Studies have shown that irrespective of the dose used, a single class of analgesic drugs cannot provide complete analgesia owing to the nature of pain transmission, which is a complex process that involves multiple pathways, mechanisms and transmitter systems (Lascelles, 1999). Moreover, over-reliance on one class of analgesic drugs, as has been the case with non-steroidal anti-inflammatory drugs (NSAIDs), may not only undermanage pain in some patients, but could also increase the possibility of side effects associated with such drugs (Epstein, 2011).

Multimodal analgesic drug therapy is a practice that is currently gaining popularity in veterinary practice due to additive analgesia effect and the use of small doses of individual drugs (Lemke, 2004; White *et al.*, 2007). This improves patient comfort and minimizes the need for high doses or prolonged use of any one particular drug (Epstein, 2011), hence minimizing the likelihood of undesirable side effects as well as the cost of treatment. However, documented scientific information on multimodal pain therapy is scanty hence more research to evaluate the safety and effectiveness of various drug combinations needs to be conducted before they can be recommended for use in veterinary patients.

A number of studies have reported on the effectiveness of analgesics on management of postoperative pain (Leece *et al.*, 2005; Dzikiti *et al.*, 2006; Larisa *et al.*, 2009) and postoperative stress in dogs (Benson *et al.*, 2000; Freeman *et al.*, 2010). Studies in human patients have

demonstrated that postoperative pain and stress are important drivers of wound infection, and delayed wound healing (Goiun and Kiecolt-Glaser, 2011). However, there is no documentation of studies in animals with reference to the effects of analgesics on surgical wound healing or the correlation between pain, stress and wound healing. It was therefore considered important to carry out a study to evaluate the effects of pain management on stress responses and wound healing in dogs using individual analgesics as well as their combination. The results of this study are useful in guiding veterinarians on the importance of effective postoperative pain management and its benefit to wound healing as well as the wellbeing of veterinary patients.

In particular, this study was designed to compare the effectiveness of single and multimodal drug therapy (NSAID-Opioid combination) in managing postoperative pain, stress response and surgical wound healing in dogs. The study was carried out with the hypothesis that optimal postoperative pain management using multimodal drug therapy minimizes postoperative stress and improves wound healing in dogs.

1.2 General objective

The general objective of this study was to evaluate the effects of butorphanol, meloxicam and their combination on postoperative pain, stress response and wound healing in dogs after ovariohysterectomy.

1.3 Specific Objectives

1. To conduct a systematic review on pain management protocols in dogs undergoing ovariohysterectomy
2. To determine the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on post-operative pain after ovariohysterectomy in dogs.
3. To determine the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on post-operative stress after ovariohysterectomy in dogs.
4. To determine the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on wound healing after ovariohysterectomy in dogs.

CHAPTER TWO

2.0 GENERAL LITERATURE REVIEW

2.1 Pain

2.1.1 Physiology of pain

There are three physiologic processes that result in the perception of pain. These three processes are transduction, transmission, and modulation of neural signals that originate in response to noxious stimuli (Lamont *et al.*, 2000). A noxious stimulus travels to the brain from the site of origin through a chain of three neurons (Figure 2-1). The first order neuron begins in the periphery and travels to the dorsal horn of the spinal cord where it synapses with the second order neuron, which crosses the spinal cord and ascends to the brain. A second synapse occurs within the thalamus and the third order neuron projects into the cerebral cortex (Lamont *et al.*, 2000; Muir and Woolf, 2001).

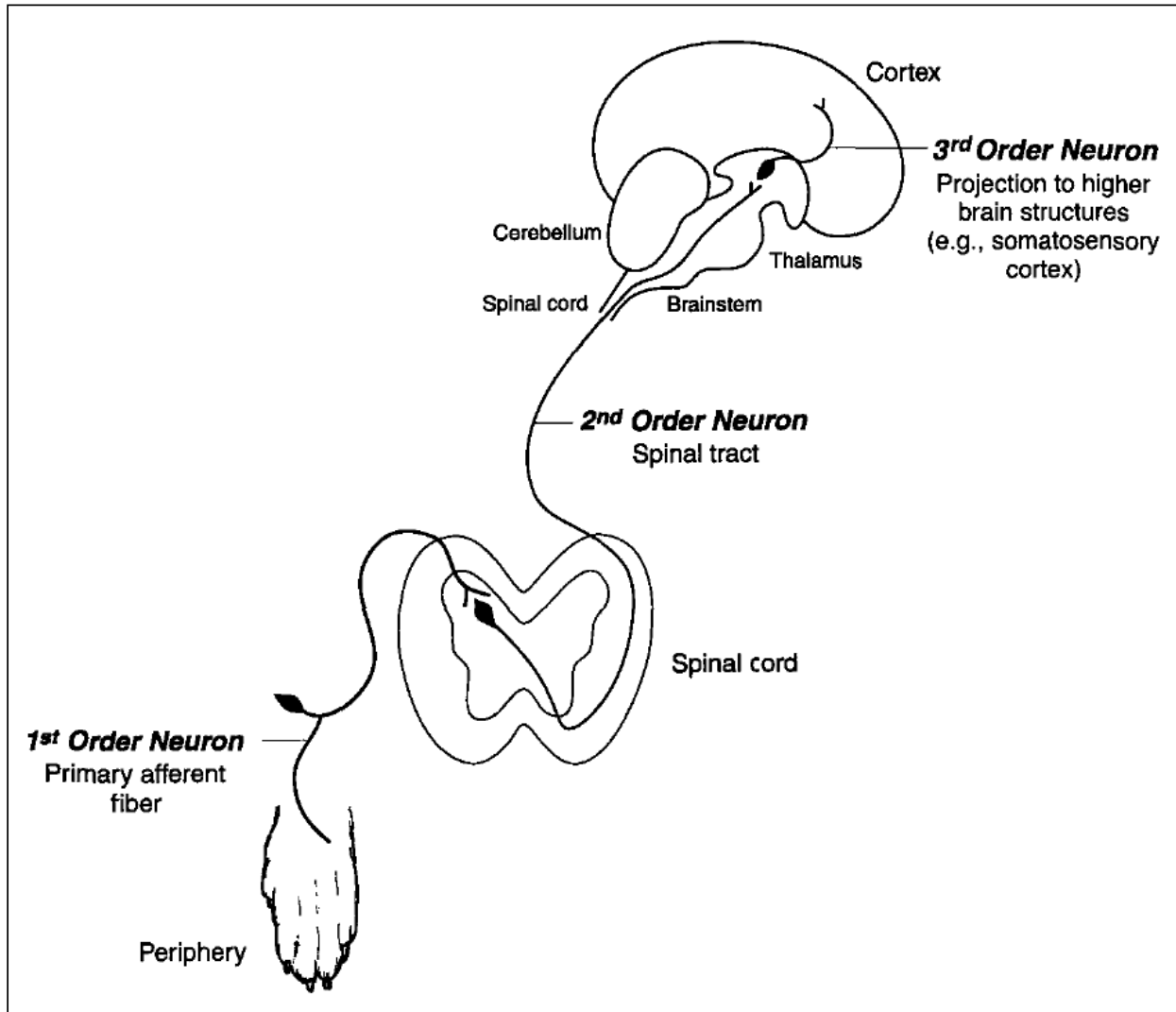


Figure 2-1: A schematic representation of the pain pathway as a chain of three neurons. (From: Lamont *et al.*, 2000).

Ascending axons of the receptors are classified as A δ and C- afferent nerve fibers. The A δ fibers are thinly myelinated large diameter axons capable of transmitting impulses quickly. They are responsible for the generation of “fast pain” (sharp, well localized, transient pain). The C-fibers are smaller unmyelinated axons that conduct impulses more slowly. These fibers contribute to “slow pain”, which can be characterized by a more diffuse burning sensation that persists after the termination of noxious stimuli (Lamont *et al.*, 2000). Both types of afferent nerve fibers extend axons that synapse with neurons located in the dorsal horn of the spinal cord. The dorsal horn of the spinal cord is organized in layers or laminae of functionally distinct cells that form columns (Muir, 2002), which extend the entire length of the spinal cord (Figure 2-2). The majority of A δ fibers form synapses in lamina I, while most C- fibers travel to lamina II (Muir, 2002). Here the axons may form synapses with one of the three types of dorsal horn neurons (interneurons, propriospinal and projection neurons). Interneurons may be excitatory or inhibitory and contribute to local modulation of the afferent signals; propriospinal neurons are involved in reflex activities while projection neurons are involved in the projection of afferent signals to supraspinal centers such as the midbrain and cerebral cortex (Lamont *et al.*, 2000).

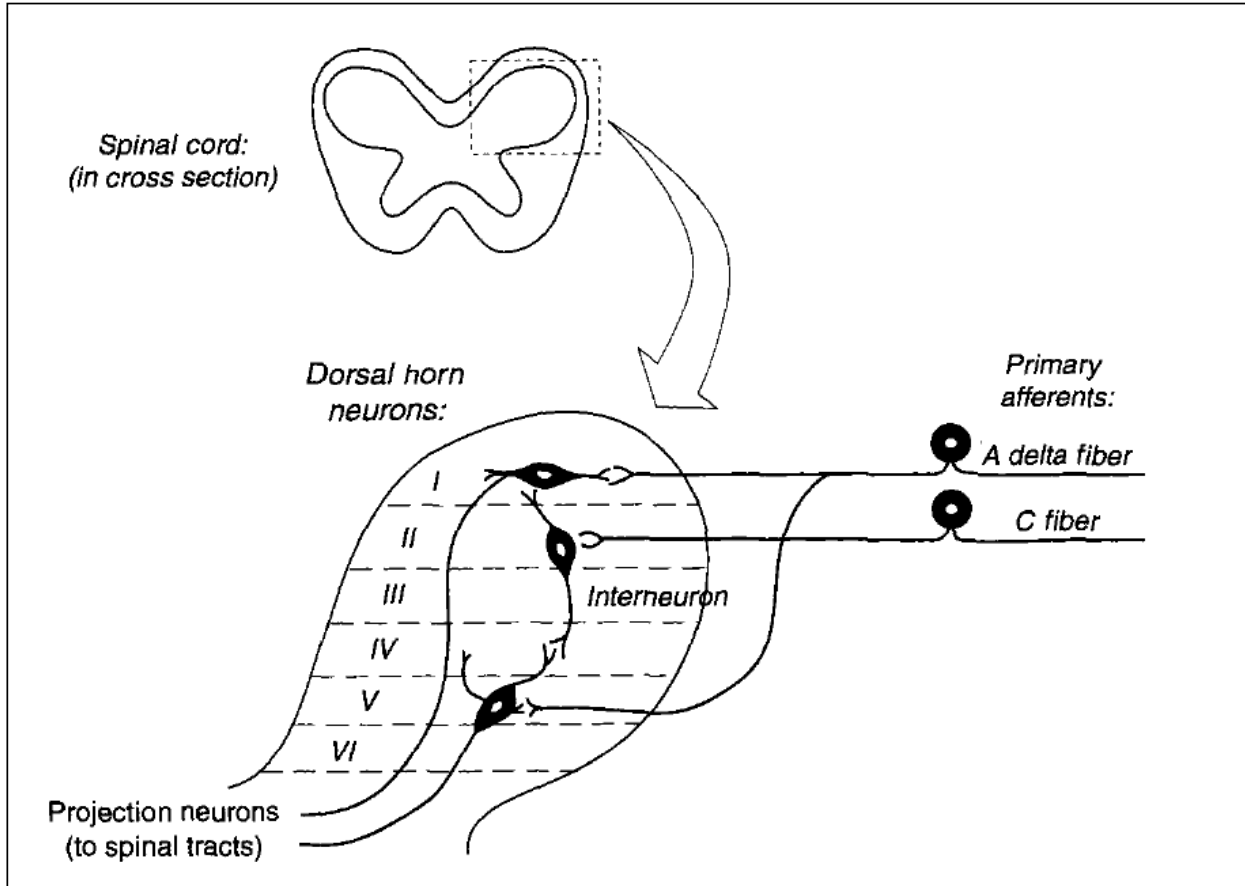


Figure 2-2: Laminar organization of the spinal cord dorsal horn. A delta fibers are shown entering in lamina I and V while C fibers enter at the second laminae. (From: Lamont *et al.*, 2000).

Communication between afferent axons and dorsal horn neurons depends upon the release of both excitatory and inhibitory neurotransmitters that are produced, stored, and released from the terminal ends of the afferent axons and dorsal horn neurons (Muir and Woolf, 2001). Input from both types of fibers (A δ and C) results in the release of the excitatory neurotransmitters, glutamate and aspartate.

Glutamate binds to μ -amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA), kainate, and N-methyl-D-aspartate (NMDA) receptors. These receptors are ligand-gated sodium and calcium channels. Therefore, once glutamate is bound to the receptor, the neuronal membrane is depolarized by the influx of positive ions (Muir and Woolf, 2001). In addition, a variety of other neuropeptides capable of eliciting depolarization of the dorsal horn neurons are released (by C-fibers in particular). These neuropeptides include substance P, vasoactive intestinal peptide, neurotensin, and cholecystinin (Muir and Woolf, 2001). The magnitude of neurotransmitter release is proportional to stimulus intensity. Intense stimulation (either thermal or mechanical) results in increased release of glutamate and substance P, which potentiate the generation of action potentials, that ascend along the spinal cord to higher processing centers (Lamont *et al.*, 2000).

From the dorsal horn of the spinal cord, afferent nociceptive information is transmitted to supraspinal centers through projection neurons that follow one of several pathways. These pathways include: spinothalamic, spinoreticular, spinomesencephalic and postsynaptic dorsal column tracts (Lamont *et al.*, 2000). While all of these tracts are involved in nociceptive transmission to some degree, their relative importance appears to vary considerably between species (Muir and Woolf, 2001). In many species, the spinothalamic tract is the most prominent

pathway in the spinal cord and serves a principal role in pain transmission. The spinothalamic tract originates from the axons in several dorsal horn laminae (I, V, VI, and VII), and from there it travels rostrally through the white matter of the spinal cord to the thalamus (Lamont *et al.*, 2000). In the thalamus, information is integrated and relayed to the somatosensory cortex as well as cortical association areas including the limbic system. These pathways appear to involve the sensory – discriminative aspects of pain as well as the motivational component of pain (including the determination of purposeful behavior) (Muir and Woolf, 2001).

Areas of the brainstem also contribute to the perception of pain through the action of the reticular system and the periaqueductal gray matter (PAG) (Lamont *et al.*, 2000). Reticular neurons modulate motivational aspects of pain through projections to the medial thalamus and limbic system, while the periaqueductal gray matter exerts its effect on pain perception through projections to the hypothalamus and thalamus (Lamont *et al.*, 2000). Signaling between ascending spinal tract projections, the thalamus and cerebral cortex is still not well understood. It is however thought that glutamate and aspartate are the principal excitatory neurotransmitters, while gamma-aminobutyric acid (GABA), glycine and the monoamines (norepinephrine, serotonin, and dopamine) function to inhibit transmission of noxious stimuli (Lamont *et al.*, 2000).

The transmission of painful stimuli can be modified by inhibitory signals that descend the three-neuron chain (Hamilton, 2003). Studies have shown that the transmission of pain is subject to inhibitory influences on four levels: cortical and thalamic structures, PAG, medulla and pons, and spinal cord dorsal horn. The most important of these four appear to be the PAG and the dorsal horn of the spinal cord (Lamont *et al.*, 2000).

Stimulation of the PAG results in outflow of opioid peptides that inhibit the transmission of painful stimuli both at the level of the brain and at the dorsal horn of the spinal cord. This outflow of opioids is thought to be mediated by the release of the inhibitory neurotransmitter GABA from an interneuron. Additionally, dorsal horn neurons have also been shown to contain dense concentrations of GABA as well as glycine, serotonin, norepinephrine, and the endogenous opioid peptides (Lamont *et al.*, 2000). Release of these neurotransmitters will effectively block transmission of noxious stimuli to supraspinal levels, thereby reducing the degree of pain perception.

2.1.2 Pain recognition and assessment

Immediate and appropriate assessment of post-operative pain, aids in optimal pain control and evaluation of analgesic efficacy (Lascelles *et al.*, 1994). However, recognition of pain in animals is a challenge (Anil *et al.*, 2002) as it relies on the interpretation of animal behavior by an observer since there is no effective means of communication (Murrell *et al.*, 2008). The challenge of pain recognition in animals is further compounded by lack of a validated method of assessing clinical pain in veterinary patients (Anil *et al.*, 2002).

Changes in non-interactive behavior in undisturbed animals coupled with responses to interaction with the patient - handling of the animal and its surgical site - have been reported to be the most effective clinical tool for rapid evaluation of post-operative pain (Lascelles *et al.*, 1994). The combination of non-interactive and interactive behavioral changes can serve as a basic template for constructing different pain scales.

Pain scales provide a subjective assessment of post-operative pain in dogs and include the visual analogue scale (VAS), numerical rating scale (NRS) and simple descriptive scale (SDS) (Lascelles *et al.*, 1997; Lemke *et al.*, 2002). Though these scales provide a reliable subjective appraisal of acute pain, lack of linearity and specificity with descriptive pain behaviours have been identified as the main drawbacks (Holton *et al.*, 1998; Hansen, 2003). However, VAS has been reported to give satisfactory measures of sedation in dogs undergoing surgery (Lascelles *et al.*, 1998; Slingsby and Waterman-Pearson, 2001).

The short form of the Glasgow composite measure pain scale (CMPS-SF) has been introduced for assessment of acute pain in a clinical setting (Reid *et al.*, 2007). The CMPS-SF involves use of a structured questionnaire completed by an observer while following a standard protocol which includes the assessment of spontaneous and evoked behaviors, interactions with the animal and clinical observations (Murrell *et al.*, 2008). The CMPS-SF is easy to use and allows a quick assessment of acute clinical pain (Morton *et al.*, 2005; Reid *et al.*, 2007). In addition, CMPS-SF is the only method that has validation data currently (Morton *et al.*, 2005; Reid *et al.*, 2007).

2.1.3 Pain management

Pain needs to be managed in a comprehensive manner peri-operatively using a wide range of therapies and techniques (Egger and Love, 2009; Epstein, 2011). Techniques that are used to control pain are based on: limitation of nociceptor stimulation, interruption of peripheral neural transmission, inhibition of nociceptive transmission at the level of the spinal cord, modulation of brain pathways, or combined use of any of these techniques (ACVS, 1996; Muir, 1998; Mogoa and Mbithi, 2004). Therapies for pain management include systemic, local and/or regional use of

various categories of drugs such as anxiolytics (phenothiazines, benzodiazepines, α -2-Adrenoceptor agonists), non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, opioids, local anaesthetics/analgesics and dissociative anaesthetics such as ketamine. These drugs can be used either alone or in various combinations (Egger and Love, 2009; Epstein, 2011).

Different drugs and techniques have been used in management of pain following ovariohysterectomy in dogs. NSAIDs and opioids are the most popular analgesics in dogs and are either administered preemptively, postoperatively and/or in multimodal therapy. There are reports on the use of NSAIDs for pain management in dogs, including use of carprofen (Lascelle *et al.*, 1998; Slingsby and Waterman-Pearson, 2001; Leece *et al.*, 2005; Dzikiti *et al.*, 2006), dipyrone (Imagawa *et al.*, 2011), meloxicam (Caulkett *et al.*, 2003; Leece *et al.*, 2005), ketorolac and flunixin (Karol *et al.*, 1996).

Meloxicam is one of the cyclooxygenase type 2 (COX-2) selective NSAIDs (Churchill *et al.*, 1996) that inhibit many of the inflammatory functions of arachidonic acid, but selectively spare the housekeeping functions of prostaglandins and thromboxanes (Engelhardt *et al.*, 1996). These functions include the regulation of gastrointestinal blood flow and gastro-protective mechanisms, the regulation of renal blood flow and control of platelet aggregation and clot formation (Engelhardt *et al.*, 1996). Consequently, meloxicam provides a prolonged superior analgesia while minimizing the undesirable side effects associated with NSAIDs (Caulkett *et al.*, 2003; Leece *et al.*, 2005). It is for this reason that meloxicam is extensively used in management of chronic pain states such as osteoarthritis and is a safe and effective drug for controlling acute postoperative pain for up to 20 hours in dogs undergoing laparotomy (Mathews *et al.*, 2001).

Opioids continue to have a crucial role in perioperative pain management for veterinary patients. The opioids widely used for pain management in dogs include: morphine (Mastrocinque and Fantoni, 2003; Dzikiti *et al.*, 2006; Pekcan and Koc, 2010; Kongara *et al.*, 2012), tramadol (Mastrocinque and Fantoni, 2003; Fajardo *et al.*, 2012; Kongara *et al.*, 2012), pethidine (Slingsby and Waterman-Pearson, 2001), fentanyl (Pekcan and Koc, 2010), butorphanol (Karol *et al.*, 1996; Caulkett *et al.*, 2003; Larisa *et al.*, 2009) and buprenorphine (Slingsby *et al.*, 2011). Studies comparing the analgesic effects of meloxicam and butorphanol in dogs following ovariohysterectomy have demonstrated that meloxicam produces analgesia of superior quality and prolonged duration compared to butorphanol (Mathewset *al.*, 2001; Caulkett *et al.*, 2003).

Butorphanol tartrate ([17-(cyclobutylmethyl) morphinan-3,14-diol D-tartrate]) is a synthetic opioid receptor agonist-antagonist with good analgesic properties in dogs (Pfeffer *et al.*, 1980). Administration at 0.2 mg/kg either intramuscularly or subcutaneously has been found to be effective in managing perioperative pain in dogs (Karol *et al.*, 1996; Caulkett *et al.*, 2003; Larisa *et al.*, 2009). Studies on the effectiveness of butorphanol, meloxicam and firocoxib for managing pain after ovariohysterectomy in dogs have demonstrated that meloxicam and firocoxib provide superior analgesia compared to butorphanol (Caulkett *et al.*, 2003; Camargoet *al.*, 2011).

2.1.4 Multimodal pain therapy

Multimodal or balanced analgesic regimens involve the use of two or more analgesic agents in combination to provide either an additive or synergistic analgesic effect (Dahl and Kehlet, 1993). The agents should act by different mechanisms in order to maximize their beneficial analgesic effects and minimize any harmful side effects (Slingsby and Waterman-Pearson, 2001). This

approach is thought to have a higher likelihood of providing optimum analgesia than the use of a single analgesic agent (Dahl and Kehlet, 1993). Combinations of opioids and non-steroidal anti-inflammatory drugs (NSAIDS) have been used successfully in human and veterinary practice.

A number of studies have reported on the use of different opioid-NSAID and other drug combinations for pain management in dogs. These include combinations of morphine and carprofen (Dzikiti *et al.*, 2006) as well as pethidine and carprofen (Slingsby and Waterman-Pearson, 2001). In addition, Kongara *et al.* (2012) used tramadol and morphine combination while Fajardo *et al.*, (2012) reported the use of intraoperative infusion of combinations of tramadol, lidocaine and ketamine as well as morphine, lidocaine and ketamine for pain management in dogs undergoing ovariohysterectomy.

Results from all these studies provide evidence that the approach of pre-operative use of analgesics and multimodal drug therapy provides analgesia of more superior quality than post-operative and single drug therapies. There is however no report on the analgesic effects of meloxicam-butorphanol combination in dogs.

2.2 Stress

Stress is a process through which both internal and external environmental demands exceed an individual's perceived ability to cope, thereby resulting in behavioral and physiological changes (Vileikyte, 2007). Stress has also been defined as a consequence of the failure of an individual to respond appropriately to physical or emotional threats (Ice and James, 2007; Solowiej *et al.*, 2009). Stressors/stress stimuli can be physical, psychological and social in origin (Solowiej *et al.*, 2009).

Perioperative stress in canine patients has been attributed to anxiety, excitement from handling, hospitalization, fear, depression, anaesthesia, tissue damage and pain (Fox *et al.*, 2000; Beilin *et al.*, 2003). Tissue damage and manipulation during surgery evoke nociceptive afferent activity resulting in stress response even in patients that are receiving adequate general anaesthesia (Benson *et al.*, 2000).

Stress can be acute or chronic in nature. Long-term stress is more harmful than short-term stress (Dhabhar, 2002). Stress activates the hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes resulting in downstream hormonal and immunological changes (Upton and Solowiej, 2010). Hormones involved include adrenocorticotrophic hormone, cortisol and catecholamines [epinephrine and norepinephrine] (Padgett and Glaser, 2003; Dickerson and Kemeny, 2004; Glaser and Kiecolt-Glaser, 2005). Cortisol stimulates production of glucose and breakdown of tissue protein; increases sensitivity of blood vessels to adrenaline, resulting to increased heart rate and blood pressure; affects lymphoid organs causing increased production of neutrophils, thrombocytes and erythrocytes (Dhabhar, 2002); and reduces the levels of pro-inflammatory cytokines like interleukin-6 and enzymes such as matrix metalloproteinases (Kudoh *et al.*, 2001; Freeman *et al.*, 2010). These pro-inflammatory cytokine and enzymes play a key role in tissue repair and when their levels are reduced post-operatively, there is a tendency for delayed wound healing (Upton and Solowiej, 2010). Studies in dogs have shown a negative correlation between levels of postoperative cortisol and pro-inflammatory cytokines at the wound site (Freeman *et al.*, 2010). It has also been demonstrated that stress-induced up-regulation of glucocorticoids suppresses tumor necrosis factor α and interleukin-6 in humans (DeRijk *et al.*, 1997). Glucocorticoid hormones also modulate a range of immune functions including cytokine

expression, adhesion molecule expression, immune cell trafficking (the distribution of cells in circulation in peripheral blood) as well as cell proliferation and differentiation (Vileikyte, 2007), consequently interfering with wound healing.

Stress has consequences to an individual's health and is a significant determinant of outcomes of surgical procedures (Glaser and Keicolt-Glaser, 2005). Studies in human patients have shown that increased postoperative stress has some association with the resulting wound infection (Beilin and Shavit, 2003), delayed wound healing (Broadbent *et al.*, 2003; Ebrecht *et al.*, 2004), prolonged hospitalization and increased cost of treatment (Morrison *et al.*, 2003). Although some studies have been done in veterinary patients to assess the relationship between postoperative pain and stress (Mastrocinque and Fantoni, 2003), there is scanty information regarding the effect of stress on wound healing in animals.

In humans, physiological, behavioral and psychological measures have been used to assess stress response perioperatively. Physiological measurements that are used to assess stress include heart rate, blood pressure, respiratory rate, galvanic skin response and cortisol (Upton and Solowiej, 2010). Behavioral measures include vocal expression, facial expression, bracing, restlessness and rubbing or massaging of the wound (Upton and Solowiej, 2010). Psychological measurements involve the use of questionnaires, which allow patients to give reports of their emotional feelings (Upton and Solowiej, 2010). These questionnaires include the Hospital Anxiety and Depression Scale [HADS] (Zigmond and Snaith, 1995), the Perceived Stress Scale [PSS] (Cohen, 1995), the State Trait Anxiety Inventory [STAI] (Spielberger *et al.*, 1970) and the General Health Questionnaire [GHQ] (Goldberg, 1995).

In dogs, both physiological and behavioral changes have been used to assess stress. Väinsänen *et al.*, (2005) used changes in heart rate, heart rate variability and behavioral patterns to evaluate pre-operative stress in dogs hospitalized for elective ovariohysterectomy. In this study, panting, yawning and snout licking coupled with elevated heart rate and lower heart rate variability were observed in dogs exposed to acute stressors. In another study, physiological parameters (rectal temperature, heart rate, respiratory rate and blood pressure), metabolic parameters (cortisol and glucose) and surgical stress markers (interleukin 6 and C-reactive protein) were used to assess stress in dogs undergoing oophorectomy (Freeman *et al.*, 2010). Cortisol and glucose concentration are indicators of metabolic responses and are attributed to perception of pain due to surgical trauma (Freeman *et al.*, 2010). Cortisol concentration increases after the start of surgery and reaches maximum levels 4-6 hours post-operatively (Desborough, 2000; Marcovich *et al.*, 2001). Studies in dogs have shown significant increase in cortisol concentration following ovariohysterectomies (Freeman *et al.*, 2010). Glucose increases for 36 hours post-operatively in dogs. However, this increase has not been shown to be significant (Marcovich *et al.*, 2001; Hancock *et al.*, 2005; Freeman *et al.*, 2010). Acute phase proteins such as interleukin-6 and C-reactive protein are early indicators of inflammation and tissue injury (Luk *et al.*, 2009). Plasma Interleukin 6 has been shown to increase for up to 12 hours post-operatively in dogs. A positive correlation between plasma interleukin 6 concentration and cortisol has been established in postsurgical patients (Kudoh *et al.*, 2001; Freeman *et al.*, 2010). Catecholamines (epinephrine, norepinephrine, adrenocorticotrophic hormone) have also been used to assess the effect of medetomidine in minimizing perioperative stress response in dogs. Their concentrations reduced significantly when medetomidine was administered pre-emptively in the dogs (Benson *et al.*, 2000).

Attenuation of the stress response perioperatively is necessary because it improves wound healing and surgical outcome (Benson *et al.*, 2000). Supportive therapy and systemic administration of analgesics can decrease stress response in patients and are most effective when administered pre-emptively (Woolf and Chong, 1993; Benson *et al.*, 2000).

