UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Evaluation of intraperitoneal ropivacaine for postoperative analgesia following ovariohysterectomy in dogs

A dissertation submitted to the
Faculty of Veterinary Science,
University of Pretoria

In fulfilment of the
requirements for the degree
Master of Science (MSc)

Researcher: Dr. Majid Khanzadeh Alishahi

Supervisor: Prof. George Frik Stegmann

Department of Companion Animal Clinical Studies
Faculty of Veterinary Science
University of Pretoria

Pretoria

September 2018

UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

DECLARATION OF ORIGINALITY

This document must be signed and submitted with every
essay, report, project, assignment, mini-dissertation, dissertation and/or thesis

Full names of student: Dr. Majid Khanzadeh Alishahi

Student number: 17231249

Declaration:

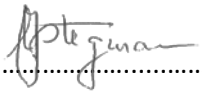
1. I understand what plagiarism is and am aware of the University's policy in this regard.

2. I declare that this thesis (e.g. essay, report, project, assignment, mini-dissertation, dissertation, thesis, etc.) is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with departmental requirements.

3. I have not used work previously produced by another student or any other person to hand in as my own.

4. I have not allowed, and will not allow, anyone to copy my work with the intention of passing it off as his or her own work.

Signature of student: 

Signature of supervisor: ..... 

DECLARATION

I, Majid Khanzadeh Alishahi, hereby declare that the research presented in this thesis, was executed by myself, under guidance from my supervisor. Neither the substance, nor any part of the thesis has been submitted in the past, or is to be submitted for a degree at the University of Pretoria or any other University. This thesis is presented for fulfilment of the requirements for degree of Master of Science (MSc).

Majid Khanzadeh Alishahi (DVM)

September 2018

DEDICATION

This thesis is dedicated, with love, to my parents,

Marzieh and Ali

ACKNOWLEDGEMENTS

I would like to acknowledge and thank my supervisor Prof. George Frik Stegmann for his continued guidance and support throughout this research project.

A special thank you is extended to Prof. Geoffry Fosgate for his assistance with the statistical procedures.

To my family, thank you for your never-ending support and encouragement. You guys provided a massive foundation of love and support, and for that I am truly thankful.

Thesis Summary

By

Dr. Majid Khanzadeh Alishahi

Supervisor: Prof George Frik Stegmann

Department: Companion Animal Clinical Studies

Degree: MSc

Objective To determine if intraperitoneal (IP) ropivacaine in conjunction with systemic analgesics improves postoperative analgesia following ovariohysterectomy (OHE) in dogs.

Study Design Randomized, blinded, clinical trial.

Animals Twenty dogs presented to the Veterinary Academic Hospital for elective OHE.

Methods Dogs were premedicated with acepromazine (0.03 mg kg^{-1}) and morphine (0.3 mg kg^{-1}) intramuscularly (IM). Anaesthesia was induced with propofol (4 mg kg^{-1}) intravenously (IV) and maintained with isoflurane (2%) in oxygen. Dogs were randomly assigned into one of two groups: group R received ropivacaine (n=10; 1 mg kg^{-1}) and group S received 0.9 % saline (n=10; 0.1 mL kg^{-1}) IP after linea alba incision. All OHE were performed by the same experienced surgeon. At completion of surgery, carprofen was administered IM at 4.4 mg kg^{-1}. Pain was assessed using a mechanical nociceptive threshold (MNT) device before premedication, 30 minutes after premedication, as well as 2, 4 and 20 hours postextubation.

Categorical data were analyzed using Fisher exact tests. Baseline quantitative data were compared between groups using Mann-Whitney U tests. Pain responses were compared between treatments using linear mixed models and post hoc comparisons were adjusted using Bonferroni correction of *p*-values.

Results There were no significant differences ($p>0.05$) between the treatment groups regarding breed, age and weight. The effect of ropivacaine treatment to pressure required to elicit a pain response was not statistically significant different from saline. No adverse effects were observed following IP ropivacaine.

Conclusions and clinical relevance IP administration of 1 mg kg^{-1} ropivacaine in conjunction with systemic analgesics did not improve postoperative analgesia following OHE in the dog.

Keywords analgesia, anaesthesia, local anaesthesia, intraperitoneal, ropivacaine, dog, ovariohysterectomy

Table of Contents

1 Chapter 1- Introduction 1
1.1 Introduction 1
1.2 Hypothesis 2
1.3 Aim of the study 2
1.4 Objective 2
2 Chapter 2 - Literature Review 3
2.1 Pain and its definitions 3
2.2 The physiology of pain 4
2.2.1 Pain pathways 4
2.2.2 Pain processing 5
2.2.3 Ascending pathways 7
2.2.4 Descending pathways 8
2.3 Pain Modulation 9
2.3.1 Desensitization 9
2.3.2 Sensitization 9
2.3.3 NMDA and AMPA receptors 11
2.4 New approaches to central pain management 11
2.5 Classification of pain 12
2.5.1 Chronic pain 12
2.5.2 Acute pain 12
2.5.3 Somatic pain 12
2.5.4 Visceral pain 13
2.5.5 Neuropathic pain 13
2.5.6 Psychological pain 13
2.5.7 Physiologic (Adaptive) pain 13
2.5.8 Maladaptive (pathological) pain 14

2.5.9 Cancer pain 14
2.6 Common analgesic medications used in perioperative period 15
2.6.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) 15
2.6.2 Opiates and opioids 15
2.6.3 NMDA antagonist – ketamine 15
2.6.4 α-2 adrenoreceptor agonists 16
2.6.5 Local anesthetics 16
2.7 Multimodal and pre-emptive analgesia 20
3 Chapter 3 – Experimental study 21
3.1 Material and Methods 21
3.1.1 Animals 21
3.2 Study design 22
3.3 Anaesthesia and surgical procedures 22
3.4 Physiological parameters 23
3.5 Pain assessment 23
3.6 Result analysis 25
4 Chapter 4 – Results 27
4.1 Demographic data 27
4.2 Age and weight 27
4.3 Intraoperative values 28
4.4 Perioperative pain scores 29
5 Chapter 5 – Discussion 33
6 Appendix 42
Appendix 6.1: Patients list 42
Appendix 6.2: Animal ethics certificate 43
Appendix 6.3: Research consent form 44
Appendix 6.4: Recording sheet 45
Appendix 6.5: Chart for recording pain scores measured by the mechanical algometer 46
Appendix 6.6: Normality assessment 48

LIST OF FIGURES

Figure 3.1 ProdPro is a hand-held Mechanical Algometer that evaluates the mechanical nociceptive threshold (MNT) to pressure by applying steadily increasing pressure. It measures the pressure in Newton (N) ranging from 0.1 to 32 N with an accuracy of ±0.5 N. 26

LIST OF TABLES

Table 2.1 Comparison of features between local anaesthetic agent groups (Dugdale, 2011)...18

Table 2.2 Physicochemical properties of some local anaesthetic agents (Dugdale 2011). 20

Table 4.1 Comparison of signalment for 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo. 28

Table 4.2 Comparison of intraoperative cardiorespiratory variables within 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo. 30

Table 4.3 Comparison of postoperative pressures (Newton) required in 20 dogs to elicit a pain response treated with either intraperitoneal ropivacaine or saline following linea alba incision during ovariohysterectomy. 32

Table 4.4 Univariate predictors of the pressure* required to elicit a pain response within 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo following linea alba incision. 33

LIST OF ABREVIATIONS

AMPA – δ-amino-3-hidroxi-5-metil-4-isoxazol propionate acid

Ca^{2+} - Calcium ion

CdLin - caudal linea alba

cm - centimeter

CrLin - cranial linea alba

FE´Iso - end-tidal isoflurane concentration

f_R – Respiration Rate

H^+ - hydrogen ion

HR – Heart Rate

IM – Intramuscular

IP – Intraperitoneal

IV – Intravenous

K^+ - potassium ion

Kg – kilogram

LA – local anaesthetic

Labd - left abdomen

Llin - left linea alba

m/s – meters per second

mg – milligram

Mg^{2+} - magnesium ion

mL – Milliliter

mm – millimeter

MNT – Mechanical Nociceptive Threshold

N – Newton

Na^{+} - sodium ion

NGF – nerve growth factor

NMDA – N-metil-D-aspartate

NSAID's – non-steroidal anti-inflammatory drugs

O^2 – oxygen

OHE – Ovariohysterectomy

$PE´CO_2$ - end-tidal carbon dioxide partial pressure

pH – hydrogenionic potential

pKa – acid dissociation constant

Rabd - right abdomen

Rlin - right linea alba

SC – Subcutaneous

α – alfa

δ – delta

κ - kappa

μ - mu

1 Chapter 1- Introduction

1.1 Introduction

Ovariohysterectomy (OHE) is a common surgical procedure performed in small animals. Mild to moderate postoperative pain is expected following OHE (Hardie et al., 1997, Carpenter et al., 2004). Surveys in Britain, Australia and Canada estimated that only 13-26 % of animals undergoing OHE received analgesics (Carpenter et al., 2004). There are two important factors associated with pain after abdominal surgery; somatic pain such as surgical trauma to the abdominal wall during incision; and visceral pain due to the mechanical changes in internal organs (Savvas et al., 2008). Postoperative pain in animals has detrimental effects on recovery because it may cause loss of appetite, self-mutilation, behavioural alterations, aggravation of protein catabolism, respiratory depression, cardiac arrhythmia and central hypersensitivity to noxious stimuli that can develop into chronic pain (Kim et al., 2012). All of these adverse effects may increase the period of hospitalization and consequent costs (Kalchofner Guerrero et al., 2016). It is also considered ethically necessary to reduce pain after surgery in animals (Kalchofner Guerrero et al., 2016). Therefore, there is a growing need to improve analgesic management during abdominal surgery in small animals, including OHE. Currently, there is interest in the combined use of systemic analgesics and local anaesthetics (Hewson et al., 2006, Savvas et al., 2008).

Local anaesthetics have a wide range of clinical use and routes of administration. They are commonly used in infiltration anaesthesia, field blocks or spinal anaesthesia. Moreover, local anaethetics have the favorable features of being inexpensive, readily available and not subject to statuary control (Carpenter et al., 2004).

Recent studies in human medicine have reported that intraperitoneal (IP) administration of local anaesthetics such as bupivacaine reduces pain scores, early postoperative analgesic needs, and time to first rescue analgesia after laparoscopic abdominal surgeries (Buck et al., 2004, Freilich et al., 2008, Arden et al., 2013, Perniola et al., 2013, Perniola et al., 2014, Roy et al., 2014). In dogs, IP bupivacaine has also been shown to be an effective treatment for dogs during OHE (Carpenter et al., 2004).

To the best of our knowledge, the use of ropivacaine as IP analgesia in dogs has not been reported. The aim of this study was to investigate the postoperative analgesic effects of intraperitoneal ropivacaine following OHE in dogs.

1.2 Hypothesis

Intraperitoneal administration of ropivacaine will be useful to reduce perioperative pain during ovariohysterectomy in dogs.

1.3 Aim of the study

To determine if intraperitoneal (IP) ropivacaine in conjunction with systemic analgesics improves postoperative analgesia following ovariohysterectomy (OHE) in dogs.

1.4 Objective

In this study we attempt to determine the effectiveness of an intraperitoneal (IP) instillation of ropivacaine for perioperative analgesia during ovariohysterectomy (OHE) in healthy female dogs.

2 Chapter 2 - Literature Review

2.1 Pain and its definitions

Pain is a unique feeling which is usually difficult to depict. It is usually considered as an unpleasant emotion, mostly associated with tissue damage. The International Association for the Study of Pain (IASP) defines pain as, “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. They expressed later “pain is always subjective. Each individual learns the application of the word through experiences to injury in early life” (Merskey, 1986).

Another interesting definition of pain is described by Molony with regard to animal pain: “An aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of tissues. It changes the animal’s physiology and behavior to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery.” (Molony and Kent, 1997)

The two previously mentioned definitions indicate that pain is not only a physical event, but it also has behavioral, psychological and intellectual characteristics too. We can see intellectual elements only in the species with more developed cerebral cortex such as primates. In order to fully understand the subject, the limbic system can be taken as an example: Some emotions like anxiety and fear are related to the limbic system which sends signal to the cerebral cortex. Accordingly, it means psychological emotions are able to raise pain perception (Dugdale, 2011). In the field of biology, the word that is associated with pain is “nociception”. Nociception is a physiological process when a noxious stimuli activates the neuron to elicit a neuronal response, which in the end leads to the perception of pain. At this stage if any factor disrupts this process,

then the stimulus will not draw out a neuronal response and analgesia is established. In another word, analgesia is the complete absence of pain or the ability to feel it. However, reaching to this point is very difficult because despite being able to stop the transmission or the perception of noxious stimuli, they still exist and they will stimulate the neurons and only “Hypoalgesia”, which is e level of decreased perception of pain, will be obtained. This stage can be achieved by using different drugs or by biological endogenous mechanisms in stressful situations, such as a predator attack. The latter is a crucial biological feature, which enables the animal to run away from a dangerous situation into safety. The inverse of hypoalgesia is also possible to happen and is called “hyperalgesia”. It occurs when there is an inflammatory response and a worsened pain reaction to noxious stimuli due to reduced receptor threshold and increased electric impulse generating. Hyperalgesia can occur at the margin or center of the lesion site continuing to perceive pain even after the stimulus is terminated. Moreover, hyperalgesia can turn into a more severe situation called “allodynia”, when even a non-noxious stimulus can trigger a pain response (Dugdale, 2011, Viñuela-Fernández et al., 2007, Tranquilli et al., 2013)

2.2 The physiology of pain

2.2.1 Pain pathways

Nociception is consecutive procedure starting at the stage of nociception receptors by conversion of chemical or physical stimuli to electrical impulses and continuing with their transition by an afferent nerve to the dorsal horn of medulla. From this point, the impulses are conducted to the spinal and supraspinal centers for modulation and perception of the stimuli. The function of nociceptive pathways is very complicated, however in order to make it more simple to explain, there are three stages of neurons by which the nociceptive stimulus is

transferred: the primary neurons, the projector neurons and the supra spinal neurons (Dugdale, 2011, D'Mello and Dickenson, 2008).

The cellular part of the primary neurons (afferent neurons) is located in the ganglia of the dorsal root of the medulla and its axons extend peripherally to their aimed organs and centrally to the dorsal horn of the medulla. The primary neurons are connected to some specific sites on the dorsa horn of medulla (six lamellae) with the purpose of processing the incoming information. Lamellae one and two are connected to neurons with specific nociceptive (information) while the deepest lamellae is linked to unspecific (D'Mello and Dickenson, 2008).

The electrical impulse coming from the medulla is received by projector neurons which they will link it to the spinal and supra spinal centers at thalamus, hypothalamus, pons, midbrain and etc. Then these centers will lead the nociceptive impulse further into the cortical ad sub-cortical centers in brain to analyze the pain (Dugdale, 2011, D'Mello and Dickenson, 2008, Lemke, 2004, Viñuela-Fernández et al., 2007).

2.2.2 Pain processing

Noxious stimuli leads to a series of events, which changes the stimuli into an electrical signal and transfer it to the top neuronal centers, where the signal is processed and a suitable response is released ((Dugdale, 2011, Tranquilli et al., 2013, D'Mello and Dickenson, 2008, Lemke, 2004).

This process can be explained in four levels as follows:

2.2.2.1 Transduction

The first level of pain perception is transduction. When a strong enough stimulus activates the nociceptor, it triggers a response by transforming the physical energy to an electrical signal (Monteiro, 2015).

2.2.2.2 Transmission

This level is about transmission of the electrical signal by the neural pathways to top neuronal sections. The is performed by different types of pain fibers such as C-fibers for chronic pain and A-delta fibers for acute pain, chiefly towards the dorsal horn of medulla, then to the thalamus, reticular system and finally to the cortex and higher centers. Nonetheless, before reaching to top centers, the pain signal will be modulated(Monteiro, 2015).

2.2.2.3 Modulation

The pain signals are subject to some alteration derived from the endogenous descending pathways at the dorsal horn of medulla and supra spinal levels. This happens at several stages and the descending pathway system is responsible for the complexity of the connection between developing of stimuli and their perceptions, as some of them might get nullified by the descending pathways' interference. The outcome of the signal modification as dependent on the balance between excitatory and inhibitory impulses (Monteiro, 2015).