Administration of morphine has been shown to decrease plasma catecholamine concentrations after orchiectomy in cats, and xylazine caused a decrease in cortisol to almost undetectable levels (Benson *et al.*, 1991). Cortisol plasma concentrations and systolic arterial blood pressure are lower in cats receiving butorphanol than in those without butorphanol, postoperatively (Smith *et al.*, 1996).

2.3 Wound healing

2.3.1 The physiology of wound healing

Wound healing is a normal biological process that consists of four highly integrated and overlapping phases namely hemostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004) [Table 2-1]. Optimal wound healing requires that the events of each phase occur in proper sequence, at specific times, and continue for a specific duration at optimal intensity (Mathieu *et al.*, 2006). Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or non-healing chronic wounds (Guo and DiPietro, 2010).

Optimal wound healing involves the following six events: rapid hemostasis; appropriate inflammation; mesenchymal cell differentiation, proliferation and migration to the wound site; suitable angiogenesis; prompt re-epithelialization (re-growth of epithelial tissue over the wound

surface); and proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue (Gosain and DiPietro, 2004; Mathieu *et al.*, 2006).

The first phase of hemostasis begins immediately after wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)- β , platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Once bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and promote inflammation (Guo and DiPietro, 2010).

Table 2-1: Showing processes involved in normal wound healing

Wound Healing Cellular and Bio-physiologic Events	
Phase	
Hemostasis	<ol style="list-style-type: none">1. Vascular constriction2. Platelet aggregation, degranulation, and fibrin formation (thrombus)
Inflammation	<ol style="list-style-type: none">1. Neutrophil infiltration2. Monocyte infiltration and differentiation to macrophage3. Lymphocyte infiltration
Proliferation	<ol style="list-style-type: none">1. Re-epithelialization2. Angiogenesis3. Collagen synthesis4. Extracellular matrix formation
Remodeling	<ol style="list-style-type: none">1. Collagen remodeling2. Vascular maturation and regression

Inflammatory phase is characterized by sequential infiltration of neutrophils, macrophages, and lymphocytes (Gosain and DiPietro, 2004; Broughton *et al.*, 2006; Campos *et al.*, 2008). A critical function of neutrophils is the clearance of invading microbes and cellular debris in the wound area, although these cells also produce substances such as proteases and reactive oxygen species (ROS), which cause some additional damage. Macrophages play multiple roles in wound healing. In the early stages of wound healing, macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thus paving the way for the resolution of inflammation. As macrophages clear these apoptotic cells, they undergo a phenotypic transition to a reparative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote tissue regeneration (Meszaros *et al.*, 2000; Mosser and Edwards, 2008). In this way, macrophages promote the transition to the proliferative phase of healing.

T-lymphocytes migrate into wounds following the inflammatory cells and macrophages, and peak during the late-proliferative/early-remodeling phase. Although the role of T-lymphocytes is not completely understood, studies suggest that delayed T-cell infiltration along with decreased T-cell concentration in the wound site is associated with impaired wound healing, while others have reported that T-helper cells (CD 4+ cells) have a positive role in wound healing and T-suppressor-cytotoxic cells (CD8+ cells) play an inhibitory role in wound healing (Swift *et al.*, 2001; Park and Barbul, 2004).

The proliferative phase generally follows and overlaps with the inflammatory phase, and is characterized by epithelial proliferation and migration over the provisional matrix within the

wound (re-epithelialization). In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury. Within the wound bed, fibroblasts produce collagen as well as glycosaminoglycans and proteoglycans, which are major components of the extracellular matrix (ECM) (Guo and DiPietro, 2010). Following robust proliferation and ECM synthesis, wound healing enters the final remodeling phase, which can last for years.

Remodelling phase of wound healing is characterized by regression of many newly formed capillaries so that vascular density of the wound returns to normal. One critical feature of the remodeling phase is ECM remodeling to an architecture that closely resembles that of the normal tissue. The wound also undergoes physical contraction throughout the entire wound healing process, which is believed to be mediated by contractile fibroblasts (myofibroblasts) that appear in the wound (Gosain and DiPietro, 2004; Campos *et al.*, 2008).

2.3.2 Factors that affect wound healing

There are many local and systemic factors that can interfere with one or more phases and thus affect the overall wound healing. Local factors are those that directly influence the characteristics of the wound itself, while systemic factors are the overall health or disease state that affects the individual's ability to heal (Guo and DiPietro, 2010). Local factors that interfere with wound healing include tissue oxygenation, infections, foreign body and blood supply while systemic factors include age, stress, diseases such as diabetes and obesity, nutrition and medications such as corticosteroids and non-steroidal anti-inflammatory drugs (Guo and DiPietro, 2010). Studies in the United States have reported that non-healing wounds in humans incur enormous health care

expenditures with the total cost estimated at more than \$3 billion per year (Mathieu *et al.*, 2006; Menke *et al.*, 2007). However, no such studies have been conducted in veterinary practices.

2.3.3 Assessment of wound healing

Assessment of wound healing in veterinary patients can be achieved through clinical appearance, histopathology and ultrasonography (Sylvestre *et al.*, 2002; Abramo *et al.*, 2004; Laiju *et al.*, 2005; Nisbet *et al.*, 2010). The clinical appearance involves scoring of surgical wounds at specific intervals based on swelling, erythema, dehiscence, and discharge (Sylvestre *et al.*, 2002). Histopathologic evaluation involves routine processing of biopsies taken from surgical wounds at specified intervals (Abramo *et al.*, 2004). Ultrasound scanning of wounds is performed at a frequency of 7.5 MHz and evaluated based on diameter and depth of the wound (Abramo *et al.*, 2004; Laiju *et al.*, 2005). Ultrasound scanning of wounds enables repeated, noninvasive, quantitative assessment of structural changes deep within wounds while histopathological assessment allows more precision, but not serial examination of wounds (Abramo *et al.*, 2004).

2.4 The interplay between pain, stress and wound healing

Although post-operative pain and stress have been associated with delayed wound healing in human and laboratory animal studies (Padgett *et al.*, 1998; Broadbent *et al.*, 2003), there is currently no study showing this interplay in veterinary patients. Studies in human and laboratory animals have provided evidence that pain contributes to stress in an individual. Consequently, stress results in the deregulation of the immune system, mediated primarily through the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary axes or sympathetic nervous system (Godbout and Glaser, 2006; Boyapati and Wang, 2007). The hypothalamic-

pituitary-adrenal and the sympathetic-adrenal medullary axes regulate the release of pituitary and adrenal hormones. These hormones include the adrenocorticotrophic hormones, cortisol and prolactin, and catecholamines (epinephrine and norepinephrine). Stress up-regulates glucocorticoids and reduces the levels of the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α at the wound site. Stress also reduces the expression of IL-1 α and IL-8 at wound sites which are chemoattractants that are necessary for the initial inflammatory phase of wound healing (Godbout and Glaser, 2006; Boyapati and Wang, 2007). In addition, glucocorticoids influence immune cells by suppressing differentiation and proliferation, regulating gene transcription, and reducing expression of cell adhesion molecules that are involved in immune cell trafficking (Sternberg, 2006). On the other hand, cortisol functions as an anti-inflammatory agent and modulates the immune mediated responses that are essential for the initial phase of healing. Thus, stress impairs normal cell mediated immunity at the wound site, causing a significant delay in the healing process (Godbout and Glaser, 2006).

Stress can also affect the remodeling phase of wound healing by regulating the production and activation of matrix metalloproteinase enzymes which are involved in degradation of collagen as well as facilitating cellular invasion and migration in the wound (Pajulo *et al.*, 1999; Broadbent *et al.*, 2003). Stress has also been reported to increase susceptibility of wounds to bacterial infection in mice, thus delaying wound healing (Rojas *et al.*, 2002).

On the other hand, stress can lead to negative emotional states, such as anxiety and depression, which may in turn have an impact on physiologic processes and/or behavioral patterns that influence health outcomes (Guo and DiPietro, 2010). In addition to the direct influences of anxiety

and depression on endocrine and immune function, stressed individuals are more likely to develop maladaptive behaviors that negatively modulate the healing process (Guo and DiPietro, 2010). The interaction between pain, stress and wound healing is illustrated in Figure 2-3.

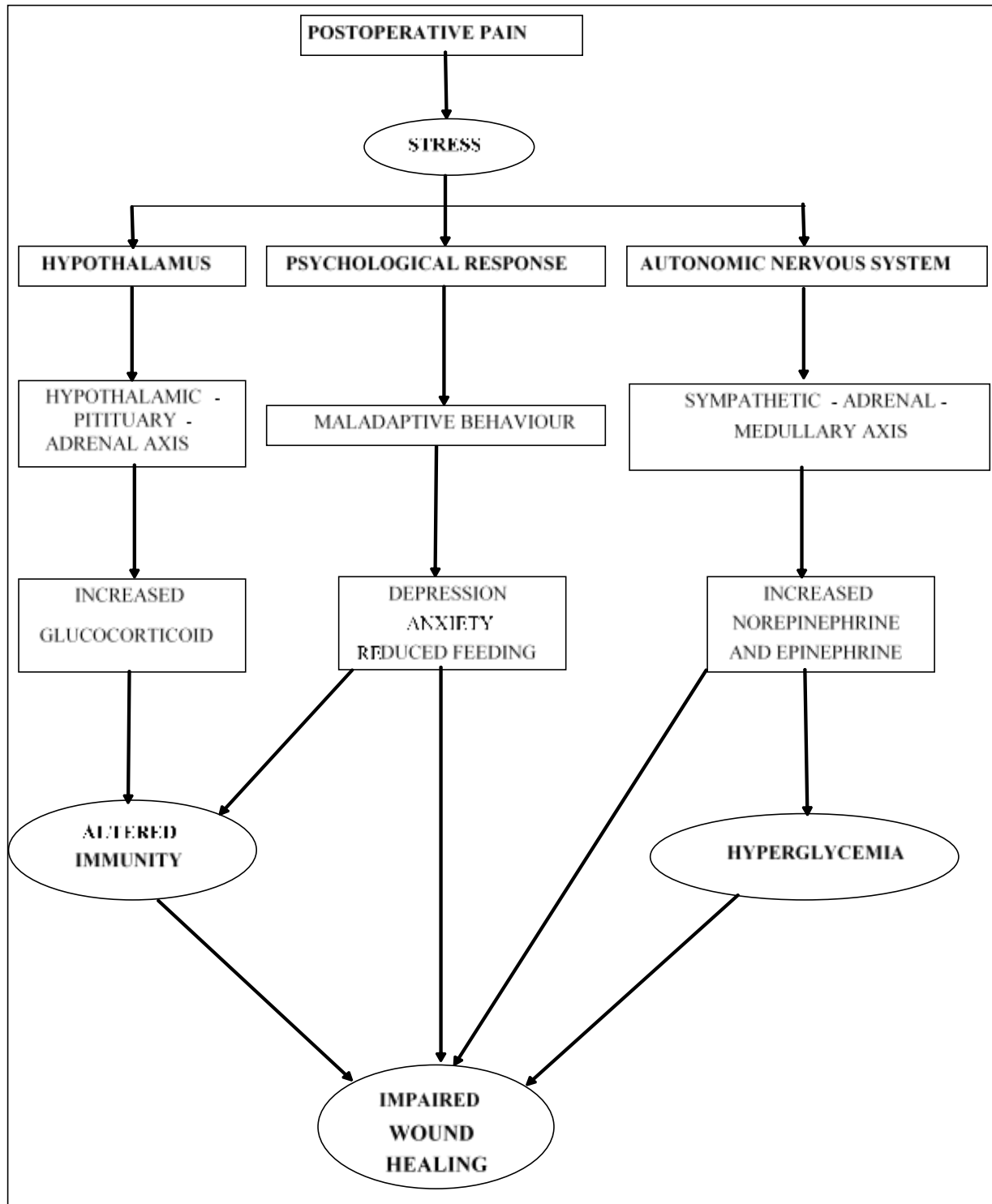


Figure 2-3:An illustration of the interaction between pain, stress and wound healing

CHAPTER THREE

3.0 ANALGESIA PRACTICES IN DOGS UNDERGOING OVARIOHYSTERECTOMY: A SYSTEMATIC REVIEW

3.1 Introduction

Ovariohysterectomy is a routine surgical procedure which is known to cause marked acute pain in dogs (Gaynor and Muir, 2002). Perioperative analgesia in surgical patients is paramount not only for humane and ethical considerations, but also for the reason that it helps minimize the deleterious physiological effects associated with pain (Hansen, 2005). These harmful effects include: increased post-operative stress, immunosuppression, increased arterial blood pressure, delayed wound healing, negative protein balance, decreased food intake and development of maladaptive behaviors including self-mutilation (Gwendolyn and Carrol, 1996; Gaynor, 1999).

The numerous analgesic drugs and techniques currently available for management of pain in animals pose a challenge to practicing clinicians with regard to the choice of the appropriate drug and technique for optimal pain management in animals. Practically, the choices are mainly influenced by the type of surgery, past experiences of the clinicians and their knowledge of the specific drug or technique, availability of the drug, associated side effects, cost and occasionally set guidelines for the clinic or hospital (Wagner and Hellyer, 2002).

This study evaluated the trends in analgesia practices in dogs undergoing ovariohysterectomy and further determined their effectiveness in managing postoperative pain. The results of this systematic review can help decision making by clinicians on the most appropriate choice of

analgesics and techniques for effective pain management, hence leading to better animal welfare and favorable surgical outcomes.

3.2 Methodology

3.2.1 Data search

Literature search was conducted to identify all trials comparing or testing efficacy of analgesics used in managing postoperative pain associated with ovariohysterectomy in dogs. Systematic searches in three databases namely Pub Med, Google Scholar and Science Direct were conducted for peer reviewed articles written in English and published between 1995 and 2015. The literature search was designed to retrieve all articles using dogs, ovariohysterectomy, pain and analgesics as the key search words. This was followed by a manual search of the references within the primary data sources to get more articles that might not have been picked during searches in the three databases.

3.2.2 Inclusion and exclusion of studies

All studies published from 1995 to 2015, written in English and assessing the effectiveness of analgesics in managing pain associated with ovariohysterectomy in dogs, were included. Studies with controlled or uncontrolled trials were included as long as the study designs were randomized. Clinical as well as experimental studies that assessed the effects of analgesics after ovariohysterectomy in dogs were included. Only complete papers were included for review. Where only abstracts were available, full papers were obtained directly from the corresponding authors through the availed email contacts. The systematic procedure followed to include and exclude articles is illustrated in Figure 3-1.

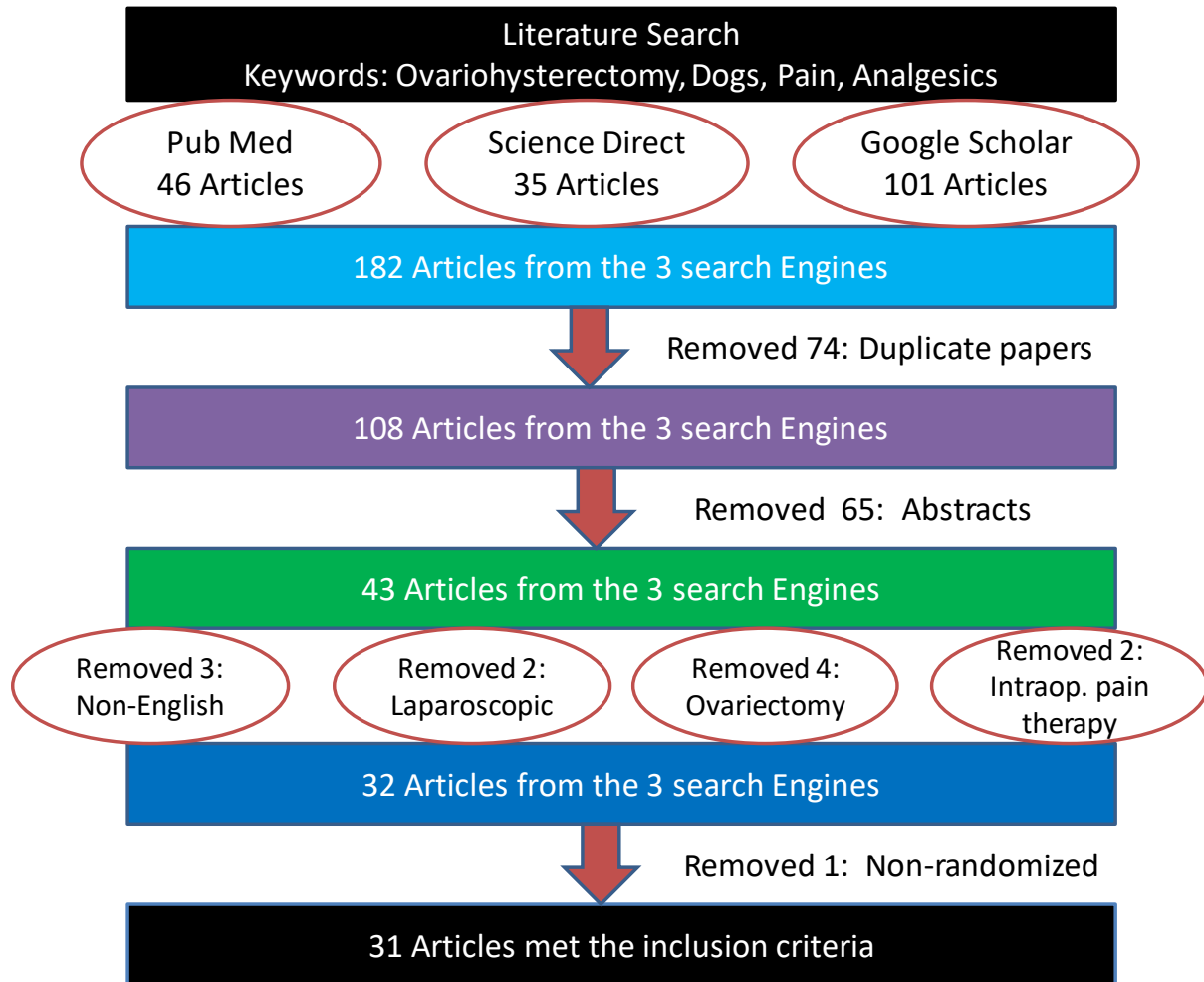


Figure 3-1: Flow chart illustrating the systematic criteria used to exclude and include articles in this study

3.2.3 Data extraction and synthesis

The articles that met the inclusion criteria were read in full, and data were extracted systematically in a predefined, standardized manner. Extracted data included: the author, year of publication, study design, objectives of the study, analgesic drugs used, technique of drug administration (multimodal versus single drug therapy, preoperative versus postoperative administration, course of drug administration, epidural versus systemic administration) and need for rescue analgesia. Quantitative data synthesis was carried out on homogenous data. Homogeneity was achieved by grouping the data into categories based on several characteristics, which included: overall goal of the study (analgesic efficacy, comparison of analgesia, timing effect, route effect and dose effect), total number of dogs used, number of dogs per group, type of analgesic groups / therapies (NSAIDs, Opioids, NSAIDs-opioids, local analgesics, acupuncture), analgesic protocols (individual or multimodal), timing of analgesic administration (preoperative, postoperative or preoperative plus postoperative), and course of analgesic therapy (once, 24 hours, 48 hours, 72 hours). The number of dogs that required rescue analgesia in each study (where available) was recorded and further comparisons carried out between different homogenous categories described above. The aim of these comparisons was to demonstrate the relative analgesic strength between the different types of drugs and their techniques of administration. The inclusion of an article into the study was performed independently by two reviewers. Any arising disagreements between the two reviewers were resolved through a discussion leading to a consensus.

3.3 Results

3.3.1 Number of studies

A total of 31 studies met the inclusion criteria for the systematic review (Table 3-1). The year with the highest number of studies that met the inclusion criteria was 2012 (22.6%) followed by 2011 (16.1%) and 2003 (9.7%). The distribution of studies as per their year of publication is illustrated in Figure 3-2.

3.3.2 Overall goals of the studies

Studies were carried out to compare various effects of analgesics after ovariohysterectomy. A total of 58.1% (n=18) of the studies compared analgesia between different pain medications, 16.2% (n=5) evaluated efficacies of different drugs, 12.9% (n=4) compared effects of various doses, 9.7% (n=3) compared the effects of route of drug administration and 3.2% (n=1) compared the effects of timing (pre-operative or post-operative) of drug administration.

3.3.3 Total number of dogs used in the studies

A total of 888 dogs were used in all the 31 studies that met the inclusion criteria. The mean number of dogs that were used per study was 28.7 ± 14.7 with the smallest number of dogs per study being 12 and the highest number being 80. The mean number of dogs per group was 10.8 ± 4.3 with the smallest number per group being 4 and the highest number being 20 dogs.

Table 3-1: A summary of the studies that met the inclusion criteria, their objectives and outcome

Author	Year of publication	Objective	Pain therapies	Dosage	Time of dosing	Course of admin.	Rescue analgesia	Outcome
Ashraf and Abu-Seida	2012	Evaluate efficacy	Diclofenac Cefotaxime Diclofenac + Cefotaxime	1.1 mg/kg 10 mg/kg 1.1 and 10 mg/kg	Postop	Once	No	Combination had similar analgesia to diclofenac alone but better analgesia compared to cefotaxime
Buhari <i>et al.</i> ,	2012	Evaluate efficacy	Tramadol IV Tramadol SQ	3mg/kg 3mg/kg	Preop	Once	No	In IV analgesia is faster but of similar efficacy compared to SQ
Camargo <i>et al.</i> ,	2011	Compare analgesia	Firocoxib Butorphanol	5mg/kg 0.2 mg/kg	Preop	Once	Yes	Firocoxib has superior analgesia than butorphanol
Campagnol <i>et al.</i> ,	2012	Compare analgesia	Incisional bupivacaine Intraperitoneal bupivacaine	1 mg/kg 5 mg/kg	Preop	Once	Yes	Intraperitoneal bupivacaine more effective than incisional bupivacaine
Carpenter <i>et al.</i> ,	2004	Compare analgesia	Bupivacaine Lidocaine	4.4 mg/kg 8.8 mg/kg	Postop	Once	Yes	Intraperitoneal / incisional bupivacaine provide better analgesia than intraperitoneal/incisional lidocaine
Cassu <i>et al.</i> ,	2012	Compare analgesia	Electroanalgesia of Acupoint EA Pre-incisional dermatome Their combination		Preop	Once	Yes	Acupoint EA and Combination have better analgesia than dermatome
Caulkett <i>et al.</i> ,	2003	Compare analgesia	Meloxicam Butorphanol	0.2 mg/kg 0.2 mg/kg	Preop	Once	Yes	Meloxicam has better analgesia than butorphanol
Dzikiti <i>et al.</i> ,	2006	Compare analgesia	Morphine Carprofen Morphine-caprofen	0.4 mg/kg 4 mg/kg 0.4 and 4 mg/kg	Preop	24 hours	Yes	Morphine, carprofen and morphine-caprofen combination have similar analgesia
Frazilio <i>et al.</i> ,	2014	Compare analgesia	Nalbuphine Nalbuphine	0.3 mg/kg 0.6 mg/kg	Preop	Once	Yes	Nalbuphine at 0.6 mg/kg provides superior and longer analgesia than at 0.3 mg/kg
Imagawa <i>et al.</i> ,	2011	Compare analgesia	Dipyron	Varying dosage 15, 25 and 35 mg/kg	Postop	48 hours	Yes	Dipyron 25 mg/kg and 35 mg/kg have similar analgesic efficacy better than achieved at 15mg/kg
Kongara <i>et al.</i> ,	2012	Compare analgesia	Morphine Tramadol Morphine-tramadol	0.5 mg/kg 3 mg/kg 0.1 and 3 mg/kg	Preop and Postop	Once	Yes	Analgesia produced by individual drugs is similar but the combination provides superior analgesia
Lascelles <i>et al.</i> ,	1998	Compare analgesia	Preoperative carprofen Postoperative carprofen	4 mg/kg 4 mg/kg	Preop and Postop	Once	No	Preoperative carprofen has better analgesia than postoperative carprofen
Leece <i>et al.</i> ,	2005	Compare analgesia	Carprofen Meloxicam	4 mg/kg SQ then 2 mg/kg oral 0.2 mg/kg SQ then 0.1 mg/kg oral	Preop and Postop	72 hours	Yes	Both drugs have satisfactory analgesia but meloxicam provides analgesia of longer duration than carprofen
Lemke <i>et al.</i> ,	2002	Evaluate efficacy	Ketoprofen	2 mg/kg	Preop	Once	Yes	Ketoprofen reduces pain postoperatively

Author	Year of publication	Objective	Pain therapies	Dosage	Time of dosing	Course of admin.	Rescue analgesia	Outcome
Mastrocinque and Fantoni,	2003	Compare analgesia	Morphine Tramadol	0.2 mg/kg 2 mg/kg	Preop	Once	Yes	Morphine and tramadol provide similar analgesia
Nunamarker <i>et al.</i> ,	2014	Compare analgesia	Buprenorphine single release Buprenorphine	0.2 mg/kg 0.02 mg/kg	Postop	72 hours	Yes	Both dosages provide similar analgesia with comparable side effects
Neves <i>et al.</i> ,	2012	Compare analgesia	Tramadol Morphine	2 mg/kg 0.1 mg/kg	Preop	Once	Yes	Extradural tramadol and morphine provide similar analgesia
Pekcan and Koc	2010	Compare analgesia	Transdermal fentanyl patch Epidural morphine	50 or 75 ug/hr 0.1 mg/kg	Preop	Once	Yes	Epidural morphine provides better analgesia than transdermal fentanyl
Rioja <i>et al.</i> ,	2012	Evaluate efficacy	Magnesium sulphate	50 mg/kg	Preop	Once	Yes	Magnesium sulphate failed to show any significant analgesic effects
Saritas <i>et al.</i> ,	2015	Evaluate efficacy	Dexketoprofen	1.0 mg/kg	Preop	Once	No	Dexketoprofen provides adequate analgesia
Salmi <i>et al.</i> ,	2009	Compare analgesia	Vedaprofen Ketoprofen Carprofen	0.5 mg/kg 2.2 mg/kg 2.2 mg/kg	Preop	Once	Yes	Vedaprofen provides similar analgesia to carprofen and ketoprofen
Shafford <i>et al.</i> ,	2002	Compare analgesia	PEMF Morphine PEMF + morphine	0.5 HZ q 20min 0.25 mg/kg 0.5 HZ q 20min and 0.25mg/kg	Postop	Once	Yes	PEMF therapy provides adequate analgesia as does morphine and the combination
Shih <i>et al.</i> ,	2008	Compare analgesia	Buprenorphine Carprofen Buprenorphine + carprofen	0.02 mg/kg 4 mg/kg 0.02 and 4 mg/kg	Preop	Once	Yes	Carprofen and the combination provide superior analgesis to that of buprenorphine; the combination has no added benefit
Singh <i>et al.</i> ,	2003	Compare analgesia	Preoperative pentazocine Postoperative pentazocine	2mg/kg 2mg/kg	Preop and Postop	24 hours	No	Pentazocine administered preoperatively has better analgesia than when given postoperatively
Slingsby <i>et al.</i> ,	2006	Compare analgesia	Varying dosages of sulfentanil Carprofen	10, 15, 25 µg/kg 4 mg/kg	Preop	Once	Yes	Sulfentanil provides better analgesia compared to carprofen; furthermore, it produces low pain score with increasing dosage
Slingsby <i>et al.</i> ,	2011	Compare analgesia	Buprenorphine Buprenorphine	20 µg/kg 40 µg/kg	Preop	Once	Yes	Both dosages provide adequate analgesia but no significant advantage on higher dosages
Stanescu <i>et al.</i> ,	2011	Compare analgesia	Robenacoxib Tramadol	2 mg/kg 2 mg/kg	Postop	72 hours	Yes	Tramadol provides longer analgesia than robenacoxib
Tavakoli <i>et al.</i> ,	2009	Evaluate efficacy	Metoclopramide	0.5 mg/kg	Preop	Once	No	Metoclopramide is effective in reducing postoperative pain
Thengchaisri <i>et al.</i> ,	2010	Compare analgesia	Carprofen Vedaprofen Tepoxalin	4.4 mg/kg 0.5 mg/kg 20 mg/kg	Postop	72 hours	No	Carprofen and tepoxalin provide better analgesia compared to vedaprofen

Author	Year of publication	Objective	Pain therapies	Dosage	Time of dosing	Course of admin.	Rescue analgesia	Outcome
Tsai <i>et al.</i> ,	2013	Compare analgesia	Meloxicam Lidocaine Meloxicam-lidocaine	0.2 mg/kg 1.0 mg/kg IV bolus then 0.025 mg/kg/hr CRI 0.2 mg/kg - 1.0 iv bolus then 0.025 mg/kg/hr CRI	Preop	Once	Yes	Lidocaine provides comparable analgesia with meloxicam; the combination has no additive advantage
Vettorato and Bacco,	2011	Compare analgesia	Pethidine Butorphanol	5 mg/kg 0.4 mg/kg	Preop	Once	Yes	Pethidine and butorphanol provide similar analgesia

KEY: PEMF= Pulse electromagnetic field; Preop=Preoperative; Postop= Postoperative; Admin= Administration; CRI= Constant rate

infusion; IV= Intravenous; SQ= Subcutaneous; EA= Symbol of a specific acupoint in dogs

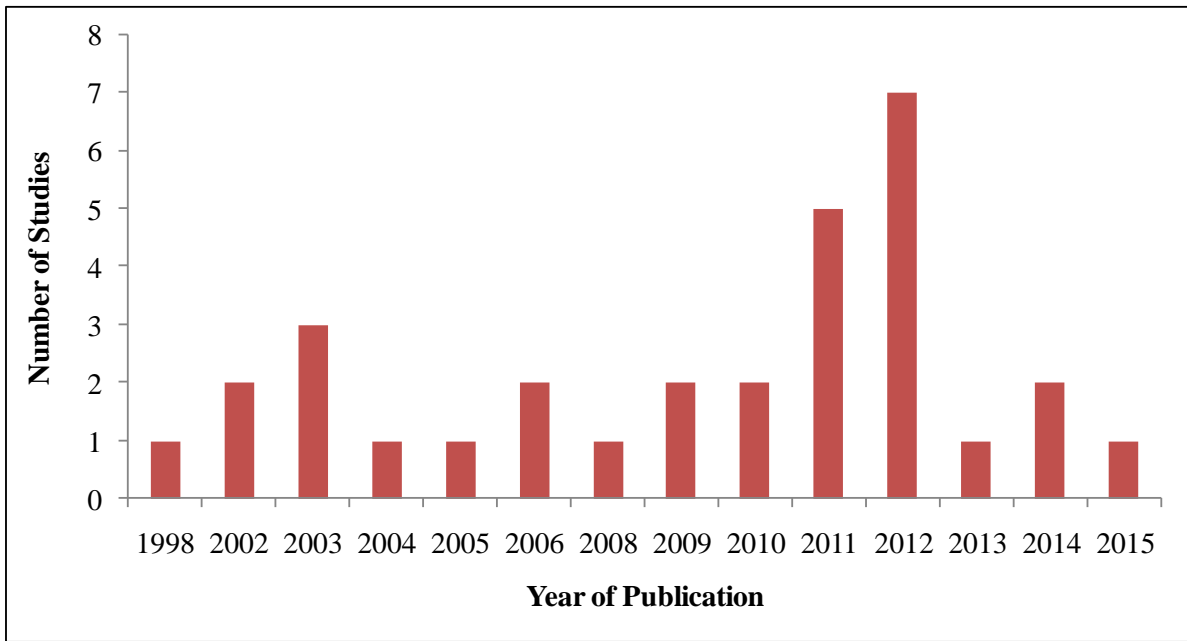


Figure 3-2: Distribution of studies that met the inclusion criteria of this systematic review based on their year of publication.