2.2.2.4 Perception

Perception of the stimulus happens when the noxious signals reach the top neuronal centers and only at this stage subjective, conscious and emotional experience of pain is felt. Therefore,

perception of a painful stimulus is completely associated with successful transmission, transduction and modification of the signal (Monteiro, 2015)

2.2.3 Ascending pathways

2.2.3.1 Afferent fibers and their nociceptors

A number of nerve axons together will form fiber networks in order to transmit the electric signal. There are three types of fibers, which are different in composition and the type of electrical signal they conduct. Not all of these fibers participate directly in pain transmission, but all of them are required to perceive the exact nature of pain. A-beta fibers are involved in non-painful stimuli, like tactile feeling. They are the fastest transmission fibers due to their large diameter and myelinated membrane. Although they not involved in normal conduction of the pain, they help to determine its nature. A-δ with a thinner diameter and myelinated membrane transmit pain impulses generated by mechano- and thermal stimuli. These fibers conduct the electrical signals at a high speed of 3 – 30 m/s to the medulla when they respond to extreme changes by mechano-thermal stimuli. Pain transmitted by these fibers is called epicritic feeling and is usually well localized, adaptive, and acute (D'Mello and Dickenson, 2008, Lemke, 2004). At last, C fibers, which are the thinnest fibers, unmyelinated and slowest in signal transmission are polymodal, which means they react to any kind of stimuli, as long as it is enough to conquer their threshold level. Transmission speed is less than 3 m/s and the pain conducted by them is slowly adaptive, unspecific and diffuse. Pain transmitted by these fibers is described as protopathic pain. Beside the A- δ and A- β fibers, the three fibers are named “pain fibers”. There are also a less known type of fibers that usually are not involved in any process, until an inflammatory reaction arises. These “silent fibers” do not react to stimuli until they are triggered

by inflammatory factors. After this time, the mechanothermal stimuli can activate these fibers because their excitement thresholds are lowered enough to be triggered. These fibers are alike C-fibers, because the pain signals conducted by them has the same features as the latter. They play a significant role in peripheral sensitization and rising the pain feeling at the wound site (Dugdale, 2011, D'Mello and Dickenson, 2008, Lemke, 2004, Viñuela-Fernández et al., 2007).

2.2.4 Descending pathways

Descending pathways transmit signals formed by the brain. These signals might be inhibitory or excitatory in their essence, to decrease or increase stimuli conduction. Inhibitory descending stimuli is sufficiently strong to stop pain signals from receiving by the brain, thus it is an interesting field to the medicine. Pavlov's studies revealed an ideal instance of the descending inhibitory pathway at field: the dogs, before giving the food, were traumatized by cuts in the paws or nose or by electrical shock. After some time they either expressed no symptom of pain feeling or ceased interpreting these stimuli as irritating, perceived them instead as before feeding sign (Lemke, 2004, Melzack and Wall, 1965).

Descending inhibitory pathways are active in four various levels: Raphe magnus nuclei in the pons, medulla oblongata plus spinal medulla, cortex and thalamus and midbrain's periaqueductal gray matter. The latter is the most important understood of all the mentioned levels (Dugdale, 2011).

2.2.4.1 Periaqueductal gray matter

This descending pathway is significant because of its high amount of opioid receptors. Its influence is most effective against pain signals at spinal stage. However, they have to be triggered by serotonin, GABA, noradrenaline, acetylcholine, adenosine or endorphins to act. By

discovering the molecules that trigger pathways, it will lead to new findings to suppress pain (Dugdale, 2011).

2.3 Pain Modulation

One of the SNC's reactions to long-term pain is "neuromodulation". Presence of painful stimuli can change the pain perception mechanisms. The two biggest phenomena which are called sensitization and desensitization can be seen because of this biologic response (Dugdale, 2011).

2.3.1 Desensitization

This is a physiological reaction that the living creature does with repeatedly low intense pain. This procedure raises the threshold to a specific type of stimuli and stops the activation of the nociceptors. This process is able to fully prevent painful stimuli to affect or only weaken its power. This feature can be simply observed when comparing pain thresholds in a young to adult animal. As the anima ages, it becomes desensitized to some stimuli which improves the animal's ability to survive. However, the reason for this phenomenon is still unknown and may not always happen (Dugdale, 2011).

2.3.2 Sensitization

This phenomenon is an inverse of desensitization. The outcome of this procedure is to increase the feeling of the painful stimuli by lowering the pain threshold of a specific stimuli and increasing its intensity. It can be classified as peripheral and central sensitization (Dugdale, 2011).

2.3.2.1 Peripheral sensitization

As the name indicates, this procedure happens peripherally, at the lesion. The inflammatory agents (serotonin, prostaglandins, substance P, histamines) and other algogenics (H^+ and K^+ ions) decrease the thresholds of the silent fibers and nociceptors, which lead to a raise in electric impulse emission. This enhancement in triggering of the nociceptors, finally lead to an increase sensation of the pain at the lesion, which also expands to the wound site's adjacent areas (Gogny, 2006).

2.3.2.2 Central sensitization

This phenomenon happens at a central level, in the CNS. Any pain can result in central sensitization but long lasting changes are mainly brought up by high intensity and prolonged stimuli. This stimuli leads to a response by the requiting receptors, which starts to react in an inordinate amount to the received impulses (wind up). The "wind up" phenomenon can be very dramatic since pain can continue even after removal of the primary cause (Gogny, 2006). Some substances play important roles in central sensitization such glutamate, which is a Neurotransmitter with the ability to attach to NMDA (N-Methyl D-Aspartate) and AMPA (α-amino-3-hidroxi-5-metyl-4-isoxazol-proprionate acid) receptors. These receptors are related to long and short duration stimuli. Central sensitization can return naturally after removing the painful stimulus or by using drugs, however in spite of the latest advances in pain management it is a difficult situation to deal with. The best solution for this problem is preventing pain (pre-emptive analgesia) (Dugdale, 2011, Gogny, 2006).

2.3.3 NMDA and AMPA receptors

AMPA receptors are associated with receiving a fundamental reaction from the medulla to non-painful and painful stimuli by running the ion changing and channels of rapid activation by glutamate. If the stimuli is sourced from C fibers and contains high frequency and intensity, then NMDA receptors are also involved (Viñuela-Fernández et al., 2007). NMDA receptors are related to non-specific cation changing channels in nervous cells (Na+ and Ca2+ influx and K+ efflux) and indirectly control receptor synthesis, cellular signaling and gene expression which can be seen in high concentration in the dorsal horn of medulla (Viñuela-Fernández et al., 2007). These receptors are unique, because of their double activation process. For being activated, these receptors have to be influenced by a persistence membrane depolarization, which will lead to changing in their structure and releasing MG2+ ions from the active region in order to allow the connection glycine and glutamate (co-agonist). Releasing a high concentration of calcium and sodium ions through the enclosed ion channels will cause depolarization in the membrane and starts cellular signaling cascades which are associated with long term neuromodulation (Dugdale, 2011, D'Mello and Dickenson, 2008, Viñuela-Fernández et al., 2007).

2.4 New approaches to central pain management

The theory that nerve growth factor (NGF) is necessary in pain signaling after lesion and inflammation, has been tested by Hefti *et al*. NGF antagonist molecules were used in some animal models with good results, indicating high effectiveness with no side effects (Hefti et al., 2006).

2.5 Classification of pain

Different types of stimuli can cause pain by activating the inflammatory mediators in the lesion site. For example excessive dilation of viscera or nerve damage can cause noxious stimuli. Duration and intensity are two significant characteristics that can be used to classify pain and distinguish pathologic or physiologic pain (Dugdale, 2011). There are different methods of classifying pain as seen below:

2.5.1 Chronic pain

A pain that lasts for a long time after the original noxious stimulus disappeared and is not possible to abolish by doing a simple treatment or analgesic medication. Usually a multimodal approach is essential to treat this type of pain. Multimodal approach consists of environment manipulation and administration of analgesic drugs along with physiotherapy, and etc. (Tranquilli et al., 2013).

2.5.2 Acute pain

A pain caused by tissue damage with thermal, chemical or mechanical agent. This type of pain begins suddenly and has a short period which can be easily eliminated through the administration of analgesic medications (Dugdale, 2011).

2.5.3 Somatic pain

Somatic pain takes place on the derm or musculoskeletal system of the body that can be simply localized and distinguished from visceral pain since these regions have a higher density of neurons with small sensitive spots (Lemke, 2004).