3.3.4 Pain management practices

3.3.4.1 Analgesia protocols

Individual analgesic protocols were used in 83.9% (n=26) of these studies for managing pain in dogs after ovariohysterectomy, compared to 16.1% (n=5) of the studies that utilized multimodal drug therapy.

3.3.4.2 Categories of analgesia drugs and techniques

Out of the 31 studies that met the inclusion criteria, opioids were used in 38.7% of the studies, NSAIDs in (19.4%), the combination of NSAIDs and Opioids in 19.4% and local analgesic in 6.5% of the studies. The remaining therapies were used in equal measure of 3.2% of the studies as shown in Table 3-2.

3.3.4.3 Timing of analgesic administration

The most preferred time for administration of analgesics was prior to surgery (preoperative), which was practiced in 64.5% (n=20) of the studies, followed by postoperative analgesia in 22.6% (n=7). In 12.9% (n=4) of the studies, analgesics were administered first preoperatively and then postoperatively (Figure 3-3). Furthermore, NSAIDs were administered mainly in the postoperative period (50%) while opioids (75%) and the NSAIDs-Opioid drug combinations (66.7%) were mainly administered prior to surgery (preoperatively) as shown in Table 3-3.

Table 3-2: Categories of analgesic drugs and techniques used in dogs undergoing ovariohysterectomy in this systematic review

Category of analgesic	Number of studies	Percentage of the number of studies (%)
Opioid	12	38.7
NSAID	6	19.4
NSAID and Opioid	6	19.4
Local analgesic	2	6.5
Acupuncture	1	3.2
Acupuncture and Opioid	1	3.2
Antiemetic	1	3.2
NMDA antagonist	1	3.2
NSAID and Local analgesic	1	3.2
Total	31	100

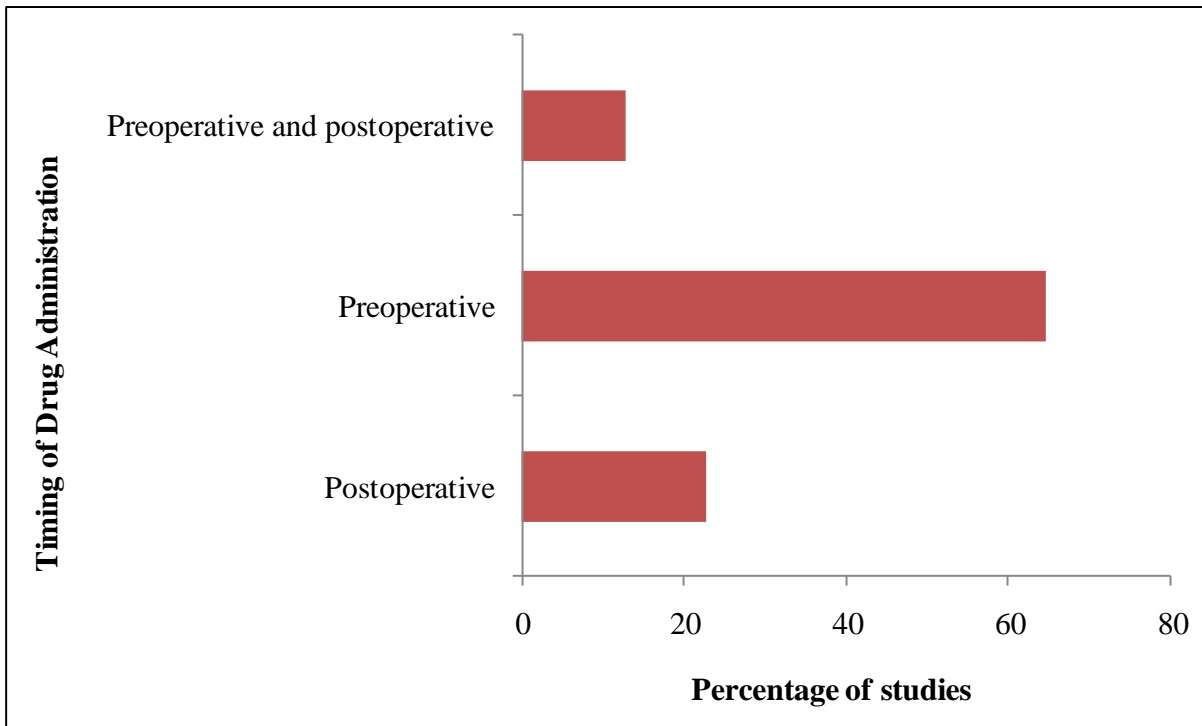


Figure 3-3: Timing of analgesic drug administration in dogs undergoing ovariohysterectomy in this systematic review

Table 3-3: Timing for analgesic drug administration according to their categories in dogs undergoing ovariohysterectomy in this systematic review

Timing of administration	Category of analgesics and percentage (%) of studies in which they were used		
	NSAID	NSAID and Opioid	Opioid
Postoperative	50.0	16.7	8.3
Preoperative	33.3	66.7	75.0
Preoperative and postoperative	16.7	16.7	16.7
Total	100.0	100.0	100.0

3.3.4.4 Postoperative course of analgesic administration

Administration of analgesics only once postoperatively, was the most common practice as reported in 77.4% (n=24) of the studies, while a 72-hour postoperative course of analgesics was reported in 12.9% (n=4) of the studies. Twenty-four hour and 48-hour courses of postoperative analgesic administration were reported in 6.5% (n=2) and 3.2% (n=1) of the studies, respectively, as show in Figure 3-4.

3.3.5 Requirement for rescue analgesia

Not all the studies assessed the need for rescue analgesia. However, a total of 713 dogs were used in the studies that assessed this parameter. Rescue analgesia was required in 25.5% (n=182) of these dogs.

3.3.5.1 Comparison of the adequacy of analgesia between the drug protocols

More dogs in control groups required rescue analgesia postoperatively (57.3%) compared to dogs under pain therapy. The likelihood that a dog under single analgesic drug therapy (21.6%) would require rescue analgesia was twice as high as for a dog under multimodal analgesic drug therapy (11.3%) as shown in Table 3-4 below.

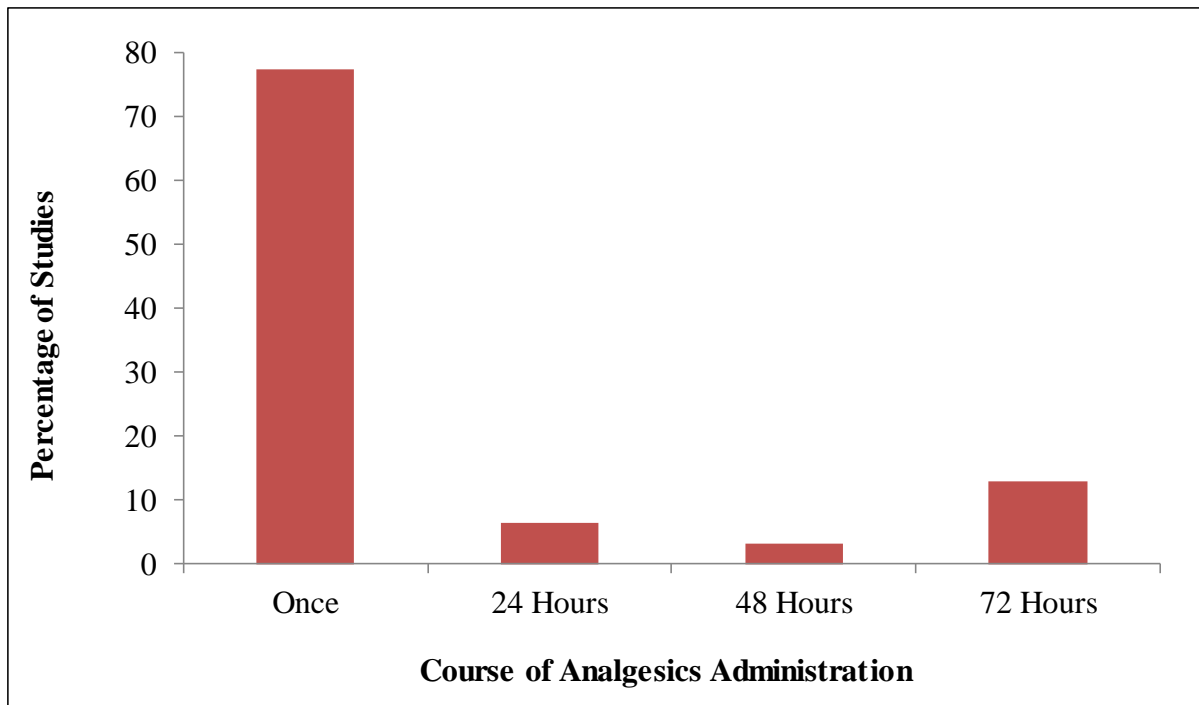


Figure 3-4: Course of analgesic administration for pain management in dogs undergoing ovariohysterectomy in this systematic review

Table 3-4: The need for rescue analgesia in dogs under control, individual and multimodal therapies in this systematic review

Category of drug therapy	Total number of dogs in each protocol	Number of dogs requiring analgesia in each protocol	% of dogs requiring rescue analgesia in each protocol
Multimodal therapy	62	7	11.3
Individual drug therapy	555	120	21.6
Control group	96	55	57.3
Total	713	182	25.5

3.3.5.2 Comparison between the categories of analgesics

The requirement for rescue analgesia was highest in dogs treated using acupuncture (43.8% of the dogs) and lowest in dogs treated using NSAID-Opioids (8.6% of the dogs) (Table 3-5). Rescue analgesia was required in 9.3% of dogs treated using NSAIDs, 26.1% of dogs treated using opioids and 28.6% of those under local analgesics.

3.3.5.3 Comparison between the times of drug administration

The percentage of dogs requiring rescue analgesia was lowest (19.2%) in categories of dogs that received pain medication both before and after surgery as compared to those that were given pain medication only prior to surgery (preoperatively) or only postoperatively. The highest percentage of dogs requiring rescue analgesia (21,0%) was witnessed in the category of dogs that was given analgesics only prior to surgery, followed by 19.6% of those dogs in the category that received analgesics only postoperatively (Figure 3-5).

3.3.5.4 Comparison between the courses of drug administration

Only two groups (One-off and 72-hours course of administration) were considered in the analysis of the requirement for rescue analgesia based on course of drug administration. This was due to low numbers of studies in the other categories (24-hour and 48-hour courses of drug administration) and the fact that these two groups represented both extremes (short duration in one-off and long duration in 72-hour). More dogs (26.4%) in the category that was given pain medication only once postoperatively required rescue analgesia as compared to 4.4% of dogs given analgesics over the course of a 72-hour period (Table 3-6).

Table 3-5: The requirement for rescue analgesia among the analgesic categories in this systematic review

Analgesic Category	Total number of dogs	Number of dogs requiring rescue analgesia	% of dogs requiring rescue analgesia
NSAID-Opioids	35	3	8.6
NSAIDs	162	15	9.3
Opioids	318	83	26.1
Local analgesic	49	14	28.6
Acupuncture	16	7	43.8

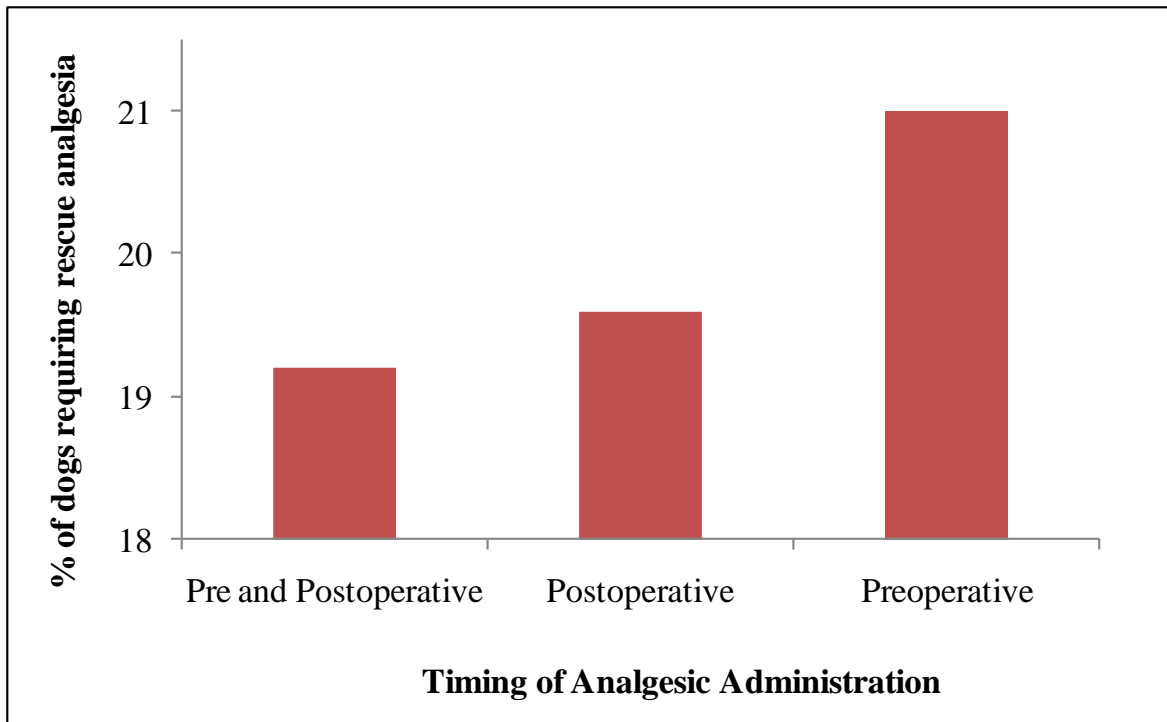


Figure 3-5: Requirement for rescue analgesia based on timing of analgesic drug administration in dogs undergoing ovariohysterectomy in this systematic review

Table 3-6: Requirement for rescue analgesia between different courses of drug administration in dogs undergoing ovariohysterectomy in this systematic review

Course of administration	Total number of dogs	Number of dogs requiring rescue analgesia	% of dogs requiring rescue analgesia
One-off	492	130	26.4
72 hours	68	3	4.4
Total	560	133	23.8

3.4 Discussion

This review indicates a general increasing trend in the number of studies focusing on postoperative pain after spay in dogs, over a 20-year period. This observation suggests that veterinarians are becoming more aware of pain and its deleterious effects in surgical patients and are further exploring better therapies which can minimize pain and hence optimize surgical outcomes. This theory is further supported by the high number of studies that sought to gain a deeper understanding of comparative analgesic efficacy of the various drugs and techniques that can optimize analgesia provided by these agents. These techniques include individual drugs, drug combinations as well as varying the dosages, route and timing of drug administration.

Opioids were the most commonly used analgesics followed by NSAIDs and NSAIDs-Opioids drug combinations. Opioids are affordable and relatively available for use in developed countries where more than 90% of the reviewed studies were conducted and this could explain in part, their widespread use in managing pain in dogs as compared to NSAIDs and other analgesics. Further observations indicated that opioids were mostly administered preoperatively and NSAIDs mostly postoperatively. Opioids are known to have good analgesia and sedative effects (Gaynor and Muir, 2002) and this could explain their widespread use for premedication. Additionally, opioids are known to cause adverse effects postoperatively compared to NSAIDs, therefore limiting their use in the postoperative period. The fact that NSAIDs have longer duration of analgesia and with no sedative effects compared to opioids (Gaynor and Muir, 2002) could have influenced their extensive use postoperatively. However, since NSAIDs are known to minimize production of prostaglandins caused by trauma like in surgery (Mathews, 1996), their use preoperatively would arguably result in better pain management as compared to when administered postoperatively. Although opioids are widely

used for postoperative pain management in dogs, their administration is restricted to the period the animal is hospitalized (Murrell and Flaherty, 2014) due to their associated side effects. This limits their use in multimodal analgesia protocols in the home environment and may lead to inadequate analgesia and consequent 'break-through' pain (Murrell and Flaherty, 2014).

This study revealed that individual drug therapy was the more frequently used technique for pain management than multimodal therapy. This is probably a reflection of inadequate information available on the latter, particularly on the drugs that can be used together, the dosages, analgesic efficacy and associated side effects. This limits veterinarians to the use of individual drugs until sufficient reliable information on multimodal drug therapy is available to them.

Results from this systematic review, show that NSAIDs had better pain relieving ability than opioids, as indicated by the number of dogs requiring rescue analgesia. A similar observation has also been made in another systematic review conducted to assess the efficacy of NSAIDs and opioids in treatment of acute renal colic in humans (Holdgate and Pollock, 2004). This finding is attributed to the fact that opioids act indirectly on the cause of pain through the opioids receptors (Reich and Hanno, 1997), while NSAIDs act directly on prostaglandin release, which is the main intermediary of pain in surgery and most pathological processes in the body (Mathews, 1996).

Local analgesics were also used in managing pain in dogs undergoing ovariohysterectomy and these included bupivacaine (Campagnol *et al.*, 2012) and lidocaine (Carpenter *et al.*, 2004; Tsai *et al.*, 2013). Interestingly, the requirement for rescue analgesia in dogs given local analgesics was almost the same as that for dogs treated using opioids. This observation can be attributed

to the fact that local analgesics were mostly administered directly at the site of nociceptor stimulation either at the skin incision (incisional) and/or at the ovarian stamps (intraperitoneal) (Carpenter *et al.*, 2004; Campagnol *et al.*, 2012) as compared to opioids that were administered systemically. Considering the cost, availability, restrictions and the side effects associated with opioids compared to local analgesics, this observation is encouraging and could stir interest, leading to possible widespread use of this technique in dogs undergoing ovariohysterectomy.

Almost half of the dogs treated using acupuncture required rescue analgesia postoperatively. In addition, variations in the outcome of analgesia treatment were observed when different acupoints or acupuncture techniques were used. For example, a study by Cassu *et al.*, (2012) demonstrated that dogs treated by electrical stimulation of acupoint EA had lower pain scores compared to dogs treated at pre-incisional dermatomes. Based on these available studies, it can be inferred that use of acupuncture for postoperative pain management, especially following ovariohysterectomy in dogs, produces variable outcomes and therefore is not as reliable as the use of proven therapies like NSAIDs, opioids and local analgesics.

More dogs in the control group required rescue analgesia more than those in which pain therapy was instituted. This observation confirms the fact that ovariohysterectomy in dogs is associated with postoperative pain, which has previously been described as acute and moderate (Gaynor and Muir, 2002). For this reason, any dog undergoing this surgical procedure must receive pain medication at least for 24 hours postoperatively so as to overcome deleterious physiological effects associated with pain and for humane reasons. Such deleterious effects include increased post-operative stress, immunosuppression, increased arterial blood pressure, delayed wound healing, negative protein balance, decreased food intake and development of maladaptive behaviors such as self-mutilation (Gwendolyn and Carrol, 1996; Gaynor, 1999). The use of

analgesics in the preoperative period in form of opioids and alpha-2 adrenergic agonists could be the reason why only 57% and not all the dogs in the control groups required rescue analgesia.

Almost double the number of dogs that were treated using individual drug therapy required rescue analgesia compared to those treated using multimodal drug therapy. Studies have shown that irrespective of the dose used, a single class of analgesic drugs cannot provide complete analgesia owing to the complex nature of pain transmission, which involves multiple pathways, mechanisms and transmitter systems (Lascelles, 1999). Multimodal drug therapy confers the advantages of using small doses of individual drugs but most importantly additive analgesia (Lemke *et al.*, 2002; White *et al.*, 2007). This improves patient comfort and minimizes the need for high doses or prolonged use of any one particular drug (Epstein, 2011) hence minimizing the likelihood of undesirable side effects. Furthermore, the widespread over-reliance on one class of drugs, as is the case with non-steroidal anti-inflammatory drugs (NSAIDs) is likely to not only under-manage some or perhaps many patients for their pain, but could increase the possibility of side effects associated with such drugs (Epstein, 2011).

Administering drugs both before and after surgery was a technique associated with better outcomes compared to giving drugs either only prior to surgery or only after surgery. Several studies exist both in human and veterinary anaesthesiology which demonstrate the beneficial effects of administering analgesics prior to surgery. For instance, carprofen administered preoperatively was shown to be more effective than when administered postoperatively in dogs undergoing ovariohysterectomy (Lascelles *et al.*, 1998). This beneficial effect can be attributed to: (1) higher plasma levels of the drug at the time of surgery, when given preoperatively; (2) higher levels of the drug in tissue fluid/inflammatory exudates when administered before surgery; (3) a positive preoperative effect in terms of either decreasing the amount of noxious

information generated at the periphery which decreases any central changes or blocking the entry of the noxious information into the spinal cord; (4) high tissue levels of drug before the surgery which promotes a more effective action against local inflammation (Lascelles *et al.*, 1998). Results from this study show that administering drugs postoperatively only, had better outcomes compared to preoperative administration. This may be attributed to the fact that since pain was assessed serially after surgery, the plasma concentration of drugs administered postoperatively was higher compared to the plasma concentration of those administered before surgery, resulting in the observed lower pain scores. Administering drugs both before and after surgery is then an innovative and effective way of managing pain as confirmed by the findings in this study. This technique utilizes the beneficial effects of each of the techniques (preoperative and postoperative) resulting into better outcomes.

The need for rescue analgesia was very low in dogs that were given analgesics for 3 days compared to those that were given a one-off pain medication, postoperatively. This observation might suggest that pain which occurs following ovariohysterectomy may be moderate but can last for several days. It could therefore make sense to administer analgesics more than once postoperatively, and if the dog is to be discharged immediately after recovery from anaesthesia, as is often common after ovariohysterectomy, then pain medications should be dispensed for the client to administer for a prescribed period, and taking into account drug use regulatory environment in each jurisdiction.

3.5 Conclusions

The following conclusions can be drawn from this study:

3.5.1 Opioids are the mainstream analgesics that are used to manage pain in dogs undergoing ovariohysterectomy and that one-time drug administration, preoperative administration of analgesics and individual drug therapy are the commonly used techniques.

3.5.2 NSAIDs are more effective in managing postoperative pain in dogs undergoing ovariohysterectomy.

3.5.3 Multimodal drug therapies, administration of analgesics before and after surgery, as well as a 72-hour course of pain therapy are the practices that provide better outcomes in managing acute postoperative pain in dogs following ovariohysterectomy.

CHAPTER FOUR

4.0 EFFECTS OF BUTORPHANOL, MELOXICAM AND BUTORPHANOL-MELOXICAM COMBINATION ON POST-OPERATIVE PAIN AFTER OVARIOHYSTERECTOMY IN DOGS

4.1 Introduction

Clinically, pain can be defined as “an aversive feeling or sensation associated with actual or potential tissue damage that may result in physiologic, neuroendocrine, and behavioral changes indicating stress response” (Merskey and Bogduk, 1994). Untreated or undermanaged pain is associated with deleterious physiological effects that interfere with patient recovery (Hansen, 2005).

Ovariohysterectomy is a routine surgical procedure in small animal practice. This surgery causes marked post-operative pain in dogs (Gaynor and Muir, 2002). Since the procedure is performed routinely on healthy and pain-free animals, the efficacy of test drugs can be reliably assessed, assuming that all postoperative pain resulted from surgery only (Slingsby *et al.*, 2006). Studies in Kenya have shown that ovariohysterectomy is the most common procedure carried out in dogs. However, only a small percentage of veterinarians administer analgesics postoperatively and when they do, it is done only once and dogs discharged without any further pain medication (Mwangi, 2013).

Immediate and appropriate assessment of post-operative pain aids in optimal pain control and evaluation of analgesic efficacy (Lascelles *et al.*, 1994). However, recognition of pain in animals is a challenge (Anil *et al.*, 2002) as it relies on the interpretation of animal behavior by an observer since there is no effective means of communication (Murrell *et al.*, 2008). The

challenge of pain recognition in animals is further compounded by lack of validated methods of assessing clinical pain in veterinary patients (Anil *et al.*, 2002). Whereas there are a number of methods of assessing pain in animals, the one that is validated and is used in assessment of acute pain in a clinical setting is the Glasgow composite measure pain scale (CMPS-SF) (Reid *et al.*, 2007). The CMPS-SF involves use of a structured questionnaire completed by an observer while following a standard protocol, which includes the assessment of spontaneous and evoked behaviors, interactions with the animal and clinical observations (Murrell *et al.*, 2008).

There is need for comprehensive management of pain in the peri-operative period through use of a wide range of therapies and techniques (Egger and Love, 2009; Epstein, 2011). Different drugs and techniques have been used in management of pain following ovariohysterectomy in dogs. Nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are the most popular analgesics in dogs and are either administered pre-operatively, postoperatively and/or through multimodal therapy. There are reports in literature on the use of a number of NSAIDs for pain management in dogs, which includes use of meloxicam (Caulkett *et al.*, 2003; Leece *et al.*, 2005), carprofen (Dzikiti *et al.*, 2006), dipyrone (Imagawa *et al.*, 2011), ketorolac and flunixin (Karol *et al.*, 1996). Meloxicam is one of the cyclooxygenase type 2 (COX-2) selective NSAIDs (Churchill *et al.*, 1996) that inhibits many of the inflammatory functions of arachidonic acid, but selectively spares the housekeeping functions of prostaglandins and thromboxanes (Engelhardt *et al.*, 1996). Consequently, meloxicam provides prolonged superior analgesia while minimizing the undesirable side effects associated with NSAIDs (Caulkett *et al.*, 2003; Leece *et al.*, 2005).

Opioids continue to have a strong place in perioperative pain management in veterinary patients. The opioids widely used in pain management in dogs include: butorphanol (Karol *et al.*, 1996; Caulkett *et al.*, 2003; Larisa *et al.*, 2009), morphine (Kongara *et al.*, 2012), tramadol (Fajardo *et al.*, 2012), pethidine (Slingsby and Waterman-Pearson, 2001); fentanyl (Pekcan and Koc, 2010), and buprenorphine (Slingsby *et al.*, 2011). Butorphanol tartrate ([17-(cyclobutylmethyl) morphinan-3,14-diol D-tartrate]) is a synthetic opioid receptor partial agonist-antagonist with good analgesic properties in dogs (Pfeffer *et al.*, 1980). A dosage of 0.2 mg/kg administered either intramuscularly or subcutaneously has been found to be effective in managing perioperative pain in dogs (Karol *et al.*, 1996; Caulkett *et al.*, 2003; Larisa *et al.*, 2009).

Studies comparing the analgesic effects of meloxicam and butorphanol in dogs following ovariohysterectomy have demonstrated that meloxicam produces analgesia of superior quality and prolonged duration compared to butorphanol (Mathewset *et al.*, 2001; Caulkett *et al.*, 2003). Furthermore, it is clear that no single class of analgesic drugs can provide complete analgesia irrespective of the dose used owing to the complex nature of pain transmission (Lascelles, 1999). Multimodal drug therapy therefore, offers an alternative and effective technique for treating pain in dogs postoperatively. Studies have reported on the use of different opioid-NSAID drug combinations in pain management in dogs. These include: morphine+carprofen (Dzikiti *et al.*, 2006) and pethidine+carprofen (Slingsby and Waterman-Pearson, 2001). There is however no report on the analgesic effects of meloxicam-butorphanol combination in dogs. The aim of this study was therefore to determine the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on post-operative pain as well as physiological parameters following ovariohysterectomy in dogs.

4.2 Materials and methods

4.2.1 Study design

This was a prospective randomized controlled study in which dogs were subjected to ovariohysterectomy. The treatments involved postoperative administration of butorphanol, meloxicam, butorphanol-meloxicam combination and a placebo. Monitoring and evaluation of various parameters was done following ovariohysterectomy and administration of analgesics/placebo.

4.2.2 The study animals

Forty-eight entire female dogs were used in the study. The dogs were acquired from clients who presented them to the University of Nairobi, Small Animal Clinic for ovariohysterectomy and were willing to have the dogs included in the study. Once acquired, the dogs were subjected to routine clinical examination to screen them for presence of any diseases. Only dogs free of diseases were selected for the study. They were dewormed (Vermic® Total, Microsules Laboratories, Uruguay), treated for ectoparasites (Frontline Plus®, Merial, Duluth-Georgia USA) and allowed 14 days to acclimatize to the new environment. During this period, dogs were subjected to weekly clinical examination and regular handling to make them get acquainted with handling and manipulation. The dogs that never accepted easy handling after the acclimatization period were excluded from the study, but were spayed and released to the owners. All the dogs excluded from the study were replaced by recruiting others that were easy to handle.

The dogs were housed individually in kennels at the Department of Clinical Studies and fed on commercial dog feed once per day but water was provided *ad libitum*. The 48 dogs were randomly assigned to 4 treatment groups of 12 dogs each. The groups were randomly generated

via computer random number table and designated as B, M, BM and C. The 4 treatment groups are described in sub-section 4.2.4 below.

4.2.3 Experimental drugs and dosages

The following analgesics were used in this study at the specified dosages:

- a. Butorphanol hydrochloride (Turbusegic®- SA, Zoetis, New Jersey- USA) (0.2 mg/kg BW) was administered subcutaneously as the test opioid analgesic drug.
- b. Meloxicam hydrochloride (Mobic®, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA) (0.2 mg/kg BW) was administered subcutaneously as the test NSAID analgesic drug.
- c. Butorphanol hydrochloride and Meloxicam hydrochloride (0.1 mg/kg and 0.1 mg/kg, respectively) were administered subcutaneously as the test opioid-NSAID drug combination.

In addition, the following drugs were used to facilitate ovariohysterectomy:

- a. Acepromazine hydrochloride 2 % (Labistress® Labiana Life Sciences SA, Barcelona-Spain) (0.1 mg/kg BW) administered intramuscularly for sedation.
- b. Propofol 1% (Propofol-Lipuro® 10mg/ml B-Braun, Melsungen-Germany) (5 mg/kg BW) administered intravenously for induction of anaesthesia.
- c. Isoflurane (Forane® Isofluranum, Abbott Laboratires Ltd, Queenborough, Kent England) inhalant anaesthetic for maintenance of anaesthesia during surgery.

4.2.4 The treatments

Treatment 1:

Dogs in group B received butorphanol hydrochloride at 0.2 mg/kg BW, injected subcutaneously.

Treatment 2:

Dogs in group M received meloxicam hydrochloride at 0.2 mg/kg BW, injected subcutaneously.

Treatment 3:

Dogs in group BM received butorphanol-meloxicam drug combination at half the dosage of each individual drug (i.e butorphanol hydrochloride at 0.1 mg/kg BW and meloxicam hydrochloride at 0.1 mg/kg BW), injected subcutaneously.

Treatment 4:

Dogs in group C served as a control and received a placebo in form of sterile saline at a dose rate of 0.5 ml/10kg BW, injected subcutaneously.

4.2.5 Experimental procedure

Food and water were withheld from the dogs 12 hours prior to surgery as a routine pre-anaesthetic preparation. The dogs were weighed each time immediately preceding the experiments.

All dogs were sedated with acepromazine hydrochloride at 0.1mg/kg BW by intramuscular injection into the lateral thigh muscles. The ventral abdominal region was shaved, scrubbed and 70% ethyl alcohol applied on the site in preparation for aseptic surgery. Propofol at 5mg/kg BW was administered intravenously as a bolus for induction of anaesthesia. After induction, dogs were intubated for maintenance of anaesthesia with isoflurane vaporized in oxygen, using a rebreathing anaesthesia circuit.

After anaesthesia and preparation, each dog was positioned on a surgical table in dorsal recumbency. The operative site was draped and routine ovariohysterectomy performed. Warm Lactated Ringers solution was administered intravenously (10ml/kg/hour) to each dog throughout the period of anaesthesia until the endotracheal tube was removed. Immediately

after placing the last skin suture, the test analgesic drugs and placebo were administered as described in treatment sub-section 4.2.4 above. All the test analgesic drugs and placebo were administered subcutaneously on the dorsal part of the neck. The drug combination was injected as a mixture in the same syringe.

4.2.6 Disposal of sharps and biological waste

A sharps container dedicated to this project was available for disposal of sharps. Disposal of the sharps and biological waste was done as per the disposal procedures followed in the Small Animal Clinic, University of Nairobi, where this work was carried out.

4.2.7 Evaluation of parameters

Evaluation of parameters was done by the investigator who is a veterinarian experienced in the use of different pain scoring systems and interpretation of signs of pain in dogs. The assessor was blinded to the analgesic treatments given to dogs in each group.

4.2.7.1 Duration of anaesthesia and surgery

The duration of anaesthesia and duration of surgery were recorded. The duration of anaesthesia was defined as the time from administration of acepromazine hydrochloride to the time of extubation. The endotracheal tube was removed upon restoration of laryngeal reflex as indicated by coughing. Duration of surgery was defined as the time from when the skin incision was made to the time when the last skin suture was placed. Duration of anaesthesia and surgery were then compared between groups.

4.2.7.2 Assessment of postoperative pain and sedation

Pain was assessed based on changes in physiological parameters and animal behavior using short form Glasgow composite measure pain scale. In addition, sedation score for each dog was evaluated since this could have affected some behavioral signs of pain.

4.2.7.3 Changes in physiological parameters

Arterial blood pressure (ABP), heart rate (HR), respiratory rate (RR) and rectal temperature (RT) were assessed before sedation (baseline scores), at 1, 2, 4, 6, 12 and 24 hours after surgery. Arterial blood pressure (mm Hg) and heart rate (number of heart beats per minute) were measured using a physiological patient monitor. Respiratory rate (number of breaths per minute) was determined by visual observation of chest excursions. Rectal temperature (in degrees Celsius (°C)) was measured using a clinical digital thermometer inserted into the rectum and held against the mucosa for at least one minute.

4.2.7.4 Changes in animal behaviour

Prior to the experiment, each animal was assessed every other day for a period of two weeks so as to establish the individual animal's normal behavior. On the day of the experiment, behavioral assessment was done before premedication (baseline scores), at 1, 2, 4, 6, 12 and 24 hours after surgery and pain scores allocated to each dog. The short form Glasgow composite pain scale shown in Table 4-1 below, which was adopted from Murrell *et al.*, (2008), was used for assessment of pain.

Table 4-1: Short form of Glasgow composite scale used to assess postoperative pain in dogs undergoing ovariohysterectomy in this study

Observation	Score	Patient criteria
Comfort	0	Happy and content or happy and bouncy
	1	Quiet
	2	Indifferent or non-responsive to surroundings
	3	Nervous or anxious or fearful
	4	Depressed or non-responsive to stimulation
Vocalization	0	Quiet
	1	Crying or whimpering
	2	Groaning
	3	Screaming
Posture	0	Comfortable
	1	Unsettled
	2	Restless
	3	Hunched or tense
	4	Rigid
Attention to the surgical wound	0	Ignoring the wound
	1	Looking at the wound
	2	Licking the wound
	3	Rubbing the wound
	4	Chewing the wound
Response to touch (Applying gentle pressure 2 inches round the surgical site)	0	Does nothing
	1	Looks round
	2	Flinches
	3	Growls or guards' area
	4	Snaps
	5	Cries
Mobility Put lead on dog and lead out of the kennel. When the dog rises/walks is it?	0	Normal
	1	Lame
	2	Slow or reluctant
	3	Stiff
	4	It refuses to move

Note: The short form Glasgow composite pain scale was adopted from Murrell *et al.*, 2008

During the assessment, the investigator approached the kennel and assessed the dog's behavior and reactions. From outside the kennel, the dog's comfort, vocalisation, posture and attention to the surgical wound were assessed. The dog was then approached, addressed vocally, and the cage door opened. The physiological parameters (arterial blood pressure, heart rate, respiratory rate and rectal temperature) were determined following which the incision wound and surrounding area of the abdomen were palpated gently. A leash was then put on the dogs and they were then led out (if they accepted to move) of the kennel for assessment of mobility.

The scores obtained for the component categories of the pain scale were finally summed up to form a total pain score. The minimum possible total pain score that could be obtained by use of this scale was 0, while the maximum possible pain score was 24.

4.2.7.5 Assessment of sedation

Sedation was assessed before premedication (baseline scores), at 1, 2, 4, 6, 12 and 24 hours after surgery and sedation scores allocated to each dog. Sedation was assessed using parameters shown in Table 4-2, as described by Tsai *et al.*, (2013).

Table 4-2: The criteria used to assess postoperative sedation in dogs undergoing ovariohysterectomy in this study

Sedation score	Criteria
0	Fully alert and able to stand and walk
1	Alert and able to maintain sternal recumbency
2	Drowsy and unable to maintain sternal recumbency
3	Fast asleep

Note: The sedation assessment criteria was adopted from Tsai *et al.*, (2013)

4.2.8 Data management and analysis

Data were entered into Microsoft Office Excel then verified and validated as correct entries based on the data collection sheets. Data were then imported into Statplus Pro 5.9.8 for computations of means or medians and p values. Data normality was tested using the Shapiro-Wilk test while statistical significance was set at a value of $p < 0.05$.

Non-parametric data (pain score and sedation score) were expressed as median. Median values were then compared between and within the four treatment groups (Butorphanol group, Meloxicam group, Butorphanol-meloxicam group and control group) at each of the assessment points using the Kruskal-Wallis rank sum test. Pooled data was used for between groups comparisons. Where statistical difference was noted, pair-wise comparison was performed using the pair wise Wilcoxon rank sum test with a Bonferroni adjustment for multiple testing.

Parametric data (duration of anaesthesia, duration of surgery, heart rate, respiratory rate, blood pressure and rectal temperature) were expressed as means \pm SD. Means \pm SD were then compared within and between the four treatment groups (Butorphanol group, Meloxicam group, Butorphanol-meloxicam group and Control group) at each of the assessment points using ANOVA for repeated measures. Pooled data were used for between group comparisons. Where significant difference was indicated by ANOVA, Fisher's least significant difference test was applied to determine statistical differences between the groups.

4.3 Results

4.3.1 General data between treatment groups

There was no significant difference in the mean body weights of dogs, duration of surgery and duration of anaesthesia as presented in Table 4-3. Duration of anaesthesia was relatively shorter for dogs in the meloxicam group (59.5 ± 15.5 minutes) and those in the butorphanol-meloxicam group (59.6 ± 16.4 minutes), as compared to that for dogs in the control group (60.8 ± 14.4 minutes), and those in the butorphanol group (63.9 ± 16.0 minutes).

Table 4-3: The mean body weight and duration of surgery and anaesthesia following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Treatment group	Body Weight	Duration of Surgery	Duration of Anaesthesia
Butorphanol	15.4±3.9	26.8±7.9	63.9±16.0
Meloxicam	15.1±3.4	25.4±8.9	59.5±15.5
Butorphanol-Meloxicam	16.0±5.1	22.6±9.2	59.6±16.4
Control (Placebo)	19.0±5.9	25.3±8.4	60.8±14.4
P-Value	p=0.89	p=0.72	p=0.91

4.3.2. Pain scores

4.3.2.1 Trends in pain scores between treatment groups at the assessment points

At 1 hour postoperatively, dogs injected with meloxicam had significantly lower pain scores compared to those injected with butorphanol ($p=0.002$), the butorphanol-meloxicam combination ($p=0.01$), as well as those in the control group ($p=0.001$). Despite dogs injected with the butorphanol-meloxicam combination having lower pain scores compared to dogs in butorphanol group and control group, the differences were not statistically significant. Dogs injected with butorphanol had relatively lower pain scores compared to those in the control group one hour postoperatively, but this difference was not significant.

At two hours postoperatively, pain scores in dogs injected with meloxicam were significantly lower than those in dogs injected with butorphanol ($p=0.004$), and those in the control group ($p=0.002$). The butorphanol-meloxicam combination produced lower pain scores in dogs than did butorphanol ($p=0.03$) and the placebo ($p=0.009$). Although the butorphanol-meloxicam combination produced lower pain scores in dogs than meloxicam did, the difference was not statistically significant. A similar observation was made on pain scores attained by dogs under butorphanol and those in the control at 2 hours postoperatively.

Comparison of pain score between the four treatment groups 4 hours postoperatively showed that dogs under butorphanol had significantly higher pain scores compared to dogs under meloxicam ($p=0.002$) and the butorphanol-meloxicam combination ($p=0.04$). Dogs in the control group had significantly higher pain scores than those in meloxicam group ($p=0.001$) and butorphanol-meloxicam combination group ($p=0.01$). The difference in pain scores between dogs in the butorphanol group and those in the control group as well as between dogs

in the meloxicam group and those in the butorphanol-meloxicam combination group, was not significant.

At 6 hours postoperatively, dogs in the control group had significantly higher pain scores compared to dogs in the butorphanol ($p=0.03$) and meloxicam groups ($p=0.01$), but relatively higher scores compared to those in butorphanol-meloxicam drug combination. On the other hand, the pain scores for dogs under butorphanol were similar to those under meloxicam ($p=0.33$) and the butorphanol-meloxicam combination ($p=0.69$). A similar trend was seen between median pain scores in dogs under the meloxicam and butorphanol-meloxicam combination ($p=0.30$). Although the median pain scores for dogs in the meloxicam (median score 2) and butorphanol-meloxicam combination (median score 2) were lower than the score for dogs in the butorphanol (median score 3) and control groups (median score 3), the differences were not statistically significant at 12 hours postoperatively. By the end of the 24-hour monitoring period, dogs in all the four treatment groups had a median pain score of one. These changes in median pain scores are shown in Table 4-4 and Figure 4-1.

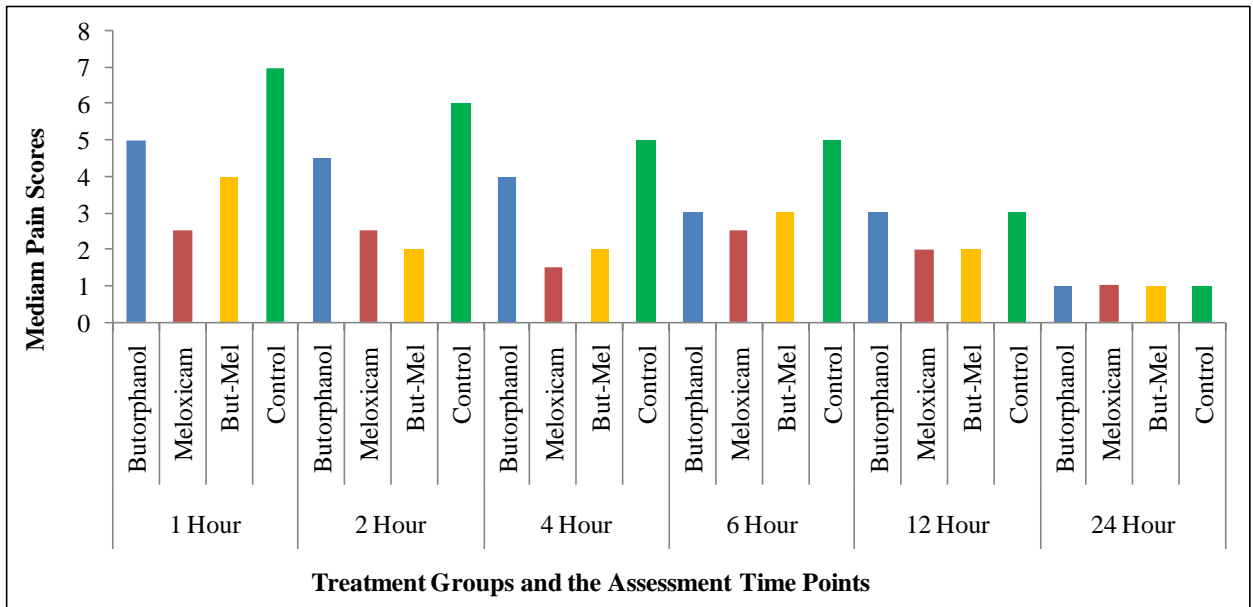


Figure 4-1: Trend in pain scores (median values) following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

4.3.2.2 Comparison of overall pain scores between the treatment groups

Pain scores for dogs in the four treatment groups showed a significant ($p=0.01$) difference between the four groups. Dogs injected with meloxicam had significantly lower pain scores compared to those under butorphanol ($p=0.01$) and those in the control group ($p=0.01$). Dogs injected with the butorphanol-meloxicam combination had significantly lower pain scores compared to those in the control group ($p=0.02$). Pain scores in dogs under butorphanol-meloxicam combination were lower than pain scores for dogs under meloxicam, but the difference was not statistically significant. Similarly, there was no significant difference in pain scores for dogs under butorphanol-meloxicam combination when compared to pain scores in dogs under butorphanol. The same observation was made when pain scores for dogs under butorphanol were compared to those for dogs in the control group.

4.3.3 Sedation scores

4.3.3.1. Within group comparison

In dogs under butorphanol, the sedation score increased significantly from a baseline score of 0 to a score of 2, one hour postoperatively (Figure 4-2). The sedation score remained significantly higher than baseline value for up to 6 hours postoperatively, and only returned to score 0 at 12 hours postoperatively. Sedation score for dogs under the butorphanol-meloxicam combination was also significantly ($p=0.01$) higher when compared to the baseline sedation score. However notably, dogs in this group had relatively higher sedation score from 2 hours through to 4 hours postoperatively, returning to baseline value at 6 hours after surgery. In the meloxicam and control groups, dogs had significantly ($p=0.000$ and $p=0.05$, respectively) higher sedation scores at 1 hour postoperatively when compared to the baseline sedation scores.

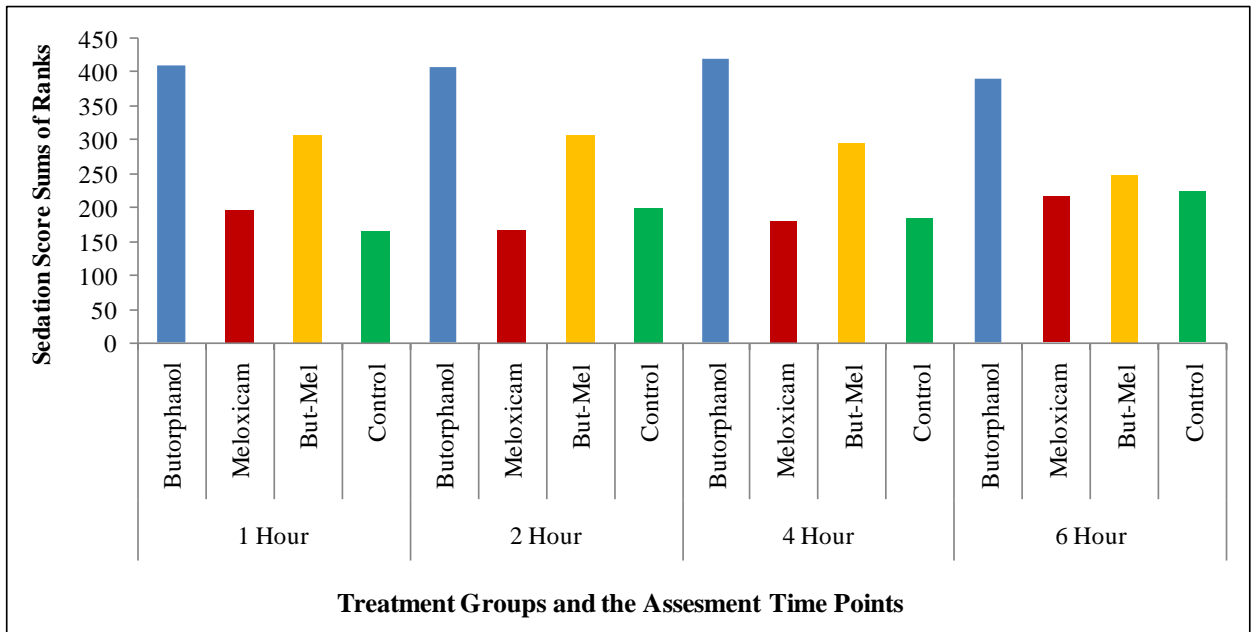


Figure 4-2: Trend in sedation scores (median values) following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Table 4-4: Changes in pain and sedation scores following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Parameter	Time	Butorphanol	Meloxicam	But-Mel	Control
Median Pain Score	Baseline	0	0	0	0
	1 Hour	5	2.5	4	7
	2 Hours	4.5	2.5	2	6
	4 Hours	4	1.5	2	5
	6 Hours	3	2.5	3	5
	12 Hours	3	2	2	3
	24 Hours	1	1	1	1
Median Sedation Score	Baseline	0	0	0	0
	1 Hour	2*	1*	1*	1*
	2 Hours	2*	0	1*	0
	4 Hours	1*	0	1*	0
	6 Hours	1*	0	0	0
	12 Hours	0	0	0	0
	24 Hours	0	0	0	0

Key: * indicates value significant at $p < 0.05$ compared to the baseline value

4.3.3.2. Between group comparisons

There was a significant difference in sedation scores between the four treatment groups at 1 hour ($p=0.000$), 2 hours ($p=0.000$), 4 hours ($p=0.000$) and 6 hours ($p=0.04$) postoperatively. At 1 hour after surgery, dogs injected with butorphanol had significantly ($p=0.000$) higher sedation scores compared to dogs injected with meloxicam and those in the control group ($p=0.000$). Similarly, dogs under the butorphanol-meloxicam combination had significantly ($p=0.03$ and $p=0.02$, respectively) higher sedation scores compared to dogs in the meloxicam and control groups. However, the sedation scores for dogs in the meloxicam and control groups were the same ($p=0.62$) and, so were the sedation scores for dogs under butorphanol and those under the butorphanol-meloxicam combination ($p=0.21$)

At 2 hours postoperatively, dogs in the butorphanol group had significantly higher sedation scores than those in meloxicam ($p=0.000$) and control group ($p=0.000$), but statistically similar sedation scores to dogs under the butorphanol-meloxicam drug combination. Butorphanol-meloxicam drug combination caused significantly more sedation compared to meloxicam ($p=0.01$) and the placebo ($p=0.05$). At this time, the median sedation score for dogs in meloxicam group was not statistically different from that for dogs in the control group.

At 4 hours postoperatively, the median sedation score for dogs in the butorphanol group was significantly higher than that for dogs in the meloxicam ($p=0.000$) and control groups ($p=0.000$), but not statistically different from that for dogs under the butorphanol-meloxicam drug combination. Dogs injected with butorphanol-meloxicam drug combination had significantly higher sedation score than those injected with meloxicam.

As at 6 hours postoperatively, dogs under butorphanol, had significantly deeper sedation than those under meloxicam ($p=0.01$) and those in control group ($p=0.04$). There were no significant differences in sedation scores between the four treatment groups at 12 hours and 24 hours, postoperatively (Table 4-4).

4.3.4 Changes in blood pressure

4.3.4.1 Trends in mean blood pressure within the treatment groups

Generally, the blood pressure in the four treatment groups decreased from baseline values to reach lowest values 1 hour postoperatively as shown in Table 4-5 and illustrated in Figure 4-3. Following this, the blood pressure rose gradually but did not return to baseline values within the 24-hour monitoring period.

In dogs in the butorphanol group, blood pressure decreased from a baseline value of 99.5 ± 25.8 mmHg to a lowest value of 85.1 ± 35.5 mmHg at 1 hour postoperatively. At 12-hour postoperatively, the blood pressure had risen again to its highest value of 95.4 ± 24.8 mmHg. However, these changes in blood pressure were not statistically significant.

In dogs under meloxicam, there was a relative decline in blood pressure from a baseline value of 105 ± 30.1 mmHg to a low value of 97.4 ± 23.3 mmHg at 1 hour postoperatively. The blood pressure then started to rise from 2 hours (99.3 ± 21.5 mmHg) after surgery and reached the highest value of 102.5 ± 22.0 mmHg at 6 hours postoperatively. However, these changes were not statistically significant when compared to baseline value.

The lowest recorded mean blood pressure of 97.7 ± 18.3 mmHg in dogs under butorphanol-meloxicam combination was recorded 1 hour postoperatively. Following this, the mean blood pressure increased to reach a high of 111.5 ± 16.5 mmHg at 6 hours postoperatively. However, these changes were not statistically significant when compared to the baseline mean blood pressure of 107.9 ± 21.8 mmHg.

Dogs in the control group had their mean blood pressure decline from a baseline value of 105.3 ± 26.4 mmHg to 97.8 ± 19.7 mmHg at 1 hour postoperatively. The blood pressure later rose to 108.8 ± 25.2 mmHg at 6 hours after surgery. These changes in mean blood pressure of the dogs in the control group were however, not statistically significant (Table 4-5).

4.3.4.2 Mean blood pressure between the treatment groups

Comparison of the mean blood pressure between the 4 treatment groups [the 24-hour mean blood pressure (75.6 ± 25.3 mmHg) in the meloxicam group was not considered since it was an outlier] showed that there was significant difference ($p=0.000$) in the mean blood pressure between the four treatment groups. Dogs injected with butorphanol had significantly lower mean blood pressure (92.0 ± 5.3 mmHg) compared to those under meloxicam (100.9 ± 2.7 mmHg $p=0.003$), those under the butorphanol-meloxicam drug combination (105.2 ± 4.4 mmHg $p=0.0003$) and those in the control group (103.1 ± 3.8 mmHg $p=0.001$). Despite the mean blood pressure in dogs under meloxicam (100.9 ± 2.7 mmHg) being lower than that in dogs under butorphanol-meloxicam drug combination (105.2 ± 4.4 mmHg) and those in the control group (103.1 ± 3.8 mmHg), the differences were not statistically significant ($p=0.07$ and $p=0.28$, respectively). A similar observation was made for the comparison between mean blood pressure in dogs in the control group (103.1 ± 3.8 mmHg) and those under the butorphanol-meloxicam drug combination (105.2 ± 4.4 mmHg; $p=0.36$).

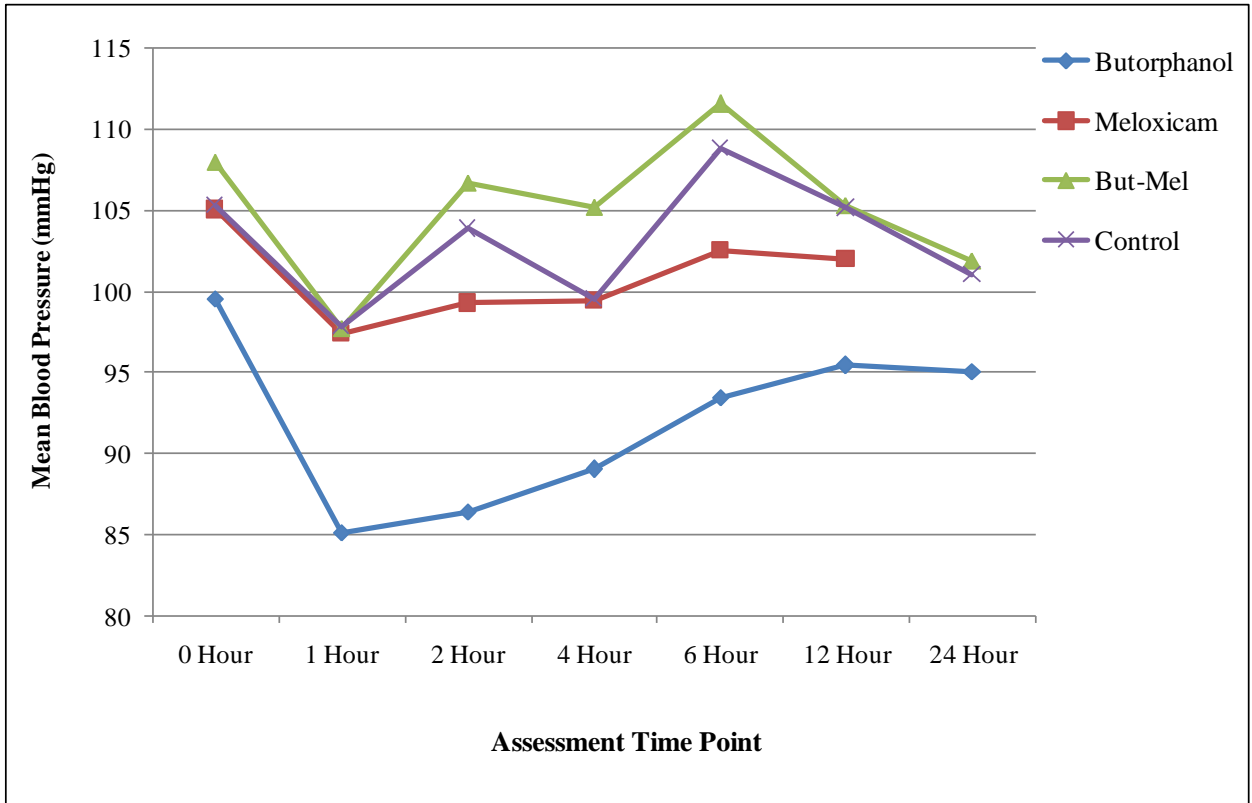


Figure 4-3: Trend in mean blood pressure following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

4.3.5 Heart rate

4.3.5.1 Mean heart rate within the treatment groups

Overall, there was an initial rise in heart rate observed in dogs in the three treatment groups which received analgesics, within the first hour of administration of the drugs. Thereafter, an up and down trend in heart rate was seen in dogs in all the treatment groups. At the end of the 24-hour monitoring period, heart rate remained below baseline values in dogs injected with meloxicam, butorphanol-meloxicam drug combination and those in the control group. However, all these changes in heart rate were not statistically significant.