2.5.4 Visceral pain

In contrast to somatic pain, visceral pain is spread and triggered by mechanical damage to the internal organs (dilatation, distention and ischemia). Pain in these organs is less specific due to the low density of receptors and each receptor is related to a larger field compared to somatic ones (Lemke, 2004).

2.5.5 Neuropathic pain

Neuropathic pain can be seen when the patient's pain cannot be reduced by normal pain medication. It is originated by nerve lesions, which result in ectopic activity due to the accumulation of sodium channels in the lesion site. This accumulation can spread to the neuronal bodies in the dorsal ganglia and to other neurons, including effector neurons. It all might lead to spontaneous pain and in increased sensitivity in the peripheral nociceptors (D'Mello and Dickenson, 2008).

2.5.6 Psychological pain

Some patients complain about feeling pain in their bodies even when there is no injury. This phenomenon can be associated with psychological pain which often happens due to severe damage to neurons and their modulation in pain pathways after extreme episodes of pain. This problem can continue even after complete therapy and vanishing from the injury site (Dugdale, 2011).

2.5.7 Physiologic (Adaptive) pain

Physiologic pain is the most common type of pain, as the name indicates, with a physiologic function. Its aim is to help the subject's survival by acting in a protective manner. This will avoid more damage to the lesion and allow the subject to recover efficiently. This kind of pain

is an adaptive pain which needs to activate high-threshold receptors (nociceptors), well focused in time and space, while its electrical impulses are conducted through A- δ fibers (Dugdale, 2011, Viñuela-Fernández et al., 2007, Lemke, 2004, Bishop, 1980b, Hellyer et al., 2007).

2.5.8 Maladaptive (pathological) pain

Pathological or maladaptive pain is an abnormality caused by a trauma or other painful incidents such as medical operations or car accidents. This type of pain is very disabling to the subject and usually is difficult to localize because besides the normal nociceptors, silent receptors which are responsible for non-painful stimuli, such as tactile sensation are also involved and even may continue after the main stimuli has ended. C fibers conduct electrical impulses responsible for pathological pain (Dugdale, 2011, Lemke, 2004, Viñuela-Fernández et al., 2007, Bishop, 1980a, Hellyer et al., 2007).

When a patient feels pain the veterinarian must do his best to relieve the pain but it is in a pathological case that his reaction is saving life since it is a situation that the pain will not end itself (Dugdale, 2011, Lemke, 2004, Viñuela-Fernández et al., 2007, Bishop, 1980b).

2.5.9 Cancer pain

“Cancer-related pain results from the treatment for cancer or from the cancer itself. Cancer-related pain depends on the type of cancer, the stage of the disease and the pain threshold (tolerance for pain) of the person with cancer mostly due to compression or infiltration of hollow organs, soft tissues, bones or nerves. But it could also be caused by the treatment or the tests done to diagnose cancer” (pain, NA).

2.6 Common analgesic medications used in perioperative period

2.6.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

This group is enormous and consists of drugs which are common in analgesic, anti-pyretic and anti-inflammatory features. NSAIDs can be classified into two groups basd on their molecular composition: carboxylic acids (salicylic, acetic, proprionic, fenamic and nicotinic acids) and enolic acids (pyrazolones, pyrazolidines and oxicams) (Dugdale, 2011).

2.6.2 Opiates and opioids

Drugs in this class have significant analgesic features and are derivates of the opium, which is obtained from the poppy flower (P*apaver somniferum*) (Dugdale, 2011). Morphine was the first introduced drug from this family and is currently the outstanding of this drug category. All other drugs from this family are compared to morphine, in order to evaluate their relative potency. All substances from this category are analgesics but are also known as narcotics, since narcosis (a sense of sedation and sleepiness) is one of their side effects. *Opiates* are the natural substances derived from opium; however, opioids are the synthetic drugs with similar action to opiates. Methadone, Buprenorphine and Tramadol are examples of opioids (Dugdale, 2011).

2.6.3 NMDA antagonist – ketamine

Ketamine is a short acting drug made from phencyclidine. Tiletamine is more long acting compare to ketamine. Ketamine is frequently used in veterinary medicine because of its dissociation feature among the limbic and thalamocortical systems. Moreover, ketamine is potent analgesic drug, which makes it very helpful to use in analgesia procedures. It is also one of the few drugs that is able to lower the central sensitization event (Riviere and Papich, 2017).

2.6.4 α-2 adrenoreceptor agonists

This drug category is very popular and used around the world by physicians and veterinarians since they are introduced in 20th century. Detomidine, medetomidine, dexmedetomidine and xylazine belong to this group. After the discovery of medetomidine and dexmedetomidine, practitioners started to use the more often because of their excellent combination of analgesic and sedative features (Kraus, 2012). Usually, α-2 agonists are used in combination with opioids, benzodiazepines or other sedative/tranquilizers for a better sedation effect, longer period of action and lowered dosages of all drugs. Opioids and α-2 agonists have synergetic influence that should be considered. At least, considering and α-2 agonists with general anesthesia will highly decrease the required dosage for achieving the appropriate stage of anaesthesia, either with inhalable or fixed substances (isoflurane, sevoflurane, propofol, thiopentone, etc.) (Monteiro, 2015).

2.6.5 Local anesthetics

2.6.5.1 Introduction and classification

It is relatively a new technique to use local anaestheics for analgesia during surgery under general anaesthesia. Up until recently these drugs have been used only in minor conscious procedures such as lameness clinical exam in horses, but never used with other analgesic drug categories for major surgeries (Jones, 2002). However, recently their usage have been upgraded to epidural anaesthesia, intraoperative, and even postoperative analgesia, through using a local infiltration method to an intramuscular or subcutaneous-placed catheter (Dugdale, 2011). Local anaesthetics effectively block sodium or other ion channels in the peripheral nerves' membrane, preventing membrane depolarization and, thus, stopping electrical signal transmission. Their

molecular structure include an aromatic structure with lipophilic characteristics, an intermediate chain composed of carbon and hydrogen atoms and an amide group with hydrophilic features. The nature of intermediate link allows the division of the drugs to an amide-linked group composed by lidocaine, bupivacaine, ropivacaine, etc. or an ester- group like cocaine, procaine, tetracaine, etc. Both groups have good local analgesia properties but they are different in several features (Table 2.1). Local anaesthetics are slightly acidic substances and have a higher concentration of ionized molecules compare to non-ionized ones. After usage, the local pH which is about 7.4 will rapidly balances the concentration, depending on the molecules' pKa. Both types of the substances are required to have the nerve blocking impact. (Riviere and Papich, 2017, Dugdale, 2011, Casati and Putzu, 2005)

Table 2.1 Comparison of features between local anaesthetic agent groups (Dugdale, 2011)

Features of Amino-Esters	Features of Amino-Amides
Poor tissue penetration	Good tissue penetration
Short duration of action (rapid metabolism)	Long duration of action (slower metabolism)
Fast elimination = decreased chance of Toxicity	Slow elimination = increased risk of toxicity
Increased chance of allergic reactions due to para-amino benzoic acid (pABA) as metabolite.	Possibility of allergic reactions due to methylparaben as preservative (can break down into pABA)

2.6.5.2 Mechanism of action

LAs' Mechanism of action is not completely discovered. These substances are very liposoluble with an alkaline pKa and are quickly absorbed by the tissues. This feature leads to a quick

attachment to the sodium channels in peripheral nerves and block their transmission. This is done by ionized and non-ionized molecules. Ionized molecules block the sodium channels from the outside of fibers, while the non-ionized molecules are absorbed into the fibers and perform the block from inside. It is important to know that use of Las to anesthetize an inflamed tissue may be with a delayed onset of action, since in this situation the local pH has changed (Dugdale, 2011). Therefore, there are three significant features associated with local anaesthetics: lipid solubility, which is associated with drugs' potency; pKa which controls the concentration of ionized and on-ionized molecules after usage, thus it regulates speed of onset of action; tissue protein binding, which influences the duration of action. A combination of these three features and also tissue situation affects the drug's tissue penetration (Dugdale, 2011).