The highest mean heart rate of 137.3 ± 32.6 beats/minute for dogs in the butorphanol group was recorded 1 hour after ovariohysterectomy, relative to a baseline value of 122.0 ± 17.9 beats/minute. The lowest mean heart rate for dogs in this group (120.3 ± 37.7 beats/minute) was recorded 6 hours, postoperatively.

Dogs in the meloxicam group had a mean heart rate of 127.7 ± 28.7 beats/minute at 1 hour postoperatively. It then fluctuated between 116.5 ± 35.0 beats/minute at 4 hours and 120.9 ± 31.8 beats/minute at 6 hours postoperatively. The lowest mean heart rate for dogs in this group was 95.9 ± 20.0 beats/minute, which was recorded 24 hours after surgery.

Dogs under the butorphanol-meloxicam drug combination recorded a high heart rate (123.6 ± 27.7 beats/minute) 1 hour postoperatively compared to a baseline value of 117.4 ± 31.8 beats/minute. After that, heart rate dropped to the lowest recorded value of 105.8 ± 17.7 beats/minute, 2 hours postoperatively. An up and down trend was then observed with a peak of 123.9 ± 18.9 beats/minute, at 12 hours and a low of 112.8 ± 23.0 beats/minute, 24 hours postoperatively.

Heart rate in dogs in the control group increased from a baseline value of 127.5 ± 18.8 beats/minute and remained consistently high up to 4 hours postoperatively, when a value of 134.1 ± 36.7 beats/minute was recorded. Thereafter, the heart rate started to decline reaching a low of 101.4 ± 39.9 beats/minute, 12 hours postoperatively. Although the heart rate started to rise as from 12 hours postoperatively, by the end of the 24-hour monitoring period, it was (111.3 ± 35.3 beats/minute) still below the baseline value (Table 4-5).

4.3.5.2 Mean heart rate between the treatment groups

Although dogs injected with butorphanol and those in the control group had relatively higher mean heart rates (125.5 ± 6.0 beats/minute and 123.1 ± 12.0 beats/minute, respectively) compared to those under meloxicam (116.4 ± 10.4 beats/minute) and those under the butorphanol-meloxicam drug combination (117.1 ± 6.7 beats/minute), these differences were not statistically significant ($p=0.20$).

4.3.6 Respiratory rate

4.3.6.1 Mean respiratory rate within treatment groups

Respiratory rate in dogs under butorphanol, meloxicam and the butorphanol-meloxicam drug combination declined significantly ($p=0.02$; $p=0.003$; and $p=0.05$, respectively) from baseline values following administration of the test drugs. However, respiratory rate in dogs in the control group decreased relatively ($p=0.59$) when compared to the baseline value (Figure 4-4 and Table 4-5).

In dogs under butorphanol, the respiratory rate reduced significantly ($p=0.01$) from a baseline value of 25.8 ± 5.4 breaths/minute to a value of 19.7 ± 5.5 breaths/minute in the first 1 hour after surgery. The respiratory rate remained significantly lower compared to baseline value from 1 hour to 6 hours postoperatively, when a value of 22.0 ± 3.2 breaths/minute ($p=0.04$) was recorded. Thereafter, the respiratory rate rose and reached a value of 24.0 ± 6.3 breaths/minute 24 hours after surgery. A similar trend was seen in dogs injected with the butorphanol-meloxicam drug combination whose respiratory rate reduced significantly ($p=0.04$) to a value of 21.5 ± 5.5 breaths/minute 1 hour postoperatively as compared to a baseline value of 27.8 ± 8.0 breaths/minute. The respiratory rate remained significantly lower through 6 hours postoperatively where a value of 21.5 ± 4.8 breaths/minute ($p=0.04$) was recorded. At 24 hours postoperatively, the recorded respiratory rate of 23.0 ± 2.8 breaths/minute was still relatively ($p=0.12$) lower than baseline value. (Figure 4-4 and Table 4-5).

The respiratory rate in dogs under meloxicam declined significantly ($p=0.03$) from a baseline value of 28.5 ± 5.8 breaths/minute to 21.7 ± 8.3 breaths/minute at 1 hour postoperatively. Respiratory rate values in these dogs remained significantly lower than baseline value up to 12 hours postoperatively where a value of 20.0 ± 3.6 breaths/minute ($p=0.000$) was recorded. After

the 24-hour monitoring period, the respiratory rate was 24.5 ± 3.3 breaths/minute, still relatively lower than baseline value.

There was no significant change in the respiratory rate in dogs in the control group. However, the respiratory rate decreased from a baseline value of 27.6 ± 11.1 breaths/minute to attain the lowest value of 21.6 ± 5.7 breaths/minute ($p=0.14$) at 12 hours postoperatively. Thereafter, the respiratory rate rose to 24.9 ± 5.6 breaths/minute at 24 hours, postoperatively (Figure 4-4 and Table 4-5).

4.3.6.2 Mean respiratory rate between the treatment groups

Dogs in the control group had relatively higher respiratory rate (23.7 ± 2.0 breaths/minute) compared to the respiratory rate in dogs under butorphanol (22.0 ± 2.3 breaths/minute), meloxicam (22.2 ± 3.3 breaths/minute) and the butorphanol-meloxicam drug combination (22.8 ± 2.3 breaths/minute). However, the differences in these rates were not statistically significant ($p=0.58$).

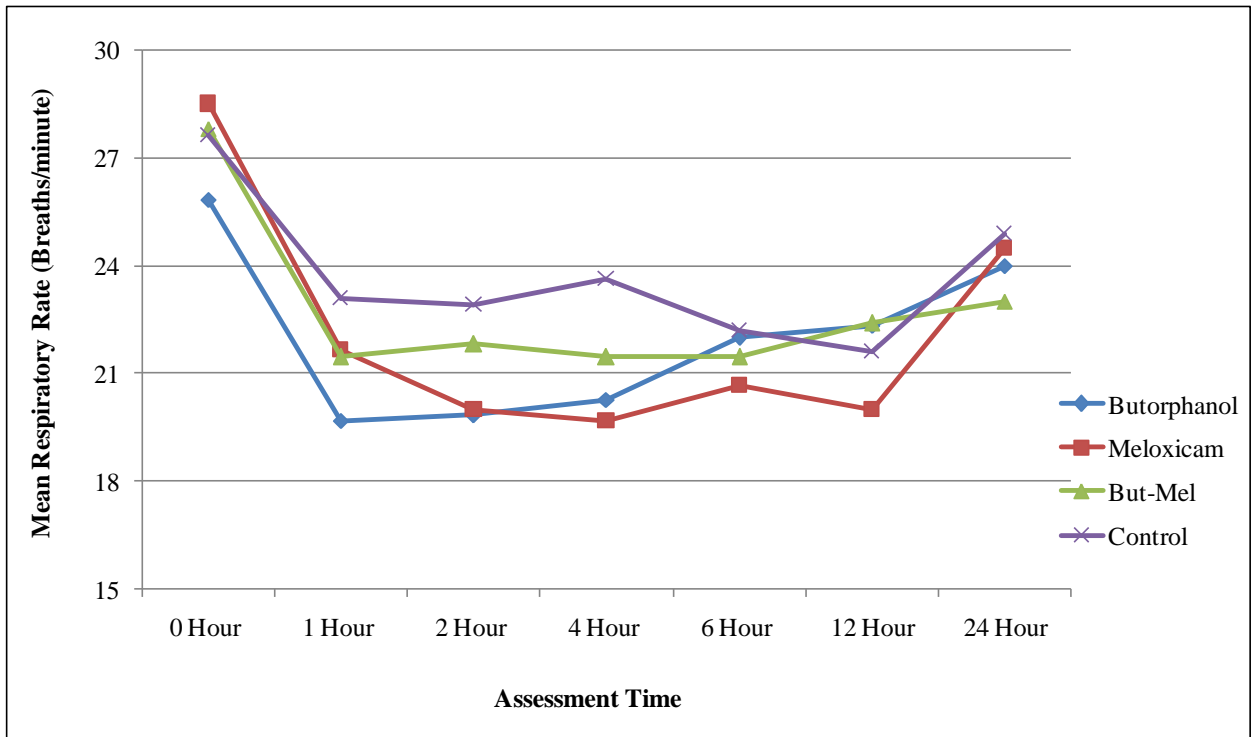


Figure 4-4: Trend in mean respiratory rate following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

4.3.7 Rectal temperature

4.3.7.1 Mean rectal temperature within treatment groups

There were significant changes in rectal temperature in dogs under butorphanol ($p=0.000$), meloxicam ($p=0.000$), the butorphanol-meloxicam drug combination ($p=0.006$) and those in the control group ($p=0.000$) following the various interventions after surgery. The general trend for temperature in dogs in all four treatment groups was a decline from baseline values to reach lowest values, 1 hour postoperatively. Thereafter the temperature started to rise, reaching the highest values 12 hours after surgery in dogs under meloxicam; 6 hours postoperatively in dogs under butorphanol and those in the control group; and 24 hours for the dogs in the butorphanol-meloxicam drug combination (Figure 4-5 and Table 4-5).

Rectal temperature in dogs under butorphanol decreased significantly ($P=0.001$) from a baseline value of 38.6 ± 0.9 °C to a lowest value of 37.3 ± 0.8 °C, one hour after surgery. The temperature in this group remained significantly ($p=0.04$) lower up to 2 hours postoperatively where a value of 37.9 ± 0.6 °C was recorded. Thereafter, the temperature started to rise and reached the highest value of 38.8 ± 0.8 °C ($p=0.55$), 6 hours postoperatively. By the end of the 24-hour monitoring period, rectal temperature in dogs in this group was relatively lower (38.5 ± 0.4 °C, $p=0.76$), compared to baseline value.

A similar trend was seen in rectal temperature in dogs under the control group whose temperature declined significantly ($p=0.000$) from a baseline value of 38.8 ± 0.7 °C to the lowest recorded value of 37.2 ± 1.0 °C, 1 hour postoperatively. Temperature in this group remained significantly ($p=0.002$) lower up to 2 hours after surgery, where a value of 37.7 ± 0.8 °C was recorded. After that, the temperature rose gradually to reach the highest recorded value of 38.5 ± 0.4 °C, 6 hours postoperatively. However, this value was not significantly ($p=0.25$)

different from baseline value. In dogs under the butorphanol-meloxicam drug combination, rectal temperature dropped significantly ($p=0.002$) from a baseline value of 38.6 ± 0.7 °C to the lowest value of 37.6 ± 0.6 °C recorded 1 hour after surgery. This was followed by a relative ($p=0.64$) increase in temperature to the highest recorded value of 38.7 ± 0.5 °C, 24 hours postoperatively (Figure 4-5 and Table 4-5).

Temperature in dogs injected with meloxicam decreased significantly ($p=0.002$) from a baseline of 38.7 ± 0.8 °C and reached the lowest value of 37.4 ± 1.0 °C, 1 hour postoperatively. Temperature in dogs in this group remained significantly lower at both 2 hours (37.7 ± 0.8 °C, $p=0.01$) and 4 hours (38.1 ± 0.5 °C, $p=0.04$), postoperatively. After this, the temperature increased gradually to reach the highest value of 38.6 ± 0.3 °C, 12 hours after surgery. By the end of the 24-hour monitoring period, rectal temperature in this group was still relatively ($p=0.20$) lower (38.3 ± 0.6 °C), compared to baseline value (Figure 4-5 and Table 4-5).

4.3.7.2 Comparison of mean rectal temperature between the treatment groups

Despite dogs in the meloxicam and control groups having lower rectal temperatures (38.2 ± 0.5 °C and 38.2 ± 0.5 °C, respectively) compared to that in dogs in the butorphanol and butorphanol-meloxicam combination groups (38.3 ± 0.5 °C and 38.4 ± 0.4 °C, respectively), these differences were not statistically significant ($p=0.84$).

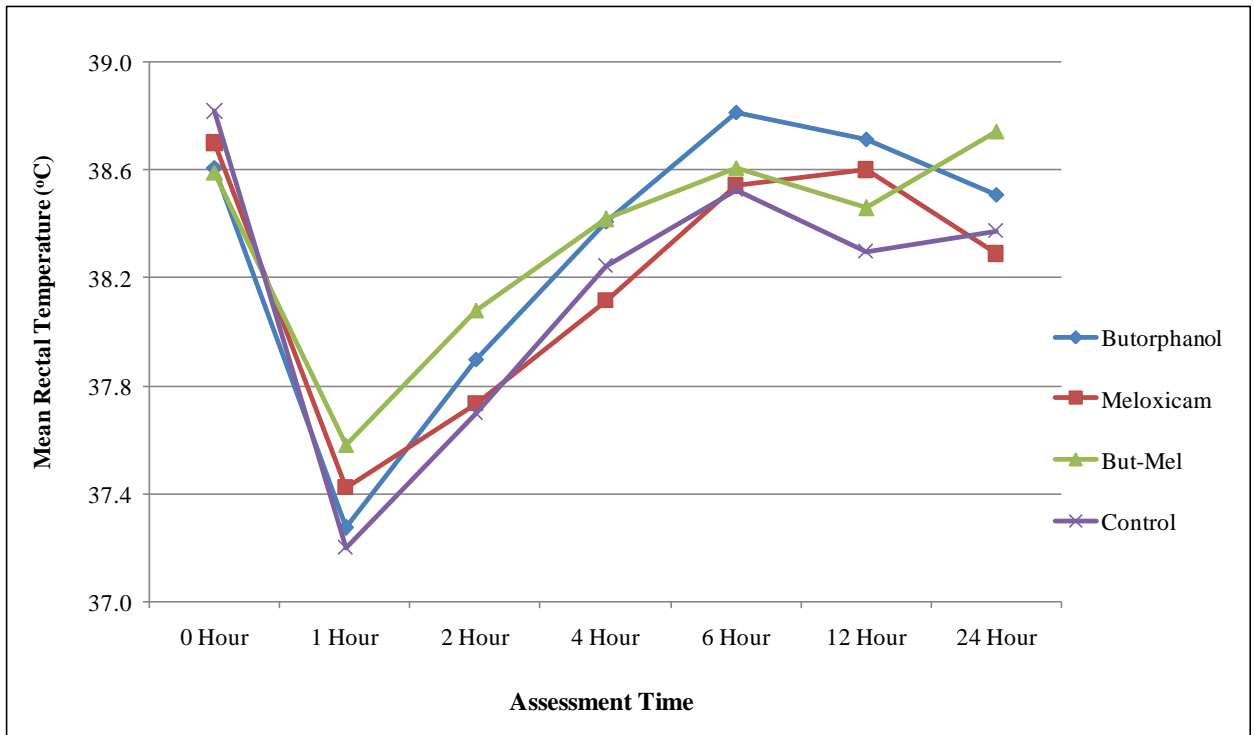


Figure 4-5: Trend in mean rectal temperature following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Table 4-5: Changes in cardiopulmonary parameters and temperature following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariectomy.

Parameter	Time	Butorphanol	Meloxicam	But-Mel	Control
Blood Pressure (Mm/Hg)	0 Hour	99.5±25.8	105±30.1	107.9±21.8	105.3±26.4
	1 Hour	85.1±35.5	97.4±23.3	97.7±18.3	97.8±19.7
	2 Hour	86.4±21.4	99.3±21.5	106.6±27.7	103.8±21.5
	4 Hour	89.0±22.5	99.4±23.4	105.2±27	99.5±26.2
	6 Hour	93.4±17.7	102.5±22.0	111.5±16.5	108.8±25.2
	12 Hour	95.4±24.8	102±30.7	105.3±24.1	105.1±32.7
	24 Hour	95.0±20.4	75.6±25.3	101.9±18.2	101.0±26.6
Heart Rate (Beats/Minute)	0 Hour	122.0±17.9	120.1±23.5	117.4±31.8	127.5±18.8
	1 Hour	137.3±32.6	127.7±28.7	123.6±27.7	127.9±39.2
	2 Hour	122.6±32.1	122.6±33.7	105.8±17.7	128.5±19.8
	4 Hour	123.8±29.3	116.5±35.0	122.1±20.6	134.1±36.7
	6 Hour	120.3±37.7	120.9±31.8	114.3±23.1	130.9±38.4
	12 Hour	129.5±37.0	111.2±29.9	123.9±18.9	101.4±39.9
	24 Hour	122.8±42.1	95.9±20.0	112.8±23.0	111.3±35.3
Respiratory Rate (Breathes/Minute)	0 Hour	25.8±5.4	28.5±5.8	27.8±8.0	27.6±11.1
	1 Hour	19.7±5.5*	21.7±8.3*	21.5±5.5*	23.1±8.6
	2 Hour	19.8±5.0*	20.0±7.4*	21.8±3.7*	22.9±4.8
	4 Hour	20.3±2.7*	19.7±4.3*	21.5±4.5*	23.6±5.8
	6 Hour	22.0±3.2*	20.7±5.6*	21.5±4.8*	22.2±8.3
	12 Hour	22.3±4.7	20.0±3.6*	22.4±4.3	21.6±5.7
	24 Hour	24.0±6.3	24.5±3.3	23.0±2.8	24.9±5.6
Temperature (°C)	0 Hour	38.6±0.9	38.7±0.8	38.6±0.7	38.8±0.7
	1 Hour	37.3±0.8*	37.4±1.0*	37.6±0.6*	37.2±1.0*
	2 Hour	37.9±0.6*	37.7±0.8*	38.1±0.5	37.7±0.8*
	4 Hour	38.4±1.1	38.1±0.5*	38.4±0.8	38.2±0.7
	6 Hour	38.8±0.8	38.5±0.4	38.6±1.0	38.5±0.4
	12 Hour	38.7±0.5	38.6±0.3	38.5±0.5	38.3±0.4
	24 Hour	38.5±0.4	38.3±0.6	38.7±0.5	38.4±0.6

Key: * indicate figures are significantly different (p<0.05) compared to their respective baseline values

4.4 Discussion

Dogs in this study were evenly distributed as body weight was statistically similar between the four treatment groups. Duration of anaesthesia and surgery were also statistically similar and thus could not have potentially influenced the observed pain scores. However, dogs in the butorphanol group had relatively longer duration of anaesthesia compared to those in the meloxicam, butorphanol-meloxicam drug combination and control groups. This observation might have been as a result of the synergistic sedation induced by both butorphanol and acepromazine.

The high pain scores observed in the first 6 hours after surgery in dogs under the control group and the observation of similar pain scores in all treatment groups by 24 hours postoperatively supports the hypothesis that ovariohysterectomy causes marked and acute postoperative pain which is nevertheless short lived (Gaynor and Muir, 2002). For ethical reasons and also to avoid the deleterious effects associated with postoperative pain, analgesics are warranted after spay. This ensures patient comfort, optimizes on wound healing and generally improves the surgical outcome (Robertson, 2002).

The significantly lower pain scores found in dogs treated with meloxicam as compared to scores in dogs under butorphanol particularly in the first 4 hours postoperatively, was similar to previous reports (Caulkett *et al.*, 2003) in which meloxicam at 0.2mg/kg was found to have better analgesia compared to butorphanol at the same dosage rate in dogs after ovariohysterectomy. Studies have shown that NSAIDs have better analgesic outcomes than opioids after ovariohysterectomy in dogs. For example, firocoxib was found to produce better analgesia than butorphanol (Carmago *et al.*, 2011). Carprofen was reported to have produced

lower pain scores than buprenorphine (Shih *et al.*, 2008). However, carprofen had similar analgesia as morphine after ovariohysterectomy (Dzikiti *et al.*, 2006).

Butorphanol is a short acting narcotic which has been shown to provide effective visceral analgesia for 23 to 53 minutes when administered at a dosage of 0.2 – 0.8 mg/kg body weight in dogs (Sawyer *et al.*, 1991). The choice of the dosage of butorphanol of 0.2 mg/kg in this study might have resulted in higher pain scores. By the time the dogs were assessed one hour postoperatively, the effective circulating dose was low and as such pain scores were high compared to meloxicam which is a long acting NSAID. To support this argument, dogs under butorphanol in this study were also found to have statistically similar pain scores to those in the control group in the first 4 hours postoperatively. Studies have shown that where butorphanol is used to manage pain postoperatively, repeat doses are warranted within 2 hours of administration (Caulkett *et al.*, 2003). This may not be practical in most clinical practices and for this reason butorphanol might not be the ideal analgesic drug for managing pain post-ovariohysterectomy in dogs. In fact, butorphanol has been shown to provide inadequate analgesia in dogs after laparotomy (Mathews *et al.*, 1996), cystotomy and splenectomy (Mathews *et al.*, 2001).

On the other hand, dogs under meloxicam were more comfortable especially at 4 hours postoperatively when the lowest median pain scores were recorded. Meloxicam ([4-hydroxy-2methyl-N-(5-methyl-2thiazolyl)-2H-1,2-benzothiazine-3carboxamide-1,1-dioxide]) is an NSAID of oxime group which preferentially blocks cyclooxygenase 2 (COX-2) enzyme resulting in antipyretic, analgesic and anti-inflammatory effects (Leeset *et al.*, 1991; Mathews, 1996). When administered subcutaneously at a dosage of 0.2mg/kg, meloxicam is bound by plasma proteins and reaches peak plasma concentration in about 3 hours post-administration

(Lees *et al.*, 1991; Mathews, 1996). The peak plasma concentration could explain why dogs under meloxicam in this study had lowest pain scores at 4 hours postoperatively. However, due to its rapid elimination, pain scores increased again at 6 hours postoperatively. Clinically this could indicate the need for top up 6 hours following initial injection. Practically this is possible as redosing will be done once or twice considering the observation that pain emanating from ovariohysterectomy seems to be short lived, lasting for about 12 hours postoperatively.

In this study, the butorphanol-meloxicam drug combination produced statistically similar analgesia to meloxicam and butorphanol. Although there is no study comparing analgesic effects of butorphanol-meloxicam combination and the individual drugs post-spay in dogs, other studies have shown similar results in reference to opioid-NSAID multimodal analgesia. In one study, morphine-carprofen drug combination was shown to have similar analgesia compared to either drug administered alone (Dzikiti *et al.*, 2006). Similar findings were also reported where buprenorphine-carprofen drug combination lacked additive analgesic benefits compared to administration of either carprofen or buprenorphine alone (Shih *et al.*, 2008). In the current study, the lack of additive analgesia in dogs under the drug combination could be in part because of the small number of animals per group, and/or the chosen method of pain assessment which may not have been sensitive enough to detect minor inter-group differences (Tsai *et al.*, 2013). It has been suggested that pain scores are designed to distinguish marked differences in awareness of pain, and not to assess diminishing normal behaviors (Hardie *et al.*, 1997). Another possibility is that both butorphanol and meloxicam produced their maximal effects to pain elicited by ovariohysterectomy such that the synergistic effects of their combination could not be perceivable (Tsai *et al.*, 2013). However, in light of pain scores being relatively lower in dogs in the butorphanol-meloxicam drug combination compared to those in animals under the individual drugs, it would then mean that multimodal therapy has

an added advantage compared to single drugs. This advantage can possibly be picked statistically if the number of animals in a group is large.

The significantly higher sedation scores observed in all the treatment groups one hour postoperatively, can be attributed to the prolonged sedative effect of acepromazine, which was used for premedication. However, it is unlikely that the dosage of acepromazine (0.10 mg/kg) used in this study could have alone contributed to the prolonged sedation that was observed in the four treatment groups. This is because acepromazine-associated-sedation is known to have a ceiling effect (Gomes *et al.*, 2011) where any further increase in dosage after sedation plateau is reached does not positively correlate to enhanced degree of sedation (Hall *et al.*, 2001). However, studies have shown that the sedative effects of acepromazine can be enhanced by combining it with an opioid (Smith *et al.*, 2001; Caulkett *et al.*, 2003; Monteiro *et al.*, 2009; Camargo *et al.*, 2011). This could explain the reason why dogs under butorphanol had a protracted duration of sedation of up to 6 hours postoperatively, compared to dogs in the meloxicam and control groups. The longer duration of sedation in dogs in the butorphanol-meloxicam combination group than in dogs in the meloxicam and control groups can be attributed to the sedative effects of butorphanol. On the other hand, the shorter duration of sedation observed in animals under the butorphanol-meloxicam drug combination as compared to that seen in dogs in the butorphanol group was due to the lower dosage of butorphanol in the drug combination.

Due to sympathetic stimulation, pain causes changes in physiological parameters like heart rate, respiratory rate, temperature, blood pressure and plasma cortisol (Smith *et al.*, 1996; Mathews, 2000; Robertson, 2014). However, these changes ought to be interpreted carefully

since their etiology is not limited to pain only but can occur in response to fear, stress, type of surgery, anaesthesia and pharmacological intervention (Smith *et al.*, 1996; Mathews, 2000).

In this study, there were no significant changes in blood pressure within the treatment groups albeit for a mild hypotension observed 1 hour postoperatively. The observed hypotension could be associated with anaesthesia more so acepromazine that was used in premedication. Acepromazine is a phenothiazine which has antagonistic action on dopamine, histamine, serotonin and catecholamine receptors (Lemke, 2007). Acepromazine associated hypotension occurs as a result of sedation, vasodilation caused by vascular smooth muscle relaxation and decreased systemic vascular resistance, depression of hypothalamic and brain stem vasomotor reflexes, peripheral α_2 -adrenergic blockade, and cardiac depression (Lemke, 2007). Intravenous injection of acepromazine at 0.1mg/kg has been shown to decrease the cardiac output and blood pressure by 20-30%. The reduction in blood pressure is dose dependant such that severe hypotension is seen at high doses of acepromazine (Boyd *et al.*, 1991). The vasodilatory effects of isoflurane can also be associated with the observed mild hypotension (Hikasa *et al.*, 1996). Dogs under butorphanol were significantly more hypotensive when compared to those under meloxicam, butorphanol-meloxicam combination and those in the control group. This difference can be explained by the synergistic effects of acepromazine and butorphanol on blood pressure as previously reported (Kojima *et al.*, 1999; Kojima *et al.*, 2002).

There were no significant changes in the heart rate within and between the treatment groups. However, an initial tachycardia was observed in dogs in all the groups 1 hour postoperatively followed by an up and down pattern, except for the dogs in the control group whose heart rate was consistently high. The observed tachycardia could have been a compensatory mechanism

for decreased systemic vascular resistance and blood pressure caused by the anaesthetics (Lemke, 2007). In addition, tachycardia one hour postoperatively could have been as a result of pain due to sympathetic nervous system stimulation (Smith *et al.*, 1996; Mathews, 2000; Robertson, 2014). This argument is supported by the fact that pain scores were highest one hour postoperatively in all the treatment groups. The same reasoning can be applied in explaining the consistently high heart rate in dogs in the control group where pain therapy was not instituted.

Studies have shown variable results regarding the effects of pain on respiration rate. In the current study, significant decrease in respiration rate was seen in dogs treated using butorphanol, meloxicam and the butorphanol-meloxicam combination, but this remained high in animals in the control group. This can be attributed to the sympathetic stimulation of respiration in response to pain in the control group (Smith *et al.*, 1996; Mathews, 2000; Robertson, 2014).

The initial significantly low rectal temperature in dogs in all the treatment groups could be associated with depressant effects of the isoflurane anaesthesia on hypothalamic thermoregulatory centre (Steagall *et al.*, 2008) and vasodilation caused by acepromazine (Lemke, 2007). Additionally, postsurgical sedation which reduces metabolism and impacts on thermoneogenesis could have contributed to the observed decrease in body temperature (Armstrong *et al.*, 2005). The prolonged low rectal temperature in the meloxicam group could be associated with its excellent anti-inflammatory effects (Leeset *et al.*, 1991; Mathews, 1996).

4.5 Conclusions

The following conclusions can be drawn from this study:

4.5.1 Ovariohysterectomy causes acute moderate to severe postoperative pain in dogs. Pain therapy is therefore warranted following this procedure so as to avoid deleterious physiological effects associated with untreated pain and to improve on surgical outcomes.

4.5.2 Meloxicam alone and the butorphanol-meloxicam drug combination produce similar levels of analgesia in dogs after ovariohysterectomy, but use of meloxicam alone requires a top-up dose at least 6 hours after the initial meloxicam injection.

4.5.3 Butorphanol produces very short-lived analgesia that is associated with severe sedation and hypotension. Thus when it is used on its own to manage pain after spay, there is need for repeated doses at least every 2 hours and careful monitoring.