2.6.5.3 Effects and pharmacokinetics

It should be considered that all tissues have sodium channels, with some difference in type and density depending on the tissue, thus if a sufficient dose of an LA is systemically absorbed, those tissue channels will be affected and cause depressant influence, especially in cardiovascular (cardiac arrest) and the CNS (sedation, seizures, depression, coma, respiratory arrest). This matter shows the complications of using high doses of Las which might inhibit the ion channels of vital organs (heart or brain) and lead to a dangerous situation for the patient. These complications are mainly observed with more lipophilic LAs, thus it is safer to use vasoconstrictor in order to delay the systemic absorption (lidocaine, i.e.), though more modern LAs already have a certain vasoconstricting effect (ropivacaine, bupivacaine and levobupivacaine) (Casati and Putzu, 2005). Allergic reaction is another side effect of these drugs class, especially reaction to ester-linked group molecules. Nerve toxicity can happen by using

these drugs due to detergent-like activity of high doses of LAs, preservatives of commercial solutions and excessive vasoconstrictor effect (Riviere and Papich, 2017, Dugdale, 2011). All LA drugs are metabolized in liver, thus care should be exercised when using LAs in patients with impaired liver function (Plumb, 2005).

Table 2.2 Physicochemical properties of some local anaesthetic agents (Dugdale 2011)**.**

	pKa	**Onset**	**Relative lipid solubility**	**Toxicity**	**Relative potency**	**Protein binding**	**Duration of action**
Lidocaine	7.9	Fast	150	Medium	2	65 %	Intermediate
Bupivacaine	8.16	Moderate	1000	High	8	95 %	Long
Ropivacaine	8.1	Moderate	400	Medium	6	95 %	Long

2.6.5.4 Example 1 – Ropivacaine

Ropivacaine is an amide linked local anaesthetic with lots of similarities to bupivacaine. There are only two differences in their structures, ropivacaine has one less carbon in the side chain and also is a pure left-isomer. Left-isomers in comparison with right-isomers are proven to be less toxic. Also ropivacaine is less lipophilic compare to bupivacaine due to the reduced number of

carbons in its side chain. There are two features that make ropivacaine usage safer than bupivacaine: less inherent cardiovascular and CNS toxicity, and less systemic absorption. Because of its decreased liposolubility, it is harder to pass the blood-brain-barrier and cause an increased duration of action (Dugdale, 2011, Casati and Putzu, 2005). It is proved that ropivacaine is 50% less potent than bupivacaine, in controlled trials. But in clinical studies, this difference is not noticeable. (Casati and Putzu, 2005)

2.7 Multimodal and pre-emptive analgesia

Several years ago, during development of analgesia as a discipline, the analgesic procedures were very simplistic. They consisted of a single drug and its dosage was titrated to effect. Sometimes, the dose would be too high that the side effects of the drug would become so serious before or during operations. After the discovery of new drugs and doing more researches, a better approach of analgesic drugs was realized and combination of different drugs was used to control pain. Surprisingly the outcome improved analgesia and it gradually became the routine. The use of combination of analgesic drugs leads to major reduction of each drug's dose and reducing the possible side effects (Gogny, 2006, Hellyer et al., 2007).

3 Chapter 3 – Experimental study

3.1 Material and Methods

3.1.1 Animals

Twenty client-owned dogs admitted for routine elective ovariohysterectomy were enrolled for the study (Appendix 6.1). The study was performed at the Onderstepoort Veterinary Academic Hospital (OVAH), Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa. The experimental protocol was submitted to and approved by the University of Pretoria Animal Ethics Committee (Project Number: V095-17, Appendix 6.2). Written consent form explaining the purpose of the study was signed by the owners (Appendix 6.3). Their health status was evaluated by physical examination and blood analysis including haematocrit, total protein, glucose, urea and creatinine. Only healthy dogs were enrolled in the study. Dogs were excluded if they were pregnant, aggressive, too sensitive to abdominal palpation or were having additional procedures. Dogs were admitted on the morning of the surgery, spent the night in hospital, and then were discharged the following day.

3.2 Study design

Dogs were randomly divided into two groups, ropivacaine (R) and saline placebo group (S) using the envelope technique. In the sealed envelope system dogs are given randomly generated treatment allocations within sealed opaque envelopes. Once a client has consented to enter the trial an envelope is opened and the dog is then offered the allocated treatment.

A single observer blinded to treatment allocation performed all pain scoring. Both groups were subjected to either IP ropivacaine (Naropin 1 mg kg^{-1}, AstraZeneca) or saline administration after linea alba incision during OHE. For the saline treatment group, the dose was identical to the calculated volume for ropivacaine based on body weight.

3.3 Anaesthesia and surgical procedures

Food, but not water, was withheld for 12 hours before anaesthesia. All dogs were premedicated with 0.03 mg kg^{-1} acepromazine maleate (Neurotranq 10 mg mL^{-1}, Virbac RSA Ltd) and 0.3 mg kg^{-1} morphine sulfate (10 mg ml^{-1}, Pharma-q Holdings Ltd) intramuscularly (IM). Thirty minutes later, a 22G intravenous (IV) catheter (Jelco®, Smiths Medical International Ltd) was aseptically placed in the cephalic vein and induction was performed using IV propofol (Fresenius Propoven 1% 10 mg mL^{-1}, Fresenius Kabi South Africa Ltd) at 4 mg kg^{-1} given to allow tracheal intubation. General anaesthesia was maintained using inhalation anaesthesia with isoflurane 2% (Isofor 250 mL, Safeline Pharmaceuticals Ltd) in oxygen. For dogs with body weight <5kg, a Mapleson D non-rebreathing circuit was used with the fresh gas flow rate set at twice the minute volume. For dogs >5kg body weight a circle rebreathing circuit was used with the fresh gas flow rate set at 15 mL kg^{-1} $minute^{-1}$. Similar anaesthetic depth was maintained for all dogs (eyes in ventromedial rotation). A balanced electrolyte solution was administered

during procedure at a rate of 10 mL kg^{-1} $hour^{-1}$. At completion of surgery, carprofen (Rimadyl 50 mg mL^{-1}, Zoetis South Africa) was administered IM at 4.4 mg kg^{-1}. Postoperative analgesia was maintained with carprofen (Rimadyl Chewable, Pfizer Animal Health, Sandton) per os at 4.4 mg kg^{-1} body weight for 3 days.

A single experienced surgeon blinded to treatment allocation was used to perform the surgery. The surgical technique consisted of a 3-5 cm skin, subcutaneous and linea alba incision on the ventral midline of the abdomen. Directly after the incision, ropivacaine or saline was instilled into the abdominal cavity. After the OHE was completed, the linea alba, subcutaneous tissues and skin were routinely sutured.

3.4 Physiological parameters

Intraoperative variables were measured with a multiparameter anaesthetic monitor (Cardiocap/5, Datex-Ohmeda, Helsinki) that included heart rate (HR, beats $minute^{-1}$), respiration rate (f_R, breaths $minute^{-1}$), oesophageal temperature (Centigrade), end-tidal carbon dioxide ($P_{E'}CO_2$, mmHg) partial pressure and end-tidal isoflurane concentration ($F_{E'}$Iso, %). Duration of surgical time and time to extubation were recorded. All variables were recorded on a patient monitoring sheet (Appendix 6.4) at 5 minute intervals during surgery (Time1 – Time5).

3.5 Pain assessment

Pre- and postoperative pain scoring was performed using a force algometer (ProdPro, Topcat Metrology Ltd), which is a hand-held device that evaluates the mechanical nociceptive threshold (MNT) to pressure by applying steadily increasing pressure to the skin of the abdominal wall until a response was observed (Figure 3.1). The pressure or force was measured in Newton (N) ranging from 0.1 to 32 N, accuracy ±0.5 N (according to the manufacturer). This algometer was

equipped with a rounded, 1 cm diameter, flat-ended probe. Pre- and postoperatively, MNT was measured in sternal recumbency with the probe applied to the left and right hand abdominal fossae (pressure points). Preoperatively in dorsal recumbency, four pressure points were evaluated at a virtual incision line. MNT was measured on the midline caudal to the umbilicus, at a cranial and caudal point, approximately 45 mm apart over the intended surgical incision line, and 10 mm lateral to the linea alba midway between the former two points. Postoperatively, MNT was measured 10 mm lateral to the skin incision and midway between suture line edges and 10 mm from the cranial and caudal suture line edges, avoiding direct pressure on the suture line. Dogs were observed for any reflex movement, vocalization, turning of the head towards the device, a sudden tense abdomen or attempts to bite in response to application of the device. Thereafter, the pressure was released and the maximum applied pressure recorded. The probe application was applied three times to each site. If a value deviated excessively from the other two values (>20%), it was rejected. The average of the three measurements was recorded as the MNT value. The interactions with the dogs were standardized and measurements were always performed in exactly the same sequence (Appendix 6.5). All dogs were restrained physically on the same examination table with the help of final year students.