CHAPTER FIVE

5.0 EFFECTS OF BUTORPHANOL, MELOXICAM AND BUTORPHANOL-MELOXICAM COMBINATION ON POST-OPERATIVE STRESS AFTER OVARIOHYSTERECTOMY IN DOGS

5.1 Introduction

Stress is a state in which both internal and external environmental demands exceed an individual's perceived ability to cope, thereby resulting in behavioral and physiological changes (Vileikyte, 2007). Perioperative stress in canine patients is prevalent and is attributed to factors like anxiety, excitement from handling, hospitalization, fear, depression, anaesthesia, tissue damage and pain (Fox *et al.*, 2000; Beilin *et al.*, 2003).

Physiologically, stress activates the hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes resulting in downstream hormonal and immunological changes (Upton and Solowiej, 2010). The hormones involved include adrenocorticotrophic hormone like cortisol and catecholamines [epinephrine and norepinephrine] (Padgett and Glaser, 2003; Dickerson and Kemeny, 2004; Glaser and Kiecolt-Glaser, 2005). Cortisol is the most important hormone as it: stimulates gluconeogenesis and catabolism; increases sensitivity of blood vessels to adrenaline resulting in increased heart rate and blood pressure; affects lymphoid organs causing increased production of neutrophils, thrombocytes and erythrocytes; reduces the levels of pro-inflammatory cytokines like interleukin-6; and affects the activity of important enzymes like matrix metalloproteinases (Kudoh *et al.*, 2001; Freeman *et al.*, 2010). These pro-inflammatory cytokines and enzymes play a key role in tissue repair and their reduction post-operatively has been associated with delayed wound healing (Upton and Solowiej, 2010).

Studies in human patients have shown that increased postoperative stress is associated with wound infection (Beilin and Shavit, 2003), delayed wound healing (Broadbent *et al.*, 2003; Ebrecht *et al.*, 2004; Goiun *et al.*, 2008), prolonged hospitalization and increased cost of treatment (Morrison *et al.*, 2003). Although some studies have been done in veterinary patients to assess the relationship between postoperative pain and stress (Mastrocinque and Fantoni, 2003; Matičić *et al.*, 2010), there is very limited information regarding the effect of stress on wound healing in animals.

Owing to these negative effects, attenuation of stress response perioperatively is necessary in minimizing morbidity like wound infections and improving overall surgical outcomes (Benson *et al.*, 2000). A holistic approach should be adopted while designing such a treatment protocol due to the multifactorial nature of factors that predispose patients to stress perioperatively. Supportive therapy and systemic administration of analgesics can decrease stress response in patients and are most effective when administered preemptively (Woolf and Chong, 1993; Benson *et al.*, 2000).

In dogs undergoing ovariohysterectomy, administration of analgesics like butorphanol (Inoue *et al.*, 2006), sulfentanil and carprofen (Slingsby *et al.*, 2006), vedaprofen, ketoprofen and carprofen (Selmi *et al.*, 2009), dexketoprofen (Saritas *et al.*, 2015), epidural morphine and fentanyl patches (Pekcan and Koc, 2010), has been shown to reduce cortisol, a key marker of stress in dogs. However, there is no documented report on the effectiveness of meloxicam, butorphanol and their combination in managing acute postoperative stress in dogs. The aim of this study was therefore to evaluate the effects of butorphanol, meloxicam and butorphanol-meloxicam combination on post-operative stress following ovariohysterectomy in dogs.

5.2 Materials and methods

The design of the study, selected animals, experimental drugs and dosages, treatments, experimental procedure and disposal of sharps and biological waste were carried out as described earlier in sections 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5, and 4.2.6, respectively.

5.2.1 Evaluation of parameters

Evaluation of parameters was done by a veterinarian experienced in the use of different pain scoring systems and interpretation of signs of pain in dogs. The assessor was blinded to the analgesic treatments given to dogs in each group to avoid being biased.

5.2.1.1 Duration of anaesthesia and surgery

The duration of anaesthesia and duration of surgery were recorded. The duration of anaesthesia was defined as the time from administration of the premedicant acepromazine hydrochloride to the time of extubation. The endotracheal tube was removed upon restoration of laryngeal reflex as indicated by coughing. The duration of surgery was defined as the time from when the skin incision was made to the time when the last skin suture was placed. Duration of anaesthesia and surgery were then compared between groups.

5.2.1.2 Assessment of postoperative stress response

Postoperative stress response was assessed by using hormonal, metabolic and immune responses.

5.2.1.2 (a) Blood sample collection

An intravenous catheter was placed in the cephalic vein of each acepromazine sedated dog. About 3 ml of blood was collected from each dog before surgery (for baseline parameters) and postoperatively at 1, 2, 4, 6 and 24 hours after surgery.

5.2.1.2 (b) Assessment of hormonal changes

Serum cortisol was measured to determine stress induced hormonal changes as described by Tsai *et al.*, (2013) with slight modifications. During each sampling period, 2.0 ml of blood was collected in vacutainer tubes with clot activator. Blood was immediately centrifuged for 10 minutes at 3500 revolutions per minute and serum was separated. Serum samples were then stored at -20 °C and assayed for cortisol at the end of collection and processing period. Cortisol was assayed by means of fluoroimmunoassay using canine serum cortisol ELISA kits (ENZO®- Enzo Life Sciences, Farmingdale, Newyork, USA) and following manufacturers recommendations.

5.2.1.2 (c) Assessment of metabolic changes

Blood glucose was measured to determine stress induced metabolic changes as described by Tsai *et al.*, (2013) with slight modifications. Blood glucose was measured immediately after sample collection using a canine glucometer (AlphaTRAK® Blood Glucose Monitoring System, Zoetis, Kalamazoo, Michigan-USA).

5.2.1.2 (d) Assessment of immunological changes

Stress induced immunological changes were determined by assessing hematological parameters and neutrophil-lymphocyte ratio as described by Dąbrowski and Wawron (2014) with slight modifications. In summary, 1.0 ml of blood was collected in vacutainer tubes with

EDTA. The blood samples were then evaluated for the following hematological parameters using an automatic cell counter (Celltac Alpha MEK-6450®, Nihon Kodhen Corporation, Tokyo-Japan): total erythrocyte count (TEC) in millions/mm³, total platelet count (TPC) in millions/mm³, total leucocytes count (TLC) in millions/mm³, packed cell volume (PCV) in %, hemoglobin concentration (HB) in g/dl and differential cell count (Neutrophils, Basophils, Eosinophils, Monocytes and Lymphocytes). Neutrophil-lymphocyte ratio was then computed at each sampling period and compared within the group and between groups.

5.2.2 Data management and analysis

Data were entered into Microsoft Office Excel then verified and validated as correct entries based on the data collection sheets. Data were then imported into R statistical software (R[®], The R Foundation for Statistical Computing, Vienna, Austria) for computation of means and *p* values. Statistical significant was set at a value of $p < 0.05$.

Parametric data (Duration of anaesthesia, duration of surgery, heart rate, respiratory rate, blood pressure, rectal temperature, plasma cortisol, blood glucose, packed cell volume, haemoglobin concentration, total erythrocyte count, total leukocyte count, total platelet count, neutrophil count, lymphocyte count, monocyte count, neutrophil-lymphocyte ratio) were expressed as Mean \pm SD. Means \pm SD were then compared within and between the four treatment groups (Butorphanol group, Meloxicam group, Butorphanol-meloxicam combination group and Control group) at each monitoring and assessment times using ANOVA for repeated measures. Pooled data were used for between group comparisons. Where significant difference was indicated by ANOVA, Bonferroni corrected student t-test was applied to determine statistical differences between the groups.

5.3 Results

5.3.1 Changes in serum cortisol

5.3.2.1 Serum cortisol levels within the treatment groups

Relative to baseline values, there was significant ($p<0.05$) increase in serum cortisol in dogs under butorphanol, meloxicam and those in the control group while only a relative increase was observed in dogs under the butorphanol-meloxicam drug combination (Table 5-1 and Figure 5-1).

In dogs under butorphanol, serum cortisol increased significantly ($p<0.05$) from a baseline value of 2.5 ± 1.3 $\mu\text{g/dl}$ to a value of 7.0 ± 1.7 $\mu\text{g/dl}$, at one hour postoperatively. Serum cortisol in dogs in this group remained significantly ($p<0.05$) higher than baseline value up to 4 hours postoperatively, when a value of 6.7 ± 0.6 $\mu\text{g/dl}$ was recorded. From 4 hours onwards, a gradual decline was observed and by the end of the 24 hours monitoring period, serum cortisol concentration (2.3 ± 0.7 $\mu\text{g/dl}$) was slightly lower than baseline value (Table 5-1 and Figure 5-1).

In dogs under meloxicam, serum cortisol concentration increased significantly ($p<0.05$) from a baseline value of 3.1 ± 1.1 $\mu\text{g/dl}$ to 6.8 ± 3.1 $\mu\text{g/dl}$, at one hour postoperatively. Following this, serum cortisol continued to increase reaching the highest value of 7.0 ± 2.5 $\mu\text{g/dl}$ at 2 hours postoperatively. Serum cortisol in dogs in this group then remained significantly ($p<0.05$) higher than baseline value through to 4 hours postoperatively when a value of 4.8 ± 1.2 $\mu\text{g/dl}$ was recorded. By the end of 24 hours monitoring period, serum cortisol concentration in this group (2.4 ± 1.0 $\mu\text{g/dl}$) had declined to below baseline value (Table 5-1 and Figure 5-1).

In dogs under the butorphanol-meloxicam drug combination, serum cortisol increased from a baseline value of 3.0 ± 1.2 $\mu\text{g}/\text{dl}$ to the highest recorded value of 4.8 ± 1.8 $\mu\text{g}/\text{dl}$ at 2 hours, postoperatively. Thereafter, there was a gradual decline in the serum cortisol concentration and by 24 hours postoperatively, the concentration (2.1 ± 0.7 $\mu\text{g}/\text{dl}$) was lower than baseline value (Table 5-1 and Figure 5-1).

Serum cortisol concentration in dogs in the control group increased significantly ($p < 0.05$) from a baseline value of 3.5 ± 2.2 $\mu\text{g}/\text{dl}$ to the highest value of 8.6 ± 4.6 $\mu\text{g}/\text{dl}$ recorded at one hour postoperatively (Figure 5-1). Serum cortisol concentration remained significantly ($p < 0.05$) higher than baseline value through to 4 hours postoperatively when a value of 6.7 ± 3.9 $\mu\text{g}/\text{dl}$ was recorded. Thereafter, the concentration followed a downward trend and by 24 hours postoperatively, serum cortisol concentration in dogs in this group (2.6 ± 1.2 $\mu\text{g}/\text{dl}$) was below baseline value (Table 5-1 and Figure 5-1).

5.3.2.2 Comparison of serum cortisol concentration between the treatment groups

Despite dogs under butorphanol-meloxicam combination having lower serum cortisol concentration (3.7 ± 1.1 $\mu\text{g}/\text{dl}$) as compared to that in dogs under butorphanol (4.9 ± 2.1 $\mu\text{g}/\text{dl}$), meloxicam (4.5 ± 1.8 $\mu\text{g}/\text{dl}$) and in those in the control group (5.5 ± 2.4 $\mu\text{g}/\text{dl}$), the differences were not statistically significant ($p = 0.36$).

Table 5-1: Changes in serum cortisol concentration following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Sampling times	Butorphanol	Meloxicam	But-Mel	Control
0 Hour	2.5±1.3	3.1±1.1	3.0±1.2	3.5±2.2
1 Hour	7.0±1.7*	6.8±3.1*	4.6±1.6	8.6±4.6*
2 Hour	7.0±1.0*	7.0±2.5*	4.8±1.8	8.4±5.8*
4 Hour	6.7±0.6*	4.8±1.2*	4.5±1.7	6.7±3.9*
6 Hour	5.2±1.6	4.2±2.0	4.0±1.3	5.2±2.7
12 Hour	3.4±1.4	2.9±1.2	2.8±0.9	3.7±1.6
24 Hour	2.3±0.7	2.4±1.0	2.1±0.7	2.6±1.2

Key: * Changes in mean cortisol levels from mean baseline values significant at $p < 0.05$.

But-Mel: Butorphanol-meloxicam combination

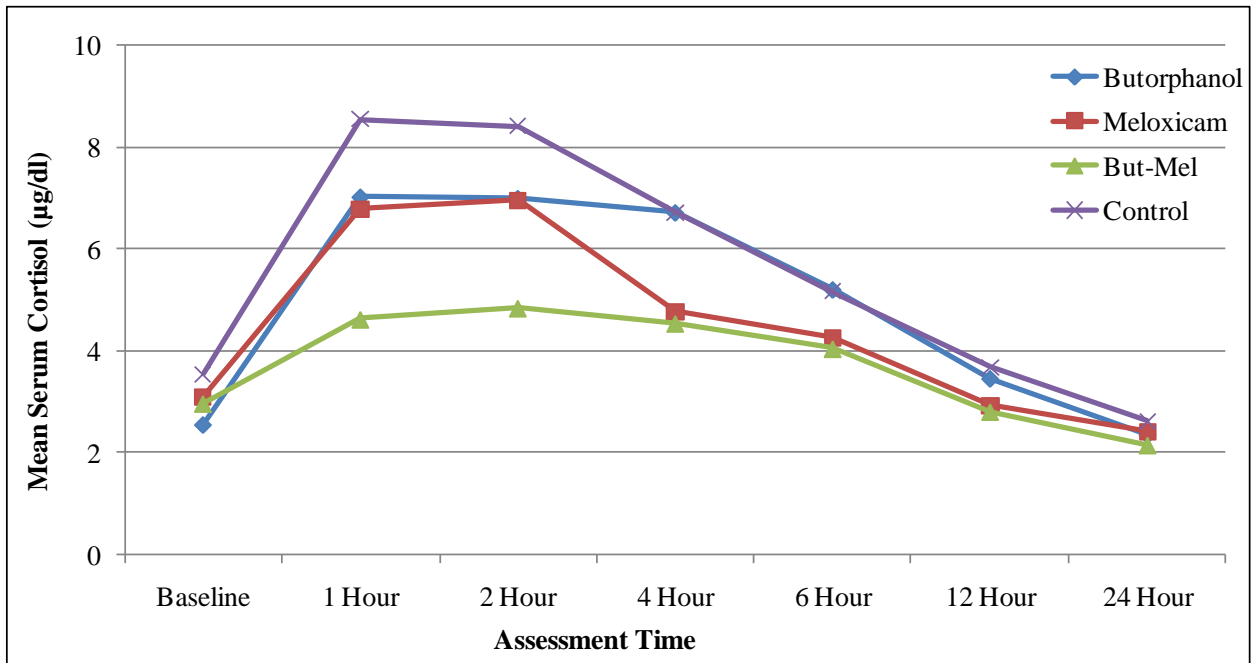


Figure 5-1: Trend in mean serum cortisol concentration following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariectomy.

5.3.2 Changes in blood glucose

5.3.2.1 Blood glucose levels within the treatment groups

Relative to baseline values, there was significant ($p<0.05$) increase in blood glucose in dogs under butorphanol, meloxicam and those in the control group. However, this increase was only relative in dogs under the butorphanol-meloxicam drug combination (Table 5-2 and Figure 5-2).

In dogs under butorphanol, blood glucose concentration increased significantly ($p<0.05$) from a baseline value of 5.0 ± 0.7 mmol/l to a value of 6.3 ± 1.3 mmol/l, at one hour postoperatively. Blood glucose concentration in dogs in this group remained significantly ($p<0.05$) higher than baseline value up to 4 hours postoperatively when a value of 5.9 ± 1.2 mmol/l was recorded. From hour 4 onwards, a gradual decline was observed and by the end of the 24 hours monitoring period, the blood glucose concentration (4.8 ± 1.1 mmol/l) was below the recorded baseline value (Table 5-2 and Figure 5-2).

In dogs under meloxicam, blood glucose concentration increased significantly ($p<0.05$) from a baseline value of 4.2 ± 0.8 mmol/l to 5.8 ± 0.6 mmol/l, at one hour postoperatively. Following this, blood glucose concentration continued to increase reaching the highest value of 5.9 ± 1.0 mmol/l at 2 hours postoperatively. Blood glucose concentration in this group then remained significantly ($p<0.05$) higher than baseline value through to 12 hours postoperatively when a value of 5.2 ± 0.7 mmol/l was recorded. By the end of 24 hours monitoring period, blood glucose concentration in dogs (4.3 ± 0.9 mmol/l) in this group had declined but was still slightly above baseline value (Table 5-2 and Figure 5-2).

In dogs under the butorphanol-meloxicam drug combination, blood glucose concentration increased from a baseline value of 4.6 ± 1.1 mmol/l to the highest recorded value of 5.4 ± 1.0 mmol/l at 1 hour, postoperatively. There after, an up and down pattern was observed with glucose concentration decreasing to 4.7 ± 1.0 mmol/l at 2 hours postoperatively, only to rise again to 4.9 ± 1.0 mmol/l at 4 hours postoperatively. Thereafter, there was a gradual decline in the blood glucose concentration and by 24 hours postoperatively, the concentration (4.0 ± 1.3 mmol/l) was below baseline value (Table 5-2 and Figure 5-2).

Blood glucose concentration in dogs in the control group increased significantly ($p < 0.05$) from a baseline value of 4.7 ± 0.7 mmol/l to the highest value of 6.6 ± 1.4 mmol/l recorded one hour postoperatively (Figure 5-2). Blood glucose concentration remained significantly ($p < 0.05$) higher than baseline value through to 6 hours postoperatively when a value of 5.6 ± 0.8 mmol/l was recorded. Although the glucose concentration followed a downward trend after this, by 24 hours postoperatively, the concentration (5.2 ± 0.6 mmol/l) was still above baseline value (Table 5-2 and Figure 5-2).

Table 5-2: Changes in mean blood glucose concentration following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Sampling times	Butorphanol	Meloxicam	But-Mel	Control
0 Hour	5.0±0.7	4.2±0.8	4.6±1.1	4.7±0.7
1 Hour	6.3±1.3*	5.8±0.6*	5.4±1.0	6.6±1.4*
2 Hour	6.1±1.0*	5.9±1.0*	4.7±1.0	6.3±1.6*
4 Hour	5.9±1.2*	5.6±0.7*	4.9±1.0	5.9±1.1*
6 Hour	5.7±0.9	5.5±0.6*	4.8±1.0	5.6±0.8*
12 Hour	5.4±1.3	5.2±0.7*	4.7±0.7	5.2±0.8
24 Hour	4.8±1.1	4.3±0.9	4.0±1.3	5.2±0.6

Key: * Mean blood glucose concentration values are significant at $p < 0.05$ when compared to the mean baseline values; **But-Mel:** Butorphanol-Meloxicam drug combination

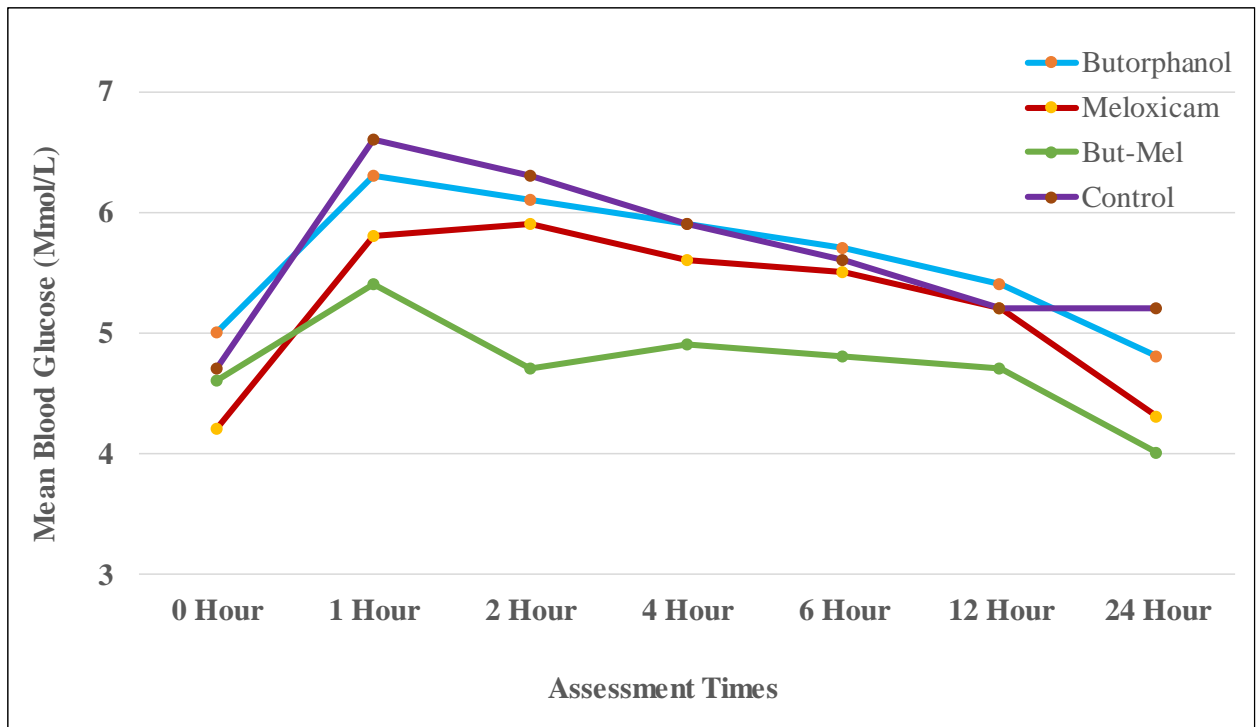


Figure 5-2: Trend in mean blood glucose concentration levels following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

5.3.2.2 Comparison of blood glucose concentration between the treatment groups

There was a significant ($p=0.03$) difference in the blood glucose concentration between the four treatment groups. Dogs under the butorphanol-meloxicam drug combination had significantly lower blood glucose concentration (4.7 ± 0.4 mmol/l) compared to those under butorphanol (5.6 ± 0.6 mmol/l, $p=0.008$) and those in the control group (5.6 ± 0.7 mmol/l, $p=0.01$). However, despite dogs under the butorphanol-meloxicam drug combination having lower blood glucose concentration as compared to those under meloxicam (5.2 ± 0.7 mmol/l), this difference was not significant ($p=0.16$). Similar observations were made for comparisons of blood glucose concentrations in dogs under meloxicam and butorphanol ($p=0.28$); meloxicam and the control group ($p=0.26$); and butorphanol and the control group ($p=0.88$).

5.3.3 Neutrophil-Lymphocyte ratio (NLR)

5.3.3.1 Trends in neutrophil-lymphocyte ratio within the treatment groups

The neutrophil-lymphocyte ratio increased significantly ($p<0.05$) from baseline values in dogs treated with butorphanol, meloxicam and those in the control group but only relatively in dogs treated with butorphanol-meloxicam drug combination (Table 5-3 and Figure 5-3).

In animals treated with butorphanol, the neutrophil-lymphocyte ratio increased from a baseline value of 4.8 ± 1.0 and reached a significantly ($p< 0.05$) higher value of 15.0 ± 6.6 at 4 hours postoperatively, as compared to baseline value. The neutrophil-lymphocyte ratio in this group remained significantly ($p< 0.05$) higher (14.8 ± 7.4) than baseline value, up to 12 hours after surgery. On the other hand, the neutrophil-lymphocyte ratio in dogs under meloxicam was significantly ($p< 0.05$) higher than baseline value (5.4 ± 1.6) at 4 hours (12.7 ± 5.5) and 6 hours (14.2 ± 8.1), postoperatively.

In dogs under the butorphanol-meloxicam drug combination, the neutrophil-lymphocyte ratio rose gradually from a baseline value of 5.8 ± 3.7 to peak at 4 hours (11.4 ± 6.5). The neutrophil-lymphocyte ratio in this group (10.3 ± 4.9) was still higher than baseline value by the end of 24-hour monitoring period (Table 5-3 and Figure 5-3).

The neutrophil-lymphocyte ratio in dogs in the control group increased from a baseline value of 4.9 ± 1.7 to reach a significantly ($p < 0.05$) higher value of 9.3 ± 2.7 at 4 hours postoperatively, when compared to baseline value. Thereafter this ratio remained significantly ($p < 0.05$) higher than baseline value through 6 hours (15.0 ± 6.2), 12 hours (16.6 ± 12.1) and 24 hours (13.7 ± 8.1), postoperatively (Table 5-3 and Figure 5-3).

5.3.3.1 Comparison of neutrophil-lymphocyte ratio between the treatment groups

There were no significant differences in the neutrophil-lymphocyte ratios in dogs between the four treatment groups ($p=0.52$). However, the mean neutrophil-lymphocyte ratios in dogs under meloxicam (9.1 ± 3.6) and butorphanol-meloxicam drug combination (9.1 ± 2.1) were relatively lower than those in dogs under butorphanol and those in dogs in the control group (11.9 ± 5.4 and 10.7 ± 4.4 , respectively).

Table 5-3: Changes in neutrophil-lymphocyte ratio following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Sampling time	Butorphanol	Meloxicam	But-Mel	Control
0 Hour	4.8±1.0	5.4±1.6	5.8±3.7	4.9±1.7
1 Hour	7.9±4.5	5.1±2.9	6.5±2.8	6.9±3.3
2 Hour	8.9±4.1	7.6±2.9	9.1±4.9	8.2±3.3
4 Hour	15.0±6.6*	12.7±5.5*	11.4±6.5	9.3±2.7*
6 Hour	20.9±8.4*	14.2±8.1*	10.3±4.9	15.0±6.2*
12 Hour	14.8±7.4*	11.2±6.1	10.2±4.8	16.6±12.1*
24 Hour	10.9±4.1	7.7±4.0	10.3±4.9	13.7±8.1*

Key: * Mean neutrophil-lymphocyte ratio values are significant at $p < 0.05$ when compared to the mean baseline values; **But-Mel:** Butorphanol-Meloxicam drug combination

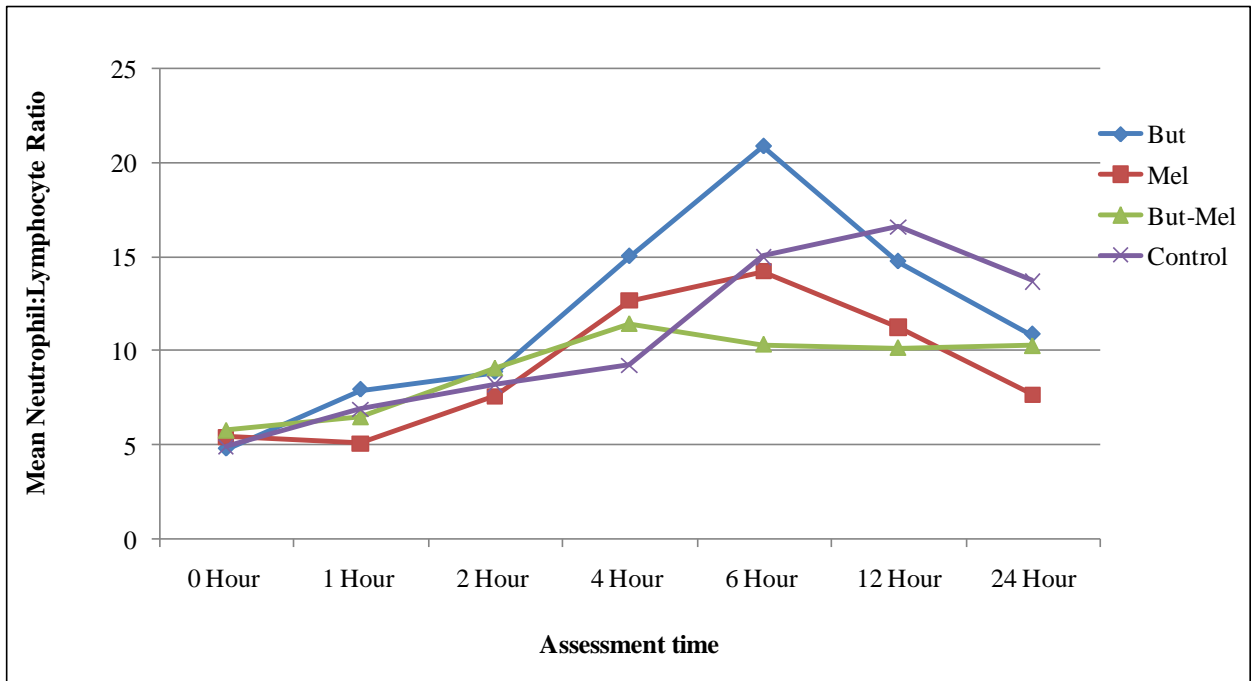


Figure 5-3: Trend in neutrophil-lymphocyte ratio following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

5.3.4 Changes in hematological parameters

Overall, administration of butorphanol, meloxicam, and the butorphanol-meloxicam drug combination in dogs in the postoperative period, resulted in significant ($p < 0.05$) changes in total leucocyte count, total erythrocyte count, neutrophil count, lymphocyte count, packed cell volume and hemoglobin concentration (Table 5-4, Table 5-5 and Table 5-6).