Nociception was evaluated at five time points: Preoperatively the day before surgery, 30 minutes after premedication, postoperatively at 2, 4 and 20 hours postextubation. The preoperative measurement (baseline) was taken as the individual limit not to be exceeded when performing the postoperative measurements.

Figure 3.1 ProdPro is a hand-held Mechanical Algometer that evaluates the mechanical nociceptive threshold (MNT) to pressure by applying steadily increasing pressure. It measures the pressure in Newton (N) ranging from 0.1 to 32 N with an accuracy of ±0.5 N.

3.6 Result analysis

Statistical analysis

Data were assessed for normality by calculating descriptive statistics, plotting histograms and performing the Anderson-Darling test (MINITAB Statistical Software, Release 13.32, Minitab Inc, State College, Pennsylvania, USA) (Appendix 6.6). Breeds were described using frequencies and 95% mid-P exact confidence intervals (CI) and compared between groups using Fisher exact tests (Epi Info, version 6.04, CDC, Atlanta, GA, USA). Age and weight were described using the median and interquartile range (IQR) due to small samples sizes and apparent violation of the normality assumption for some variables. Baseline quantitative data were compared between groups using Mann-Whitney U tests. HR, RR, $ETCO_2$, and ETISO were compared between treatment groups using Student t tests after performing appropriate data transformations when necessary. Pressure data (the force required to elicit a pain response) were rank transformed prior to statistical analysis. A general linear modelling approach that

incorporated adjustment for the repeated sampling of dogs was used to analyze the pressure data. Linear mixed models were fit including fixed effect terms for treatment, time of sampling, and other covariates. A random effect term for dog was included in all models (variance components correlation structure) to account for repeated measurements. Post hoc comparisons were adjusted using Bonferroni correction of P values. Statistical models were fit using commercially available software (IBM SPSS Statistics Version 25, International Business Machines Corp., Armonk, NY, USA) and results interpreted at the 5% level of significance.

4 Chapter 4 – Results

4.1 Demographic data

Twenty purebred dogs (Appendix 6.1) were enrolled that consisted of Yorkshire Terrier (n=12), Jack Russel Terrier (n=2), Toy French Poodle (n=1), Scottish Terrier (n=1), Basset Hound (n=1), Dachshund (n=1), Chihuahua (n=1), and Weimaraner (n=1).

4.2 Age and weight

Ages ranged from 7 months to 7 years and weights ranged from 2.2 kg to 28 kg. Average values for ropivacaine group were: age= 12 month and weight = 4.4 kg, and for placebo group were: age= 21 month and weight = 5 kg. There were no significant differences between the R- and S-treatment groups regarding breed (p = 1), age (p = 1) and weight (p = 0.529) (Table 4.1).

4.1 Comparison of signalment for 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo.

Variable	Ropivacaine		Saline		P value†
	n	PE (95% CI or IQR*)	n	PE (95% CI or IQR)	
Breed					
Yorkshire terrier	6	0.60 (0.29, 0.86)	6	0.60 (0.29, 0.86)	1.0
Other breed	4	0.40 (0.14, 0.71)	4	0.40 (0.14, 0.71)	
Dog age (months)	10	12 (9, 48)	10	21 (9, 39)	1.0
Dog weight (kg)	10	4.4 (3.0, 4.9)	10	5.0 (2.6, 10.3)	0.529

PE = point estimate as proportion or median. CI = confidence interval. IQR = interquartile range,
*95% CI presented for proportions and range presented for medians.
†Based on Fisher exact tests for categorical data and Mann-Whitney U tests for quantitative data.

4.3 Intraoperative values

Data were not normally distributed (appendix 6.6). HR was significantly higher (p=0.007) in the R-group compare to S-group (R-group 123, S-group 87 beats min^{-1}) at 5 minutes (Time 1) (Table 4.2). During the other time points the differences were minimal. f_R was statistically significant different (p=0.047) between the groups (R-group 29 (18, 68) and S-group 15 (13, 23) breaths min^{-1}) at Time 4. This tendency towards lower rates in the S-group were also observed during the other time points although not statistically significant (p>0.05). FE´Iso values were not statistically significant different (p>0.05) between treatment groups. There were also no significant differences (p>0.05) between treatment groups for PE´CO_2 over time. No adverse effects from ropivacaine were observed during the investigation.

4.2 Comparison of intraoperative cardiorespiratory variables within 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo.

	Ropivacaine		Placebo		
Time point	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**P value***
Heart rate (bpm)					
Time 1	10	123 (98, 151)	10	87 (73, 98)	0.007
Time 2	10	121 (90, 133)	10	100 (79, 122)	0.206
Time 3	10	107 (93, 126)	10	98 (81, 123)	0.456
Time 4	10	105 (84, 120)	10	98 (70, 114)	0.590
Time 5	10	100 (80, 111)	10	93 (71, 106)	0.546
Respiratory rate (bpm)					
Time 1	10	38 (17, 48)	9	20 (19, 33)	0.187
Time 2	10	31 (23, 49)	9	15 (11, 30)	0.096
Time 3	10	24 (15, 65)	9	20 (15, 23)	0.204
Time 4	10	29 (18, 68)	9	15 (13, 23)	0.047
Time 5	10	40 (19, 50)	9	18 (8, 25)	0.077
$ETCO_2$ (%)					
Time 1	8	36 (34, 39)	5	30 (6, 41)	0.280
Time 2	8	35 (28, 37)	5	33 (6, 41)	0.560
Time 3	7	35 (11, 39)	5	32 (15, 45)	0.916
Time 4	7	29 (18, 37)	5	38 (21, 48)	0.315
Time 5	2	34 (31, 37)	4	39 (12, 51)	0.879
ETISO (%)					
Time 1	10	2.0 (1.4, 2.3)	10	1.7 (1.5, 1.9)	0.619
Time 2	10	1.9 (1.6, 2.2)	10	1.8 (1.5, 2.2)	0.960
Time 3	10	1.9 (1.6, 2.1)	10	1.6 (1.4, 2.2)	0.959
Time 4	10	1.8 (1.5, 2.0)	8	1.6 (1.4, 2.2)	0.865
Time 5	3	1.9 (1.2, 2.0)	4	2.0 (1.5, 2.3)	0.584

IQR = interquartile range.

*Based on Student t tests comparing data between ropivacaine and placebo treatment groups. Respiratory rate data were transformed using the natural logarithm and $ETCO_2$ data were rank transformed prior to statistical analysis.

4.4 Perioperative pain scores

The recorded pressure varied by anatomical location (p=0.002) and over time (p=0.001) (Table 4.3). The required pressure (Newton) to elicit a reflex response varied over time within both

treatment groups (Table 4.3). Post hoc tests identified significant differences at Postop2, 4 and 20 hours when compared to the Preop values. For the S-group, significant differences were identified at Postop2, 4 and only for Llin at Postop20 compared to Preop values. Dog age was not significantly associated with pressure measurements (p=0.425) but the effect of weight was significant (p=0.022) (Table 4.4). Treatment was not significantly associated with the measured pain response (p=0.417) (Table 4.4).

4.3 Comparison of postoperative pressures (Newton) required in 20 dogs to elicit a pain response treated with either intraperitoneal ropivacaine or saline following linea alba incision during ovariohysterectomy.

Treatment	n	Preoperative Median (IQR)	n	Premedication Median (IQR)	n	Postop 2 hours Median (IQR)	n	Postop 4 hours Median (IQR)	n	Postop 20 hours Median (IQR)
Ropivacaine										
Rabd	10	25.0 (19.5, 27.0)	10	26.5 (18.5, 28.1)	10	13.0† (9.0, 17.7)	10	15.4† (10.5, 18.0)	8	17.5† (9.5, 21.5)
Labd	10	27.0 (25.0, 28.0)	10	28.0 (20.0, 28.9)	10	11.5† (8.1, 14.7)	10	14.0† (10.4, 17.1)	8	13.0† (10.8, 20.0)
CrLin	10	27.0 (20.7, 28.5)	10	26.5 (21.0, 28.5)	10	11.6† (7.2, 18.8)	10	13.4† (4.9, 18.3)	8	8.3† (6.4, 19.5)
CdLin	10	23.5 (11.0, 27.3)	10	22.5 (13.6, 28.5)	10	4.4† (3.0, 12.3)	10	3.2† (2.0, 6.6)	8	5.1† (3.0, 6.4)
RLin	10	21.5 (10.0, 27.3)	10	21.2 (9.5, 28.5)	10	4.8† (3.4, 7.8)	10	2.9† (2.0, 6.7)	8	4.8† (3.3, 5.8)
Llin	10	22.0 (10.4, 27.0)	10	20.3 (8.4, 28.5)	10	4.3† (2.7, 10.0)	10	4.6† (2.9, 6.7)	8	5.0† (4.1, 5.0)
Placebo										
Rabd	10	25.0 (14.1, 27.0)	10	24.9 (12.7, 30.0)	10	6.8† (4.9, 14.1)	10	8.1† (3.6, 12.4)	4	14.0 (8.8, 19.5)
Labd	10	23.5 (14.4, 27.0)	10	21.0 (14.8, 30.0)	10	7.9† (4.0, 14.5)	10	7.8† (3.8, 14.0)	4	16.0 (8.3, 20.8)
CrLin	10	23.5 (13.1, 28.0)	10	24.0 (19.8, 28.5)	10	9.4† (4.1, 20.9)	10	8.4† (2.2, 11.5)	4	10.6 (8.5, 16.3)
CdLin	10	10.3 (7.3, 27.0)	10	14.5 (8.7, 28.5)	10	3.8† (1.8, 7.3)	10	2.2† (1.3, 7.2)	3	7.0 (4.0, 11.0)
RLin	10	10.8 (8.7, 27.0)	10	16.5 (7.9, 28.5)	10	4.0† (1.2, 7.4)	10	2.6† (2.2, 6.0)	3	7.0 (6.0, 12.5)
Llin	10	11.4 (8.7, 27.0)	10	13.5 (7.4, 28.5)	10	4.0† (2.2, 10.0)	10	3.8† (1.9, 6.6)	4	5.5† (3.4, 10.7)

IQR = interquartile range. Rabd = right abdomen. Labd = left abdomen. CrLin = cranial linea alba. CdLin = caudal linea alba. Rlin = right linea alba. Llin = left linea alba. Medians with an adjacent † are statistically significantly different ($p < 0.05$) from preoperative values.

4.4 Univariate predictors of the pressure* required to elicit a pain response within 20 dogs undergoing elective ovariohysterectomy receiving intra-abdominal ropivacaine or saline placebo following linea alba incision.

Variable	**Level**	$\hat{\beta}$ **(95% CI)**	**P value†**
Ropivacain treatment	Yes	30.2 (-46.3, 106.7)	0.417
	No	Referent	
Breed	Yorkshire	-7.5 (-86.9, 72.0)	0.846
	Other breed	Referent	
Location	Rabd	117.7 (80.1, 155.3)	<0.001
	Labd	112.6 (75.0, 150.2)	
	CrLin	111.3 (73.7, 148.8)	
	CdLin	0.3 (-37.4, 38.0)	
	RLin	-1.0 (-38.7, 36.7)	
	Llin	Referent	
Time	Post-op 3	-172.3 (-201.9, -142.7)	<0.001
	Post-op 2	-216.8 (-241.4, -192.1)	
	Post-op 1	-196.2 (-220.9, -171.6)	
	Pre-med	8.3 (-16.4, 33.0)	
	Pre-op	Referent	
Age of animal (months)	Continuous	0.72 (-1.13, 2.57)	0.425
Weight of animal (kg)	Continuous	6.62 (1.08, 12.17)	0.022

Rabd = right abdomen. Labd = left abdomen. CrLin = cranial linea alba. CdLin = caudal linea alba. Rlin = right linea alba. Llin = left linea alba.

*Rank transformed prior to statistical analysis.

†Based on mixed-effects linear regression including a random effect for dog.

5 Chapter 5 – Discussion

Discussion

There was no improvement in analgesia in the postoperative period from the IP administration of ropivacaine after OHE. Morphine and carprofen were co-administered perioperatively in this study suggesting that these treatments were sufficient and no additional benefit from the ropivacaine could be detected. The tendency for higher intraoperative HR could be the result of systemic ropivacaine absorption (Knudsen et al., 1997). The use of IP local anesthesia for postoperative analgesia is an attractive method due to its simplicity and low cost. In the present study, IP ropivacaine was used since it is expected to be equally effective to bupivacaine for postoperative pain relief (Hansen, 2004). Furthermore, ropivacaine has less toxicity and a lower rate of cardiac and neuronal complications due to its vasoconstricting properties, which may reduce absorption into the systemic circulation (Hansen, 2004, Kang and Kim, 2010). Nevertheless, ropivacaine has the potential to have some adverse effects (Scott et al., 1989, Kang and Kim, 2010). To limit the adverse effects, in this study, the authors decided to administer ropivacaine at a rate of 1 mg kg^{-1}, which is 33% of the recommended dosage (Clarke et al., 2014) and may be another possible reason for the lack of effect observed in this study. In addition, IP absorption may be associated with a higher absorption rate due to its exposure to the large peritoneal surface. “Toxicity is more common after performing some blocks, such as intercostal and interpleural blocks, which have a high degree of systemic absorption” (Clarke et al., 2014).

Up to now, there have been no reports on the use of IP ropivacaine in dogs. Nonetheless, in humans, ropivacaine decreases the need for postoperative analgesics such as opioids in women

undergoing caesarean delivery (Bamigboye and Justus, 2008). The use of other analgesics was reduced in the ropivacaine group (Bamigboye and Justus, 2008). In their study, ropivacaine was sprayed directly onto the surgical wound as well as instilled into the peritoneum. Systemic analgesics were only administered on patient demand. The use of laparoscopic surgery compared to conventional open surgery results in less postoperative pain in humans (Enes et al., 2011, Kozol et al., 1997, Anderson et al., 2006). In dogs, bupivacaine administered subcutaneous (SC) and IP was associated with positive benefits (Campagnol et al., 2012, Carpenter et al., 2004, Savvas et al., 2008). In the Campagnol study, incisional or IP bupivacaine was given in combination with systemic butorphanol. In the Carpenter study, bupivacaine or lidocaine were administered IP and SC before wound closure in addition to systemic butorphanol (Carpenter et al., 2004). In the Savvas study, bupivacaine was administered SC and IM, and dogs were excluded if they required additional analgesia (Savvas et al., 2008).

The effectiveness of bupivacaine is controversial. It has been reported that pre- and postoperative incisional bupivacaine with or without lidocaine were not beneficial for postoperative analgesia after OHE in dogs (Fitzpatrick et al., 2010, McKune et al., 2014, Kalchofner Guerrero et al., 2016). In the Guerrero study, bupivacaine or saline were administered as a subcutaneous 'splash' before skin closure. Conflicting results on the use of local anaesthetics could be due to differences in dose, location, type and timing of instillation (Ng and Smith, 2002).

In retrospection, the large variability of the results in this study could have been minimized by restricted patient selection. Psychological factors such as demeanor, anxiety, breed and body size appeared to influence the reaction of an individual animal to the application of the algometer. Anxiety (Hellyer et al., 2007) and aggressive behavior might cause a reflex reaction

to the algometer even after minimal pressure during the preoperative and postoperative periods – thus scoring a false reaction to pressure. As this was a clinical investigation, as opposed to a laboratory model, the choice of subjects was limited to dogs presented to the facility for OHE. Based on the investigation of Savvas (Savvas et al., 2008), ropivacaine could have been administered SC at the surgical line of incision and IM into the linea alba. However, in this instance the efficacy of the IP route was investigated. In addition, the surgery was performed by an experienced surgeon thus limiting the extent of surgical trauma, and thus pain from the abdominal incision during the postoperative period. Local anaesthetic infiltration of tissue subjected to surgical incision is expected to reduce pain in the immediate postoperative period. Routine analgesic drugs (i.e. morphine and carprofen) were administered that may have masked the beneficial effect from ropivacaine; however, the aim of this study was to evaluate local anaesthesia as part of multimodal analgesia, not as a sole analgesic method. Another possible reason for the failure to show an analgesic effect could be rapid dilution of local anaesthetics in the peritoneal cavity (Schulte-Steinberg et al., 1995).

Overall, the authors believe it would be beneficial to perform a similar study with a higher ropivacaine dosage and with only systemic analgesics used as a rescue intervention.