5.3.4.1 Total leucocytes count (TLC)

An initial significant ($p < 0.05$) decrease in TLC values was observed in dogs treated with butorphanol one hour postoperatively. The TLC decreased from a baseline value of 15716.7 ± 4044 million/ mm^3 to 11275.0 ± 3573 million/ mm^3 at one hour after surgery. Thereafter, the TLC increased to 26350.0 ± 5374 million/ mm^3 , at 6 hours postoperatively, a value significantly ($p < 0.05$) higher than baseline value. The TLC value in this group remained significantly ($p < 0.05$) higher than baseline value up to 24 hours postoperatively, when a value of 29091.7 ± 7780 million/ mm^3 was recorded. A similar trend was observed in dogs under meloxicam where TLC declined from a baseline of 13827 ± 3296 million/ mm^3 to reach the lowest value of 10070 ± 2728 million/ mm^3 , at one hour after surgery, a value significantly ($p < 0.05$) lower than baseline reading. The TLC in this group of dogs then started to rise, to record a value of 19833 ± 4486 million/ mm^3 at 4 hours postoperatively, a figure significantly ($p < 0.05$) higher than baseline value. From 4 hours onwards, TLC in this group remained significantly ($p < 0.05$) higher than baseline reading up to the end of the 24 hours monitoring period when a value of 22990 ± 8363 million/ mm^3 was recorded. In dogs under the butorphanol-meloxicam drug combination, TLC values were significantly ($p < 0.05$) higher than baseline value at 6, 12 and 24 hours postoperatively. Similarly, TLC values in animals in the control group were significantly ($p < 0.05$) higher compared to the baseline value (13436 ± 3804 million/ mm^3) from

4 hours (18327 ± 4989 million/ mm^3) through to 24 hours (25050 ± 10653 million/ mm^3), postoperatively (Table 5-4).

5.3.4.2 Total platelet count (TPC)

There were no significant changes in total platelet count in dogs in the four treatment groups as shown in Table 5-4

5.3.4.3 Total erythrocyte count (TEC)

There was significant ($p < 0.05$) decline in TEC in dogs in all four treatment groups as shown in Table 5-4.

In animals under butorphanol, TEC significantly ($p < 0.05$) decreased from a baseline of 7.1 ± 0.9 million/ mm^3 to reach a low value of 5.1 ± 0.8 million/ mm^3 at one hour postoperatively. The TEC remained significantly lower than baseline level up to 24 hour (5.8 ± 1.2 million/ mm^3), postoperatively. A similar trend in TEC was observed in dogs in the control group, where significantly ($p < 0.05$) lower TEC values were recorded at 1 hour (5.4 ± 1.4 million/ mm^3) and 24 hours (6.2 ± 1.2 million/ mm^3) postoperatively, when compared to the baseline value of 7.4 ± 0.9 million/ mm^3 .

In dogs injected with meloxicam, TEC decreased significantly ($p < 0.05$) from a baseline of 7.5 ± 1.2 million/ mm^3 to reach a low value of 5.4 ± 1.1 million/ mm^3 at one hour postoperatively. The TEC remained significantly lower than baseline value (6.2 ± 1.1 million/ mm^3) up to 12 hours after surgery. A similar trend in TEC was observed in animals under the butorphanol-meloxicam drug combination whose TEC values were significantly ($p < 0.05$) lower than baseline value (7.2 ± 1.1 million/ mm^3) at 1 hour (5.1 ± 0.9 million/ mm^3) through to 12 hours (5.9 ± 0.9 million/ mm^3), postoperatively.

5.3.4.4 Neutrophil count

Significant ($p < 0.05$) changes in neutrophil count were observed in dogs in all the four treatment groups (Table 5-5). In dogs injected with butorphanol, an initial significant ($p < 0.05$) drop in neutrophil count (to 9245 ± 3074 million/ mm^3) was observed relative to baseline values at one hour postoperatively. This was followed by a gradual increase reaching values significantly ($p < 0.05$) higher than baseline values (12661 ± 3669 million/ mm^3) at 4 hours (16755 ± 4481 million/ mm^3) through to 24 hours (26101 ± 7801 million/ mm^3), postoperatively.

A similar trend was observed in dogs under meloxicam, in which the neutrophil count declined from a baseline value of 12221 ± 4955 million/ mm^3 to 9350 ± 4813 million/ mm^3 at one hour, and then rose to 17492 ± 4396 million/ mm^3 at 4 hours, and 19977 ± 8279 million/ mm^3 at 24 hours, postoperatively. The neutrophil count in dogs under the butorphanol-meloxicam drug combination followed a similar trend but the increase was significant ($p < 0.05$) only from 6 hours (18103 ± 4933 million/ mm^3) through to 24 hours (19731 ± 6387 million/ mm^3), postoperatively, when compared to a baseline value of 12217 ± 3778 million/ mm^3 (Table 5-5).

In animals in the control group, the neutrophil count was significantly ($p < 0.05$) higher than baseline value (10784 ± 3650 million/ mm^3) from 4 hours (15857 ± 4761 million/ mm^3) through to 24 hours (22533 ± 10320 million/ mm^3), postoperatively.

Table 5-4: Changes in means of total leucocyte count (TLC), total platelet count (TPC) and total erythrocyte count (TEC) following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Hematological parameter	Sampling time	Butorphanol	Meloxicam	But-Mel	Control
TLC (Millions/mm ³)	0 Hour	15716.7±4044	13827±3296	15100±4338	13436±3804
	1 Hour	11275.0±3573*	10070±2728*	12209±4141	10600±4863
	2 Hour	13100.0±3704	14827±3171	15136±5530	13491±3987
	4 Hour	18750.0±4528	19833±4486*	17127±5612	18327±4989*
	6 Hour	26350.0±5374*	23708±5779*	21160±5093*	23409±5308*
	12 Hour	31941.7±9346*	24282±9020*	22373±6737*	27291±9080*
	24 Hour	29091.7±7780*	22990±8363*	22600±6575*	25050±10653*
TPC (Millions/mm ³)	0 Hour	309.0±176.2	274.4±199.7	192.1±174.7	367.8±205.7
	1 Hour	198.5±127.7	235.9±180.6	134.4±149.9	250.9±161.7
	2 Hour	201.4±136.0	197.6±157.7	106.5±88.4	276.2±185.0
	4 Hour	211.8±140.0	169.4±132.5	85.2±82.1	271.9±199.8
	6 Hour	237.8±136.9	208.7±127.8	79.2±49.6	233.5±122.3
	12 Hour	204.7±108.4	195.8±128.3	148.4±160.0	281.5±178.8
	24 Hour	224.6±127.4	262.3±190.5	155.2±139.3	336.5±171.2
TEC (Millions/mm ³)	0 Hour	7.1±0.9	7.5±1.2	7.2±1.1	7.4 ±0.9
	1 Hour	5.1±0.8*	5.4±1.1*	5.1±0.9*	5.4 ±1.4*
	2 Hour	5.1±0.8*	5.5±1.1*	5.2±0.9*	5.1 ±1.3*
	4 Hour	5.3±0.9*	5.6±1.2*	5.0±0.9*	5.3 ±1.2*
	6 Hour	5.4±1.0*	5.6±1.1*	5.3±0.5*	5.4 ±1.2*
	12 Hour	5.5±0.8*	6.2±1.1*	5.9±0.9*	6.0 ±1.4*
	24 Hour	5.8±1.2*	6.9±1.4	6.3±1.0	6.2 ±1.2*

KEY: * Indicate values that are significant at p<0.05 when compared to the mean baseline values; **But-Mel:** Butorphanol-Meloxicam drug combination

5.3.4.5 Lymphocyte count

Significant ($p < 0.05$) changes in lymphocyte count in the postoperative period were observed only in dogs injected with butorphanol, meloxicam and those in the control group (Table 5-5). In these three treatment groups, the mean lymphocyte counts were significantly ($p < 0.05$) lower from one hour to 6 hours postoperatively, when compared to the respective baseline values (Table 5-5).

5.3.4.6 Monocytes count

Significant ($p < 0.05$) changes in monocyte count were observed only in dogs in the control group. In this group, monocyte count increased from a baseline value of 497.9 ± 187.5 million/ mm^3 to significantly ($p < 0.05$) higher values of 851.6 ± 433.7 million/ mm^3 at 4 hours and 828.1 ± 332.0 million/ mm^3 at 6 hours, after surgery (Table 5-5).

5.3.4.7 Packed cell volume (PCV)

Significant changes in packed cell volume were observed in dogs in all the four treatment groups. In animals injected with butorphanol, PCV was significantly ($p < 0.05$) lower starting at one hour ($30.7 \pm 4.9\%$) through to 24 hours ($35.8 \pm 7.4\%$) postoperatively, when compared to a baseline value of $43.4 \pm 5.4\%$. A similar trend was observed in dogs in the control group whose PCV at one hour was $32.9 \pm 6.7\%$, while that at 24 hours was $37.9 \pm 6.6\%$, against a baseline value of $45.1 \pm 4.3\%$ (Table 5-6).

Dogs injected with meloxicam were found to have significantly ($p < 0.05$) lower PCV values from 1 hour ($32.0 \pm 5.4\%$) through to 12 hours ($37.9 \pm 5.8\%$) postoperatively, when compared to the baseline value ($45.2 \pm 5.2\%$). A similar trend was seen in dogs under the butorphanol-meloxicam drug combination where significantly ($p < 0.05$) lower PCV values were recorded at 1 hour ($31.9 \pm 4.8\%$) through to 12 hours ($37.6 \pm 5.2\%$) postoperatively, when compared to a baseline value of $44.2 \pm 4.7\%$ (Table 5-6).

Table 5-5: Changes in means of neutrophils, lymphocytes and monocytes following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Hematological parameter	Sampling Time	Butorphanol	Meloxicam	But-Mel	Control
Neutrophils (Millions/mm ³)	0 Hour	12661±3669	12221±4955	12217±3778	10784±3650
	1 Hour	9245±3074*	9350±4813*	10057±3893*	8715±4133
	2 Hour	11014±3277	13846±5283	12995±5545	11448±3505
	4 Hour	16755±4481*	17492±4396*	15008±5668	15857±4761*
	6 Hour	23973±4614*	20907±5393*	18103±4933*	21019±5424*
	12 Hour	28492±8269*	21471±8692*	19473±6718*	24766±9455*
	24 Hour	26101±7801*	19977±8279*	19731±6387*	22533±10320*
Lymphocyte (Millions/mm ³)	0 Hour	2307±791.0	2233±622.8	2170±901.1	2097±764.8
	1 Hour	1403±625.4*	1581±637.6*	1568±741.1	1384±638.2*
	2 Hour	1460±656.8*	1649±642.1*	1490±668.6	1408±609.0*
	4 Hour	1276±512.6*	1299±263.1*	1391±623.0	1583±559.1
	6 Hour	1088±473.7*	1631±681.1*	1970±726.6	1528±415.3*
	12 Hour	1990±652.5	1750±394.9	2143±792.3	1833±677.9
	24 Hour	2091±787.4	2386±788.7	1908±535.3	1869±696.8
Monocytes (Millions/mm ³)	0 Hour	631.7±185.8	545.6±286.8	563.8±250.5	497.9±187.5
	1 Hour	588.0±355.2	417.1±165.5	500.0±249.8	373.0±153.9
	2 Hour	501.3±252.4	526.5±184.5	565.2±344.1	599.1±301.8
	4 Hour	577.4±259.8	693.5±381.2	659.3±412.6	851.6±433.7*
	6 Hour	534.2±387.1	723.4±356.7	1035.0±460.6	828.1±332.0*
	12 Hour	686.5±285.3	595.9±291.8	463.5±100.1	467.8±114.8
	24 Hour	652.7±403.4	544.2±283.4	519.0±191.2	591.2±380.8

KEY: * Indicate values that are significant at p<0.05 when compared to the mean baseline values; **But-Mel:** Butorphanol-Meloxicam drug combination

5.3.4.8 Hemoglobin concentration (Hb)

Significant changes in hemoglobin concentration were observed in dogs in all the four treatment groups (Table 5-6). In dogs injected with butorphanol, Hb concentration was significantly ($p<0.05$) lower at one hour (10.1 ± 1.6 g/dl) and remained so, up to 24 hours (12.2 ± 1.4 g/dl) postoperatively, when compared to the baseline value of 14.1 ± 1.8 g/dl. A similar trend was observed in dogs in the control group whose Hb concentration at one hour was 10.2 ± 2.3 g/dl while that at 24 hours, was 12.5 ± 2.3 g/dl, against a baseline value of 14.7 ± 1.6 g/dl (Table 5-6).

Dogs injected with meloxicam were found to have significantly ($p<0.05$) lower Hb concentration values from 1 hour (10.5 ± 1.7 g/dl) through to 12 hours (12.3 ± 1.9 g/dl) postoperatively, compared to the baseline value (14.9 ± 1.8 g/dl). A similar trend was seen in animals under the butorphanol-meloxicam drug combination where significantly ($p<0.05$) lower Hb concentration values were observed from 1 hour (11.0 ± 2.5 g/dl) through to 12 hours (12.1 ± 1.9 g/dl) postoperatively, when compared to a baseline value of 14.5 ± 1.7 g/dl (Table 5-6).

5.3.4.9 MCH, MCV AND MCHC

There were no significant ($p>0.05$) changes recorded in mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) in dogs in all four treatment groups in the postoperative period (Table 5-6).

Table 5-6: Changes in means of packed cell volume (PCV), hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Hematological parameter	Sampling Time	Butorphanol	Meloxicam	But-Mel	Control
PCV	0 Hour	43.4±5.4	45.2±5.2	44.2±4.7	45.1±4.3
	1 Hour	30.7±4.9*	32.0±5.4*	31.9±4.8*	32.9±6.7*
	2 Hour	31.5±4.7*	33.0±5.6*	32.5±4.8*	31.0±6.6*
	4 Hour	32.7±5.5*	33.5±5.3*	31.3±5.4*	32.5±6.0*
	6 Hour	33.6±6.0*	34.3±5.0*	33.6±3.9*	33.9±6.8*
	12 Hour	34.2±3.8*	37.9±5.8*	37.6±5.2*	36.8±7.2*
	24 Hour	35.8±7.4*	41.3±7.0	39.7±7.4	37.9±6.6*
	Hb	0 Hour	14.1±1.8	14.9±1.8	14.5±1.7
1 Hour		10.1±1.6*	10.5±1.7*	11.0±2.5*	10.9±2.2*
2 Hour		10.3±1.5*	10.8±1.8*	10.7±1.6*	10.2±2.3*
4 Hour		10.6±1.8*	10.9±1.7*	10.3±1.7*	10.7±2.1*
6 Hour		10.9±2.0*	11.1±1.7*	11.0±1.1*	10.9±2.1*
12 Hour		11.2±1.4*	12.3±1.9*	12.1±1.9*	12.1±2.5*
24 Hour		12.2±1.4*	13.6±2.5	13.1±2.5	12.5±2.3*
MCH		0 Hour	19.9±1.7	19.9±1.6	20.6±1.4
	1 Hour	19.9±1.2	19.7±1.5	20.6±1.6	20.3±1.5
	2 Hour	20.0±1.2	19.9±1.6	20.5±1.4	20.4±1.6
	4 Hour	20.0±1.4	19.9±1.6	20.6±1.5	20.5±1.3
	6 Hour	20.1±1.4	19.8±1.8	20.7±1.3	20.3±1.2
	12 Hour	20.5±1.5	19.8±1.5	20.9±1.6	20.3±1.2
	24 Hour	20.2±1.3	19.8±1.5	20.6±1.3	20.4±1.3
	MCV	0 Hour	61.3±4.0	60.5±4.9	62.9±4.6
1 Hour		60.6±3.9	60.2±5.3	62.9±4.6	61.9±4.9
2 Hour		61.4±4.3	60.6±4.9	62.5±4.3	61.9±4.9
4 Hour		61.5±4.0	60.8±4.7	62.5±3.9	62.5±4.7
6 Hour		62.1±4.0	61.5±4.3	63.3±4.6	63.1±4.7
12 Hour		62.8±4.8	60.9±4.3	63.9±5.2	62.0±4.5
24 Hour		61.9±4.7	60.1±4.9	62.7±4.0	61.7±4.8
MCHC		0 Hour	32.6±0.9	32.8±0.9	32.8±0.9
	1 Hour	32.9±0.5	32.9±0.9	32.7±0.9	32.8±0.6
	2 Hour	32.6±0.7	32.9±0.4	32.9±0.9	33.0±1.1
	4 Hour	32.5±0.7	32.7±0.5	33.0±1.0	32.8±0.7
	6 Hour	32.4±0.9	32.3±0.7	32.7±1.4	32.2±1.2
	12 Hour	32.7±1.2	32.6±0.7	32.7±1.2	32.9±0.8
	24 Hour	32.7±0.7	33.0±0.8	32.8±0.6	33.0±0.6

KEY: * Indicate values that are significant at $p < 0.05$ when compared to the mean baseline values; **But-Mel:** Butorphanol-Meloxicam drug combination

5.4 DISCUSSION

In this study, maximum cortisol and glucose concentrations were recorded in the immediate postoperative period and this increase was significant in dogs injected with butorphanol, meloxicam and those in the control group, but relative in dogs under butorphanol-meloxicam drug combination. Similar results have been reported previously in dogs undergoing routine ovariohysterectomy (Inoue *et al.*, 2006; Selmi *et al.*, 2009). In the study conducted by Inoue *et al.*, (2006), anaesthesia was achieved using propofol and isoflurane while that of Selmi *et al.*, (2009), dogs were anaesthetized using propofol and halothane before ovariohysterectomy. This is comparable to the current study where propofol and the inhalant anaesthetic isoflurane were used for anaesthesia.

One possible explanation for this observation is that stress postoperatively is not only influenced by pain but also other factors that include surgical stress emanating from tissue handling and anaesthesia (Mathews, 1996; Inoue *et al.*, 2006). This could be the reason why cortisol and glucose were sustained in higher values in all the treatment groups up to 2 hours after surgery. The anaesthesia recovery period is particularly very important in small animal surgical patients and is characterized by hypothermia. The body compensates for hypothermia by increasing metabolism and initiating shivering response (Diaz and Becker, 2010). This is true for dogs in this study as shown in Chapter 4 in the current study, where significantly low temperatures were observed in dogs in all the four treatment groups. Hypothermia could have considerably increased stress response in the immediate postoperative period.

It has been previously reported that pain induced stress may persist for up to 5 hours after surgery in dogs if effective analgesia is not instituted (Fox *et al.*, 1998; Inoue *et al.*, 2006). This study has demonstrated in Chapter 4 that the butorphanol-meloxicam drug combination

provided better analgesia compared to individual drugs and saline. This may explain why dogs in this group did not have significant changes in cortisol and glucose concentration postoperatively.

In this study, hematological results showed a typical stress leukogram pattern characterized by lymphopenia and neutrophilia. Stress-induced reduction in circulating lymphocyte numbers is a result of glucocorticoid-induced alterations in the redistribution/trafficking of lymphocytes from the blood to other body compartments (Dhabhar, 2002). In response to glucocorticoid hormones, circulating lymphocytes adhere to the endothelial cells that line the walls of blood vessels. Subsequently, lymphocytes undergo transmigration from circulation into other tissues like lymph nodes, spleen, bone marrow and skin, where they are sequestered (Dhabhar, 2002). This migration of lymphocytes from the blood causes a significant reduction in their circulating numbers. In contrast, glucocorticoid hormones stimulate an influx of neutrophils into the blood from bone marrow and attenuate neutrophils migration from the blood to other compartments, resulting in an overall neutrophilia (Bishop *et al.*, 1968). These changes are thought to ensure that the different types of cells are routed to where they are needed most during stress (Dhabhar *et al.*, 1994; Dhabhar *et al.*, 1996).

Neutrophil-lymphocyte ratio (NLR) is an important composite measure and reliable indicator of stress response in mammals. Increase in NLR has been positively correlated to stress hormones in cattle (Anderson *et al.*, 1999), sows (Kranendonk *et al.*, 2005) and boars (Bilandzic *et al.*, 2006). Further, studies have shown that exposure of goats, pigs and horses to variable stressors resulted in significant increase in stress hormones and neutrophil-lymphocyte ratio (Obernier and Baldwin, 2006).

In the current study, the neutrophil-lymphocyte ratio increased significantly in dogs in the control and individual drug groups but this increase was not significant in dogs under the butorphanol-meloxicam drug combination. This is a further prove that the changes in leucocytes, following exposure to a stressful event as it was the case for dogs in this study, is driven by glucocorticoid hormones. This augment is supported by the observation that dogs under the butorphanol-meloxicam drug combination had lower glucose concentration, reflecting lower stress levels, compared to those in the control, and those under butorphanol and meloxicam.

Interestingly, compared to glucose, neutrophil-lymphocyte ratios in the four groups took longer to reach maximum levels and were still higher than baseline values at the end of the 24 hours monitoring period. This observation is consistent with the results from other studies (Davis, 2005; Lindströmet *et al.*, 2005; Swan and Hickman, 2014) and signifies that glucocorticoid and neutrophil-lymphocyte ratio are better indicators of acute and chronic stress, respectively (Davis *et al.*, 2008; Demir *et al.*, 2015).

In this study, there was a significant change in total erythrocyte count in all treatment groups. This could be attributed to sequestration of erythrocytes in the spleen and other organs like the liver, skeletal muscles and skin subsequent to anaesthesia induced vasodilation (Wilson *et al.*, 2004; Dhumeaux *et al.*, 2012). The observed significant changes in packed cell volume and hemoglobin concentration are as a result of reduction in circulating erythrocytes (Biermann *et al.*, 2012).

5.5 Conclusions

The following conclusions can be made from the current study:

5.5.1 Butorphanol and meloxicam as individual drugs were unable to effectively control postoperative stress in dogs following ovariohysterectomy.

5.5.2 Meloxicam produced relatively better postoperative stress management in dogs following ovariohysterectomy than butorphanol.

5.5.3 The butorphanol-meloxicam drug combination was effective in minimizing stress response in dogs following ovariohysterectomy.

5.5.4 Better stress management as indicated by lower cortisol, glucose, and neutrophil-lymphocyte ratio was observed in meloxicam and butorphanol-meloxicam drug combination treated dogs than in those in the control group, indicating that postoperative pain management is important in controlling postoperative stress.

CHAPTER SIX

6.0 EFFECTS OF BUTORPHANOL, MELOXICAM AND BUTORPHANOL-MELOXICAM COMBINATION ON WOUND HEALING AFTER OVARIOHYSTERECTOMY IN DOGS

6.1 Introduction

Wound healing is a normal biological process that consists of four highly integrated and overlapping phases namely homeostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). Studies have shown that delayed wound healing in humans and laboratory animals can be associated with post-operative pain and stress (Padgett *et al.*, 1998; Broadbent *et al.*, 2003). This is usually a biological cycle that starts by postoperative pain causing stress. Stress negatively influences the inflammatory phase of wound healing by reducing pro-inflammatory cytokines, which are supposed to function by attracting phagocytes to the wound site for clearance of infectious agents and for preparation of the site for new tissue growth (Barbul, 1990; Broadbent *et al.*, 2003). This position is supported by a previous report in mice showing that stress increased susceptibility of wounds to bacterial infection, hence delaying wound healing (Rojas *et al.*, 2002). Stress can also affect the remodeling phase of wound healing by regulating production and activation of matrix metalloproteinase enzymes, which are involved in degradation of collagen as well as facilitation of cellular invasion and migration into the wound (Pajulo *et al.*, 1999; Broadbent *et al.*, 2003).

Assessment of wound healing in veterinary patients can be achieved through clinical appearance, histopathology and ultrasonography (Sylvestre *et al.*, 2002; Abramo *et al.*, 2004;

Laiju *et al.*, 2005; Nisbet *et al.*, 2010). Ultrasound scanning of wounds enables repeated, noninvasive, quantitative assessment of structural changes deep within wounds, while histopathological assessment allows more precision but not serial examination of wounds (Abramo *et al.*, 2004). The effects of pain on wound healing following surgery in dogs and the resulting quality of wound healing have not been elucidated. It was therefore considered essential to evaluate these effects by managing pain using single and multimodal pain therapies following ovariohysterectomy in dogs. The drugs used for management of pain in this study were butorphanol and meloxicam, either alone or in their combination. There is also no available literature relating the inter-relationship of pain, stress and postoperative wound healing in dogs.

6.2 Materials and methods

The design of the study, selected animals, experimental drugs and dosages, treatments, experimental procedure and disposal of sharps and biological waste are outlined in sections 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5, and 4.2.6, respectively.

6.2.1 Evaluation of parameters

Following ovariohysterectomy and each treatment, every dog was subjected to assessment of parameters and the findings were recorded. The parameters assessed are outlined below.

6.2.1.1 Assessment of wound healing

Wound healing was assessed using clinical appearance and histopathology of the wound as described below.

6.2.1.1 (a) Clinical appearance of the surgical wound

Clinical appearance of the surgical wound was scored on day 1, 2, 3 and 8 postoperatively. The surgical wound was scored by the investigator based on swelling, erythema, dehiscence, and discharge (exudation) as outlined in Table 6-1. This scoring system is adapted from Sylvester *et al.*, (2002).

6.2.1.1 (b) Histopathological evaluation of the surgical wound

Histopathological evaluation was done by taking a biopsy of the surgical wound on day 1, 2, 3 and 8 postoperatively. Three dogs were systematically chosen from the 12 dogs in a group at each sampling time (24 hours, 48 hours, 72 hours and 8 days). The 3 dogs were anaesthetized as described in section 4.4.5 and a full thickness biopsy (extending from the skin to the peritoneum) of the surgical wound and part of the surrounding tissues collected. The

dimensions of the collected biopsy were 1 cm wide and 6 cm long. The wound created after collection of the biopsy was sutured routinely in three layers and meloxicam administered subcutaneously at 0.2mg/kg everyday for 3 days.

The biopsy samples were placed in appropriately labeled eppendorf tubes and fixed in 10% buffered formalin. The samples were then processed routinely, cut and mounted on microscope slides as described by Nisbet *et al.* (2010). The tissue sections were examined under a light microscope and photo-micrographs taken using a digital camera coupled to a microscope.

The histopathology parameters that were assessed are: the population of neutrophils, macrophages and fibroblasts; the extent of neovascularization; collagen lay-down; and epithelialization. Subjective measures/scores used in the current study for collagen, epithelialization and fibroblast population as well as the counts for neovascularization in wound healing are as reported by Nisbet *et al.* (2010) and these are given in Table 6.-2

Table 6-1: Parameters used as criteria for scoring the appearance of surgical wounds in dogs

Parameters	Descriptions
Swelling	Wound edges thicker than the surrounding tissues. Measurement from cranial, mid and caudal section of the wound to be taken and averaged to get the final wound swelling score.
Erythema	Redding of the skin around the wound. Measure the distance from the wound margins. Measurement from cranial, mid and caudal section of the wound to be taken and averaged to get the final erythema score.
Dehiscence	Percentage of sutures removed by the dog. Record taken of the total number of sutures used to close the skin incision. At each examination period, record the number of sutures removed. Calculate the percentage of sutures removed in each dog.
Discharge	Any serous, serosanguinous and purulent discharge observed from the surgical wound at each examination period recorded.

Table 6-2: Scoring system for histopathological tissues evaluation of various parameters in wound healing

Parameter	Score			
	0	1	2	3
Collagen	None	Scanty	Moderate	Abundant
Epithelialization	None	Partial	Complete but immature/thin	Complete and mature with keratinization
Neovascularization	None	Up to 5 vessels/ HPF	6-10 vessels/ HPF	>10 vessels/ HPF
Fibroblast	None/minimal	Few	Moderate fibroblast	Predominant
Macrophages	None	Up to 20 macrophages/ HPF	20-40 macrophages/ HPF	>41 macrophages/ HPF
Neutrophils	None	Few	Moderate number	Predominant

Key: HPF-High Power Field

Note: Histopathology scoring system is adopted from Nisbet *et al.*, 2010

6.2.2 Data management and analysis

Data were entered into Microsoft Office Excel, verified and validated as correct entries based on the data collection sheets. Data were then imported into StatPlus Pro 5.9.8 statistical software for computation of means and p values. Statistical significance was set at $p < 0.05$.

Non-parametric data were expressed as median and parametric data as means \pm SD for analysis and comparison within and between the four treatment groups. The median values were compared using the Kruskal-Wallis rank sum test. Where statistical differences were observed, Mann Whitney rank sum test was used as a post-hoc test. Means \pm SD values were compared using ANOVA for repeated measures. Where significant difference was indicated by ANOVA, a Bonferroni corrected student t-test was applied to determine statistical differences between treatments.

6.3 Results

6.3.1 Clinical wound appearance

6.3.1.1 Wound Swelling

Wound swelling was observed in all dogs in the four treatment groups after ovariohysterectomy. The swelling increased gradually beginning 24 hours postoperatively, with maximal swelling at 48 hours for dogs in butorphanol, butorphanol-meloxicam drug combination and the control groups, but at 72 hours for those in meloxicam group. Measurements of the wound swellings (means±sd) in the four treatment groups are shown in Table 6-3.

Wound swelling was significantly ($p<0.05$) more in dogs treated with butorphanol at 48 hours (3.1 ± 3.2 cm) and 72 hours (3.0 ± 0.0 cm) when compared to the swelling at 24 hours (0.2 ± 0.4 cm). Wound swelling was still present on day 8 after surgery, but this was not significant when compared to what was observed at 24 hours. Similar observations were made in dogs treated with the butorphanol-meloxicam combination, in which wound swelling increased significantly from a value of 0.2 ± 0.3 cm recorded 24 hours postoperatively to 3.0 ± 0.0 cm at 48 hours and 2.4 ± 1.6 cm at 72 hours, postoperatively. There was still swelling on day 8 postoperatively (0.3 ± 0.6 cm), but the size of the swelling was not significantly different from what was observed at 24 hours after surgery.

Dogs in the control group had significantly ($p<0.05$) more wound swelling at 48 hours (3.3 ± 1.0 cm), 72 hours (3.3 ± 0.8 cm) and on day 8 (2.4 ± 0.5 cm) postoperatively, compared to a value of 0.7 ± 1.2 cm recorded at 24 hours postoperatively. In dogs treated with meloxicam, wound swelling increased relatively from a baseline value of 0.1 ± 0.2 cm, reaching a peak of 2.0 ± 2.7

cm at 72 hours postoperatively, but reducing to zero (no swelling at all) by day 8, postoperatively. There were no significant ($p=0.32$) differences in wound swelling between the treatment groups. However, among the dogs treated with analgesics, the least wound swelling was in the meloxicam-treated group (0.7 ± 0.9 cm) and the most was in the control group (2.4 ± 1.2 cm).

6.3.1.2 Wound Erythema

Wound erythema was a clinical feature observed in all dogs in the four treatment groups. Generally, wound erythema was observable from 24 hours postoperatively, and its extent increased with increasing time such that at 48 hours and 72 hours, the extent was relatively more than what was observed at 24 hours postoperatively (Table 6-3). The most extensive wound erythema was observed in dogs in the control group, where the highest value of 0.90 ± 0.9 cm was observed 72 hours, postoperatively. The least extent of wound erythema was observed in the meloxicam-treated group where its size increased from a baseline value of 0.08 ± 0.2 cm at 24 hours to a value of 0.17 ± 0.4 cm at 48 hours, postoperatively. Dogs in the butorphanol-meloxicam group and those in the butorphanol group had a moderate extent of wound erythema. In dogs treated with meloxicam, wound erythema peaked at 48 hours, while in dogs treated with butorphanol, butorphanol-meloxicam and in the control group, wound erythema was at its peak at 72 hours postoperatively (Table 6-3). In dogs treated with meloxicam, wound erythema had cleared completely by day 8, postoperatively.

When the extent (means \pm sd) of wound erythema in dogs in the four treatment groups was compared, it was established that dogs treated with meloxicam had significantly less extensive wound erythema (0.08 ± 0.1 cm) as compared to that observed in dogs in the control group (0.59 ± 0.3 cm).

6.3.1.3 Wound Dehiscence

More wound dehiscence (as measured by the percentage suture removal) was observed in dogs in the control group (24.8±16.9%), followed by dogs in the butorphanol group (14.3±13.2%), then meloxicam group (6.4±7.9%) and butorphanol-meloxicam drug combination group (2.3±1.9%) - Table 6-3 and Figure 6-1. However, there were no significant differences in wound dehiscence between the different treatment groups (p=0.07).



Figure 6-1: Pictorial representation of the surgical wounds in the four treatment groups 8-days postoperatively. A demonstrates wound swelling in a dog treated with butorphanol, B demonstrates a wound in one of the dog under meloxicam that had healed without dehiscence, erythema or swelling, C demonstrates slight wound erythema in a dog treated with butorphanol-meloxicam drug combination and D demonstrates complete wound dehiscence in a dog in the control group. Also notice the wound swelling (Blue arrows).

Table 6-3: Clinical wound appearance following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Clinical Features	Observation time-points	Treatment Groups			
		Butorphanol	Meloxicam	Butorphanol-Meloxicam	Control
Wound Swelling (in centimeters)	24 Hours	0.2±0.4	0.1±0.2	0.2±0.3	0.7±1.2
	48 Hours	3.1±3.2*	0.6±0.6	3.0±0.0*	3.3±1.0*
	72 Hours	3.0±0.0*	2.0±2.7	2.4±1.6*	3.3±0.8*
	8 Days	0.8±0.7	0.0±0.0	0.3±0.6	2.4±0.5*
Wound Erythema (in centimeters)	24 Hours	0.10±0.2	0.08±0.2	0.04±0.1	0.39±0.9
	48 Hours	0.41±0.4	0.17±0.4	0.34±0.5	0.75±0.7
	72 Hours	0.64±0.6	0.08±0.1	0.53±0.7	0.90±0.9
	8 Days	0.33±0.4	0.00±0.0	0.26±0.3	0.33±0.6
Wound Dehiscence (% of sutures removed)	24 Hours	2.8±8.3	0.4±1.4	2.0±6.3	3.3±10.5
	48 Hours	11.1±18.2	8.3±17.7	4.6±8.5	23.8±37.1
	72 Hours	10.0±22.4	16.7±40.8	2.8±6.8	27.8±39.0
	8 Days	33.3±57.7	0.0±0.0	0.0±0.0	44.4±50.9

KEY: * Indicate value is significantly higher compared to the respective 24-hour value

6.3.2 Histopathological findings of the wounds

6.3.2.1 Collagen score

There was a significant ($p < 0.05$) difference in the wound collagen score from 24 hours through to 8 days postoperatively in all the treatment groups as shown in Table 6-4. The amount of collagen in wounds of butorphanol-treated dogs and those in the control group, was significantly ($p < 0.05$) higher at 72-hours and day 8 monitoring time-points (Score 2) as compared to baseline score (Score 0). In meloxicam treated dogs, the amount of collagen in wounds increased significantly ($p < 0.05$) from a baseline score of 1 to a score of 2 at 48-hours and to scores of 3 at 72-hour as well as day 8 of monitoring. Butorphanol-meloxicam drug combination-treated dogs also had the amount of collagen in their wounds increasing significantly ($p < 0.05$) to scores of 3 at 72-hours and day 8 of monitoring, from a score of 1 at 24 hours, postoperatively.

6.3.2.2 Epithelialization score

There were no significant differences in the levels of wound epithelialization in dogs across the four treatment groups. However, epithelialization increased towards day 8 of monitoring postoperatively and the epithelialization was relatively more complete in wounds of dogs treated with butorphanol, meloxicam and butorphanol-meloxicam drug combination as compared to what was observed in dogs in the control group (Table 6-4).

6.3.2.3 Neovascularization

The number of blood vessels in wounds of dogs treated with butorphanol increased significantly ($p < 0.05$) from baseline values (median score of 0) through to day 8 (score of 3), postoperatively. In dogs treated with the butorphanol-meloxicam drug combination, wound neovascularization increased significantly ($p < 0.05$) from baseline values through to day 8 postoperatively (median score of 1 to median score of 2 at 72 hours and median score of 3 at day 8). Comparisons of neovascularization scores between the treatment groups did not reveal any significant differences.

6.3.2.4 Fibroblasts

The number of fibroblasts in wounds of dogs in the control group increased significantly ($p < 0.05$) from the baseline value through day 8 postoperatively (score 1 and to scores 2 at 72 hours and day 8 of postoperative monitoring). The fibroblast scores at various monitoring time-points in wounds of dogs treated with butorphanol, meloxicam and butorphanol-meloxicam drug combination were not significantly different from their respective baseline values (Table 6-4).

6.3.2.5 Macrophages

The number of macrophages in wounds of dogs generally increased from their baseline values to reach the peak at 48 hours postoperatively, then declined towards day 8 of monitoring, in all treatment groups, except for those in the control group. In the control group, the median macrophage score increased from a baseline value of 0.5 to a score of 2 at 48 hours and remained at that level through day 8 of monitoring (Table 6-4). When compared between the treatment groups, all these changes in macrophage numbers were not significant.

6.3.2.6 Neutrophils

There was significant decrease ($p < 0.05$) in the number of neutrophils (median neutrophil score) in wounds of dogs treated with butorphanol and butorphanol-meloxicam. In these two groups, median neutrophil scores decreased from a baseline score of 2 to score 0 at 72-hour and day 8, postoperatively. Unlike in the other groups where an initial decrease in neutrophil numbers was observed, the number of neutrophils (neutrophil score) in the control group increased from a median score of 1 recorded at 24 hours to 3 at 48 hours after surgery. Thereafter, the neutrophil count started to decrease and reached score 1 at 72 hours, remaining so up to day 8, postoperatively (Table 6-4).

Changes in the amount of collagen, degree of epithelialization, neovascularization, number of fibroblasts, macrophages and neutrophils in the wounds of dogs were not significantly different when compared between the four treatment groups at all sampling time-points, postoperatively.

Table 6-4: Median scores for histopathological parameters following administration of butorphanol, meloxicam, butorphanol-meloxicam drug combination and placebo in dogs after ovariohysterectomy.

Histopathological Parameters	Assessment Time-point	Treatment groups			
		Butorphanol	Meloxicam	But-Mel	Control
Collagen	24 Hours	0	1	1	0
	48 Hours	1	2*	1	0
	72 Hours	2*	3*	3*	2*
	8 Days	2*	3*	3*	2*
Epithelialization	24 Hours	2	2	2	1
	48 Hours	2	2	2.5	1
	72 Hours	2	2	3	2
	8 Days	2.5	3	3	3
Neovascularization	24 Hours	0	2	1	0
	48 Hours	1	2	2	1
	72 Hours	1.5	2.5	2*	1
	8 Days	3*	3	3*	1.5
Fibroblasts	24 Hours	1	1	0.5	1
	48 Hours	2	2	1.3	1
	72 Hours	2	3	2	2*
	8 Days	2	3	3	2*
Macrophages	24 Hours	2	2	1	0.5
	48 Hours	3	3	3	2
	72 Hours	1	2	2	2
	8 Days	1	1	1	2
Neutrophils	24 Hours	2	1	2	1
	48 Hours	1	1	1	3
	72 Hours	0*	0	0*	1
	8 Days	0*	0	0*	1

KEY:* indicates that the value is significantly different at $p < 0.05$ compared to the 24-hour baseline value

6.4 Discussion

The results of this study indicate no significant differences in the clinical effects of the individual drugs, butorphanol and meloxicam and their combination, on wound healing in dogs following ovariohysterectomy. The butorphanol-meloxicam combination only showed a slight advantage over butorphanol on its own. The finding that meloxicam-treated dogs had significantly less extensive wound erythema, less swelling and dehiscence than those treated with butorphanol as well as those in the control group can be explained by meloxicam's preferential blockade of cyclooxygenase 2 (COX-2) enzyme, which results in antipyretic, analgesic and anti-inflammatory effects (Lees *et al.*, 1991; Mathews, 1996).

Oedema and soft tissue swelling that characterize inflammation, continuously stimulate nerve endings as well as nociceptors and cause increasingly more pain and stress. Inflammation-related pain can also be caused by production of neuropeptides that include substance P, neurokinin A, bradykinin and prostaglandins (Woo, 2012). The pain is likely to cause wound mutilation and pulling out of sutures by the dog, resulting in wound dehiscence, contamination and possible infection. Hence, the reason for the more extensive wound erythema and increased incidence of dehiscence in the wounds of dogs in the control group compared to those in groups treated with analgesics. This finding further shows the advantage of pain management in enhancing postoperative wound healing.

The finding that the combination of butorphanol and meloxicam did not demonstrate any significant additive benefit over the individual drugs, can probably be attributed to the small number of dogs per group which was low for detection of minor inter-group differences as previously observed (Tsai *et al.*, 2013). It could also be due to failure of butorphanol and meloxicam to exert their maximal effects on pain (Tsai *et al.*, 2013), which may probably be

attributable to use of half of their individual dosages when the two analgesics were combined. The fact that meloxicam has more anti-inflammatory effects than butorphanol explains the lower scores of clinical wound parameters in butorphanol-meloxicam combination-treated dogs than in those treated with butorphanol. Thus, meloxicam or its combination with other analgesics can be used to not only provide better pain relief in dogs undergoing ovariohysterectomy, but also enhance wound healing.

The persistent slightly high neutrophil and macrophage counts in the control group indicated that inflammation phase remained fairly active in the wound tissues throughout to day 8 postoperatively, compared to that in dogs treated with the analgesic drugs, in which these inflammatory cells diminished towards the 72-hour and day 8 of evaluation. Inflammation is essential for wound healing with neutrophils and macrophages functioning at the local wound-level to destroy bacteria and debride the wound in preparation for neovascularization and regeneration (Walburn *et al.*, 2009). These cells also release substances such as interleukin-1 (IL-1 α , IL-1 β), interleukin-6, interleukin-8, tumor necrosis factor and matrix metalloproteinases that are vital for tissue healing (Loo *et al.*, 2007). However, studies have shown that excessive and prolonged inflammation causes significant delay in wound healing (Kiecolt-Glaser *et al.*, 1995; Padgett *et al.*, 1998; Mercado *et al.*, 2002). Further, studies suggest that the main factor influencing inflammation-related delay in wound healing is neutrophilia (Sroussi *et al.*, 2009). This is due to consumption of large amounts of oxygen during neutrophil activation, which when coupled with low blood supply contributes to wound hypoxia (Gajendrareddy *et al.*, 2005; Sroussi *et al.*, 2009) and these consequently delay wound healing.

The better scores for fibroblasts, epithelialization, neovascularization and collagen in the wounds of meloxicam-treated and butorphanol-meloxicam combination-treated dogs than in

butorphanol-treated and control group dogs, suggest that the former analgesia protocols have more effective pain management outcomes than the latter. This also suggests that when pain is well managed, stress is minimized and subsequently wound healing would be faster and possibly of more superior quality. The effects of analgesic pain management on histopathologic responses of operative wounds have not been reported previously in dogs. The mechanisms through which pain and associated stress may negatively affect wound healing have been described (Woo, 2008 and 2012). This includes response to painful stimuli by C sensory nerve fibers to release neuropeptides like substance P, which activate leukocytes and other immunoactive cells, such as glial cells to release pro-inflammatory cytokines. These proinflammatory cytokines have been shown to play a role in augmenting pain signals and stress response.

Consequent to stress, there is overproduction of glucocorticoids, specifically cortisol and catecholamines through stimulation of ACTH on the anterior pituitary gland and adrenal medulla (Blackburn-Munro, 2004; Bomholt *et al.*, 2004). These hormonal changes negatively affect wound healing as a result of changes in immune system as well as the resulting tissue hypoxia (Kiecolt-Glaser *et al.*, 1995).

Slow healing of dermal biopsy wounds was observed in human patients with higher cortisol levels (Ebrecht *et al.*, 2004). Furthermore, the relationship between stress and skin barrier recovery from damage caused by tape stripping was found to be significant in a study carried out in human subjects, indicating that high stress levels, slowed the skin barrier recovery rate (Garg *et al.*, 2001).

Glaser *et al.* (1999) examined psychological stress and the levels of pro-inflammatory cytokines in experimentally induced skin blisters on the forearm of 36 women. Women who reported more stress on the Perceived Stress Scale produced significantly lower levels of interleukin-1 and interleukin-8. Keicolt-Glaser *et al.* (1995) demonstrated that the rate of complete biopsy punch wound closure increased by 24% or 9 days longer in caregivers stressed from providing care for their relatives with Alzheimer disease compared to those in control group. Further, blood leukocytes from stressed caregivers exhibited a diminished ability to express interleukin-1 gene in response to lipopolysaccharide stimulation *in vitro*. Broadbent *et al.* (2003) investigated the relationship between psychological stress and wound repair in 36 patients following inguinal hernia operation. They reported that perceived stress before the operation was a significant predictor of low interleukin-1 levels in wound fluids accounting for 17% of the variance. In contrast, worry about the operation significantly predicted lower levels of matrix metalloproteinase 9 in the wound fluid as well as increased pain over the first 20-hours postoperatively. Interleukins play an important role of protecting the host against infection and preparing injured tissue for repair by enhancing phagocytic cell recruitment and activation (Glaser and Keicolt-Glaser, 2005).

This study has also shown in Chapter 5 that dogs not treated for pain (control group) have higher cortisol, glucose and neutrophil-lymphocytes ratios as compared to those treated with butorphanol, meloxicam, and the butorphanol-meloxicam drug combination. This further reinforces the important interaction and the negative impact of stress response on wound healing, considering that dogs in the control group had poor wound healing parameters. Thus to enhance patient comfort and improve on surgical outcomes, treatment of pain and minimizing perioperative stress is imperative.

6.5 Conclusions

The following conclusions can be made from the current study:

6.5.1 There was no significant difference in wound healing response between butorphanol-meloxicam drug combination-treated dogs and those treated with either meloxicam alone or butorphanol alone. Despite this, the butorphanol-meloxicam drug combination gave clinically better wound healing outcome than butorphanol alone.

6.5.2 Better response to wound healing was elaborated by more wound collagen, better epithelialization and neovascularization, more fibroblasts and gradual diminishing levels of neutrophil and macrophage numbers in dogs treated with analgesics in the postoperative period than in those in the control. This indicates an imperative interplay between pain, stress response and wound healing in dogs, thus justifying the use of these analgesics in pain therapy postoperatively.

CHAPTER SEVEN

7.0 GENERAL DISCUSSION

Pain is an unpleasant sensory or emotional experience associated with actual or potential tissue damage or described in terms of such damage (Merskey and Bogduk, 1994; Short, 1995; Muir, 1998). Pain occurs when nociceptors are stimulated by thermal, mechanical, or chemical stimuli and impulses sent to the central nervous system for interpretation and modulation (ACVA, 2006). In veterinary surgical patients, the main sources of pain include trauma, surgical procedures and anaesthesia induced muscle ischemia.

Pain can be controlled by interventions targeted at different points in the pain transmission pathway. Techniques that are used to control pain are based on limitation of nociceptor stimulation, interruption of peripheral neural transmission, inhibition of nociceptive transmission at the level of the spinal cord, modulation of brain pathways or combined use of any of these techniques (ACVS, 1996; Muir, 1998; Mogoia and Mbithi, 2004). Current trends for holistic approach to pain management in animals advocate for a more comprehensive approach perioperatively, by using a wide range of therapies including several categories of drugs administered through local, regional or systemic techniques. This allows for blocking of pain at several different places along the nociceptive pathways (Epstein, 2011).

The numerous analgesic drugs and techniques currently available for management of pain in animals pose a challenge to practicing clinicians with regard to the choice of the appropriate drugs and techniques for optimal pain management in animals. Because of this challenge, postoperative pain due to procedures like ovariohysterectomy is poorly managed. Practically, the choice of analgesic drugs for management of pain is mainly influenced by the type of surgery, past experiences of the clinicians and their knowledge of the specific drug or

technique, availability of the drug, associated side effects, cost and occasionally set guidelines for the clinic or hospital (Wagner and Hellyer, 2002).

In the current study, the systematic review on analgesia practices in dogs undergoing ovariohysterectomy established that opioids and NSAIDs are the most commonly used drugs for management of pain associated with this procedure, and were more effective when administered in combination rather than individually. Multimodal drug therapy confers the advantages of using small doses of individual drugs but most importantly additive analgesia (Lemke *et al.*, 2002; White *et al.*, 2007). This improves patient comfort and minimizes the need for high doses or prolonged use of any one particular drug (Epstein, 2011) hence minimizing the likelihood of undesirable side effects.

Even though the systematic review established that analgesics were mostly administered either before or after surgery, effective pain management is realized when drugs are administered both preoperatively and postoperatively. Since opioids cause sedation, induce shorter duration of analgesia and are associated with numerous side effects (Gaynor and Muir, 2002) as compared to NSAIDs, it is postulated that optimal pain management can be attained by administering opioids in the preoperative period and NSAIDs postoperatively. This way, the technique not only utilizes the sedative effects of opioids and augments intraoperative analgesia, but also at the same time provides good postoperative pain management and minimizes side effects, hence decreasing morbidity and mortality. An interesting finding from this study is that analgesia induced by local anesthetics is comparable to that produced by opioids. From a clinical point of view, this observation is encouraging considering the cost, availability, restrictions and the side effects associated with opioids as compared to local

analgesics. This could stir interest, leading to possible widespread use of local analgesics in dogs undergoing ovariohysterectomy.

Ovariohysterectomy is a routine surgical procedure which is known to cause marked acute pain in dogs (Gaynor and Muir, 2002). Perioperative analgesia in surgical patients undergoing this procedure is therefore paramount not only for humane and ethical considerations, but also for the reason that it helps minimize the deleterious physiological effects associated with pain (Hansen, 2005).

The choice of ovariohysterectomy as the study procedure in the current study was influenced by the fact that it is the most commonly performed surgery in dogs in Kenya (Mwangi, 2013) and since the procedure is performed on healthy patients, it was deemed to provide the most ideal controlled clinical model for testing effects of analgesics as it can be assumed that any observed pain is emanating solely from the procedure (Slingsby *et al.*, 2006).

The current study was designed to simulate analgesic practices amongst veterinarians in Kenya. The choice of the test drugs – meloxicam and butorphanol - in the current study was based on the finding that these are the most commonly used analgesics in dogs in Kenya (Mwangi, 2013). The dosages used are known to provide satisfactory analgesia in dogs post-ovariohysterectomy (Lemke, 2007). In drug combination, the dosage of each drug was reduced by half since it was hypothesized that the two drugs will augment analgesia. In addition, reducing the dosage is known to minimize adverse side effects produced by individual drugs (Lemke, 2004; White *et al.*, 2007). A control group was warranted so as to simulate the practice where veterinarians fail to administer analgesics postoperatively. Further, the resultant trends in pain score in the control group were meant to shed more light on the duration and intensity

of pain emanating from ovariohysterectomy. Although administering analgesics preoperatively has better analgesic outcomes (Lascelles,1999) drugs in the current study were injected postoperatively principally to simulate the common practices amongst veterinarians (Mwangi, 2013).

The findings of the current study showed that dogs in the control group, that did not receive analgesics, had high pain scores in the first 6 hours after surgery as compared to those under analgesics. This supports the hypothesis that ovariohysterectomy causes marked and acute postoperative pain which is nevertheless short lived (Gaynor and Muir 2002). For ethical reasons and also to avoid the deleterious effects associated with postoperative pain, analgesics are warranted after spay.

The findings that dogs injected with meloxicam had significantly lower pain scores compared to those under butorphanol is in agreement with previous reports which showed that use of NSAIDs leads to better analgesic outcomes than opioids after ovariohysterectomy in dogs (Caulkett *et al.*, 2003; Dzikiti *et al.*, 2006; Shih *et al.*, 2008; Carmago *et al.*, 2011). In this study, the butorphanol-meloxicam drug combination produced similar analgesia to meloxicam and butorphanol. Although there is no study comparing analgesic effects of butorphanol-meloxicam combination and the individual drugs post-spay in dogs, other studies have shown similar results in reference to opioid-NSAID multimodal analgesia (Dzikiti *et al.*, 2006; Shih *et al.*, 2008). However, in light of pain scores being relatively lower in dogs in the butorphanol-meloxicam drug combination compared to those dogs under butorphanol and meloxicam, it would then mean that multimodal therapy has an added advantage compared to the individual drugs. This supports finding established through the systematic review.

Stress is a state in which both internal and external environmental demands exceed an individual's perceived ability to cope, thereby resulting in behavioral and physiological changes (Vileikyte, 2007). Perioperative stress in canine patients is prevalent and is attributed to factors like anxiety, excitement from handling, hospitalization, fear, depression, anaesthesia, tissue damage and pain (Fox *et al.*, 2000; Beilin *et al.*, 2003).

Stress and pain are directly related in that pain activates the hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes resulting in downstream hormonal, cellular and immunological changes (Upton and Solowiej, 2010) which are key markers of stress response.

In dogs, stress can be assessed using behavioral changes (panting, yawning and snout licking) Väinsänen *et al.*, (2005); physiological parameters (rectal temperature, heart rate, respiratory rate and blood pressure); metabolic parameters (cortisol and glucose) and surgical stress markers (interleukin 6 and C-reactive protein) (Freeman *et al.*, 2010). Neutrophil-lymphocyte ratio (NLR) is also an important composite measure and reliable indicator of stress response in mammals. Increase in NLR has been positively correlated to stress hormones in cattle (Anderson *et al.*, 1999), sows (Kranendonk *et al.*, 2005) and boars (Bilandzic *et al.*, 2006).

In dogs undergoing ovariohysterectomy, administration of analgesics like butorphanol (Inoue *et al.*, 2016), sulfentanil and carprofen (Slingsby *et al.*, 2006), vedaprofen, ketoprofen and carprofen (Selmi *et al.*, 2009), dexketoprofen (Saritas *et al.*, 2015), epidural morphine and fentanyl patches (Pekcan and Koc, 2010), has been shown to reduce cortisol, a key marker of stress in dogs.

Results from the current study showed a link between postoperative pain and stress. Higher stress response scores as indicated by cortisol, glucose, and neutrophil-lymphocyte ratio were observed in dogs that had higher pain scores, particularly those in butorphanol and control groups. This link has previously been demonstrated in animals (Mastrocinque and Fantoni, 2003; Matičić *et al.*, 2010) and is associated with activation of the hypothalamic-pituitary-adrenal and the sympathetic-adrenal medullary axes by pain resulting in downstream hormonal, cellular and immunological changes (Upton and Solowiej, 2010). These changes, particularly increased cortisol stimulates gluconeogenesis and catabolism; increases sensitivity of blood vessels to adrenaline resulting in increased heart rate and blood pressure; affects lymphoid organs causing increased production of neutrophils, thrombocytes and erythrocytes; reduces the levels of pro-inflammatory cytokines like interleukin-6; and affects the activity of important enzymes like matrix metalloproteinases (Kudoh *et al.*, 2001; Freeman *et al.*, 2010).

Wound healing is a normal biological process that consists of four highly integrated and overlapping phases namely homeostasis, inflammation, proliferation, and tissue remodeling or resolution (Gosain and DiPietro, 2004). However, the outcome in the healing process of any wound is as a result of the interplay between many local and systemic factors. Local factors that interfere with wound healing include tissue oxygenation, infections, foreign body and blood supply while systemic factors include age, stress, diseases such as diabetes and obesity, nutrition and medications such as corticosteroids and non-steroidal anti-inflammatory drugs (Guo and DiPietro, 2010). Assessment of wound healing in the current study was done using standard wound assessment protocols including: clinical appearance and histopathology (Sylvestre *et al.*, 2002; Abramo *et al.*, 2004; Nisbet *et al.*, 2010).

The findings of the current study demonstrated less epithelialization, minimal collagen lay down and poor vascularization of wounds in dogs whose pain was not managed using analgesics, as compared to those where analgesics were administered postoperatively. This observation is attributed to the interplay that exists between pain and stress, which results in cellular changes especially neutrophils and macrophages and immunological changes including reduced pro-inflammatory cytokines and metalloproteinases which play a key role in tissue repair (Upton and Solowiej, 2010). Studies in human patients have shown that increased postoperative stress is associated with wound infection (Beilin and Shavit, 2003), delayed wound healing (Broadbent *et al.*, 2003; Ebrecht *et al.*, 2004; Goiunet *al.*, 2008), prolonged hospitalization and increased cost of treatment (Morrison *et al.*, 2003). This finding provides evidence that indeed pain management is not only paramount for reason of animal welfare, but it also impacts on healing. Therefore, appropriate perioperative pain management is imperative in patients undergoing any surgical procedure.

The current study clearly shows the linkage between pain, stress and wound healing. It shows that there are benefits in managing postoperative pain in dogs following ovariohysterectomy, utilizing existing analgesic drugs either as individuals or in their combination. It goes further to provide more evidence of the superiority of multimodal pain therapy in postoperative management of pain and stress in dogs following ovariohysterectomy.

CHAPTER EIGHT

8.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 Preoperative and one-time administration of analgesics were the most commonly used practices of managing postoperative pain in dogs undergoing ovariohysterectomy. However, administering analgesics both before and after surgery and for 72-hours postoperative provided better results as opposed to preoperative and one-time drug administration, respectively. It is therefore recommended that veterinarians be informed and encouraged to adopt the practices of administering analgesic both before and after surgery and for at least 72-hours postoperatively while managing pain in dogs undergoing ovariohysterectomy.

8.2 This study established that opioids are the mainstream analgesics during ovariohysterectomy in dogs and NSAIDs are the most effective drugs in managing postoperative pain in dogs undergoing ovariohysterectomy. However, the use of opioids and NSAIDs in combination is a more effective approach of managing postoperative pain than their administration as individual drugs. It is therefore recommended that both opioids and NSAIDs, be inculcated in pain management protocols for dogs undergoing ovariohysterectomy with opioids being administered preoperatively and NSAIDs being administered postoperatively.

8.3 Ovariohysterectomy causes acute moderate to severe pain in dogs, lasting for about 6 hours postoperatively. For ethical reasons and also to avoid the deleterious effects associated with postoperative pain and stress, it is recommended that comprehensive pain therapy be instituted for all dogs undergoing ovariohysterectomy.

8.4 Meloxicam alone and butorphanol-meloxicam combination produce similar levels of analgesia in dogs after ovariohysterectomy while butorphanol produces very short acting analgesia associated with severe sedation and hypotension. Meloxicam and the butorphanol-meloxicam drug combination as administered in this study are therefore recommended for use in management of acute postoperative pain in dogs, especially pain associated with procedures like ovariohysterectomy.

8.5 Combining butorphanol and meloxicam at half the dosage of the individual drugs mitigates postoperative stress response better than butorphanol or meloxicam administered individually. It is therefore recommended that butorphanol and meloxicam be administered together rather than individually, in order to attain optimal management of postoperative stress.

8.6 Better response to wound healing was observed in dogs treated with analgesics in the postoperative period than those in the control group. Butorphanol-meloxicam drug combination equally resulted in better wound healing than seen with individual drug therapies and particularly butorphanol. This study therefore recommends that pain therapy, more so using the drug combination, be instituted in all dogs undergoing ovariohysterectomy as this will not only improve on their welfare but also hasten healing of the surgical wound.

8.7 Results from this study document for the first time that there is an existing interplay between postoperative pain, stress response and wound healing in dogs undergoing ovariohysterectomy. The study however, recommends a more focused study using a large number of animals in order to quantify this relationship.

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