Conclusion

IP administration of 1 mg kg^{-1} of ropivacaine was not effective in lowering postoperative pain scores following OHE in the dog when administered in conjunction with systemic analgesics.

References

ANDERSON, M., MATHISEN, L. & OYEN, O. 2006. Postoperative pain and convalescence in living kidney donors—Laparoscopic versus open donor nephrectomy: A randomized trial. *Am J Transplant,* 6**,** 1438.

ARDEN, D., SEIFERT, E., DONNELLAN, N., GUIDO, R., LEE, T. & MANSURIA, S. 2013. Intraperitoneal instillation of bupivacaine for reduction of postoperative pain after laparoscopic hysterectomy: a double-blind randomized controlled trial. *Journal of minimally invasive gynecology,* 20**,** 620-626.

BAMIGBOYE, A. A. & JUSTUS, H. G. 2008. Ropivacaine abdominal wound infiltration and peritoneal spraying at cesarean delivery for preemptive analgesia. *International Journal of Gynecology & Obstetrics,* 102**,** 160-164.

BISHOP, B. 1980a. *Pain, Its Physiology and Rationale for Management,* American Physical Therapy Association.

BISHOP, B. 1980b. Pain: its physiology and rationale for management: part I. Neuroanatomical substrate of pain. *Physical Therapy,* 60**,** 13-20.

BUCK, L., VARRAS, M. N., MISKRY, T., RUSTON, J. & MAGOS, A. 2004. Intraperitoneal bupivacaine for the reduction of postoperative pain following operative laparoscopy: a pilot study and review of the literature. *Journal of Obstetrics and Gynaecology,* 24**,** 448-451.

CAMPAGNOL, D., TEIXEIRA-NETO, F. J., MONTEIRO, E. R., RESTITUTTI, F. & MINTO, B. W. 2012. Effect of intraperitoneal or incisional bupivacaine on pain and

the analgesic requirement after ovariohysterectomy in dogs. *Veterinary anaesthesia and analgesia,* 39**,** 426-430.

CARPENTER, R. E., WILSON, D. V. & EVANS, A. T. 2004. Evaluation of intraperitoneal and incisional lidocaine or bupivacaine for analgesia following ovariohysterectomy in the dog. *Veterinary anaesthesia and analgesia,* 31**,** 46-52.

CASATI, A. & PUTZU, M. 2005. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? *Best Practice & Research Clinical Anaesthesiology,* 19**,** 247-268.

CLARKE, K. W., HALL, L. W. & TRIM, C. M. 2014. Veterinary anaesthesia. Eleventh edition. ed. Edinburgh :: Elsevier/Saunders.

D'MELLO, R. & DICKENSON, A. H. 2008. Spinal cord mechanisms of pain. *British journal of anaesthesia,* 101**,** 8-16.

DUGDALE, A. 2011. *Veterinary anaesthesia: principles to practice*, John Wiley & Sons.

ENES, H., SEMIR, I., ŠEFIK, H., HUSNIJA, M. & GORAN, I. 2011. Postoperative pain in open vs. laparoscopic cholecystectomy with and without local application of anaesthetic. *Medicinski Glasnik,* 8.

FITZPATRICK, C. L., WEIR, H. L. & MONNET, E. 2010. Effects of infiltration of the incision site with bupivacaine on postoperative pain and incisional healing in dogs undergoing ovariohysterectomy. *Journal of the American Veterinary Medical Association,* 237**,** 395-401.

FREILICH, D. A., HOUCK, C. S., MEIER, P. M., PASSEROTTI, C. C., RETIK, A. B. & NGUYEN, H. T. 2008. The effectiveness of aerosolized intraperitoneal bupivacaine in reducing postoperative pain in children undergoing robotic-assisted laparoscopic pyeloplasty. *Journal of pediatric urology,* 4**,** 337-340.

GOGNY, M. 2006. Manejo da dor nos pacientes críticos. *Waltham Focus,* 16**,** 2-8.

HANSEN, T. G. 2004. Ropivacaine: a pharmacological review. *Expert Review of Neurotherapeutics,* 4**,** 781-791.

HARDIE, E. M., HANSEN, B. D. & CARROLL, G. S. 1997. Behavior after ovariohysterectomy in the dog: what's normal? *Applied Animal Behaviour Science,* 51**,** 111-128.

HEFTI, F. F., ROSENTHAL, A., WALICKE, P. A., WYATT, S., VERGARA, G., SHELTON, D. L. & DAVIES, A. M. 2006. Novel class of pain drugs based on antagonism of NGF. *Trends in pharmacological sciences,* 27**,** 85-91.

HELLYER, P., RODAN, I., BRUNT, J., DOWNING, R., HAGEDORN, J. E., ROBERTSON, S. A. & MEMBERS, A. A. P. M. G. T. F. 2007. AAHA/AAFP pain management guidelines for dogs and cats. *Journal of Feline Medicine & Surgery,* 9**,** 466-480.

HEWSON, C. J., DOHOO, I. R. & LEMKE, K. A. 2006. Perioperative use of analgesics in dogs and cats by Canadian veterinarians in 2001. *The Canadian Veterinary Journal,* 47**,** 352.

JONES, R. S. A history of veterinary anaesthesia. Anales de veterinaria de Murcia, 2002. Editum Ediciones de la Universidad de Murcia, 7-15.

KALCHOFNER GUERRERO, K. S., CAMPAGNA, I., BRUHL-DAY, R., HEGAMIN-YOUNGER, C. & GUERRERO, T. G. 2016. Intraperitoneal bupivacaine with or without incisional bupivacaine for postoperative analgesia in dogs undergoing ovariohysterectomy. *Veterinary anaesthesia and analgesia,* 43**,** 571-578.

KANG, H. & KIM, B. G. 2010. Intraperitoneal Ropivacaine for Effective Pain Relief after Laparoscopic Appendectomy: A Prospective, Randomized, Double-blind, Placebo-controlled Study. *Journal of International Medical Research,* 38**,** 821-832.

KIM, Y. K., LEE, S. S., SUH, E. H., LEE, L., LEE, H. C., LEE, H. J. & YEON, S. C. 2012. Sprayed intraperitoneal bupivacaine reduces early postoperative pain behavior and biochemical stress response after laparoscopic ovariohysterectomy in dogs. *The Veterinary Journal,* 191**,** 188-192.

KNUDSEN, K., BECKMAN SUURKÜLA, M., BLOMBERG, S., SJÖVALL, J. & EDVARDSSON, N. 1997. Central nervous and cardiovascular effects of iv infusions of ropivacaine, bupivacaine and placebo in volunteers. *British journal of anaesthesia,* 78**,** 507-514.

KOZOL, R., LANGE, P. M., KOSIR, M. & ET AL. 1997. A prospective, randomized study of open vs laparoscopic inguinal hernia repair: An assessment of postoperative pain. *Archives of Surgery,* 132**,** 292-295.

KRAUS, B. H. 2012. Getting Friendly with Dexmedetomidine. *Retrieved March,* 8**,** 2014.

LEMKE, K. A. 2004. Understanding the pathophysiology of perioperative pain. *The Canadian veterinary journal = La revue veterinaire canadienne,* 45**,** 405-413.

MCKUNE, C. M., PASCOE, P. J., LASCELLES, B. D. X. & KASS, P. H. 2014. The challenge of evaluating pain and a pre-incisional local anesthetic block. *PeerJ,* 2**,** e341.

MELZACK, R. & WALL, P. D. 1965. *Pain mechanics.A new theory, science [online] 150: 171-179.*

MERSKEY, H. E. 1986. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. *Pain.*

MOLONY, V. & KENT, J. E. 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of animal science,* 75**,** 266-272.

MONTEIRO, V. A. S. 2015. *Effectiveness of ropivacaine blocks in elective ovariohysterectomy in dogs for control of post-operative pain*.

NG, A. & SMITH, G. 2002. Editorial I: Intraperitoneal administration of analgesia: is this practice of any utility? : Oxford University Press.

PAIN, C. NA. *DEFINITION OF CANCER PAIN* [Online]. Available: http://www.change-pain.com/grt-change-pain-portal/change_pain_home/chronic_pain/indication/cancer_pain/definition/en_EN/310700133.jsp [Accessed].

PERNIOLA, A., FANT, F., MAGNUSON, A., AXELSSON, K. & GUPTA, A. 2013. Postoperative pain after abdominal hysterectomy: a randomized, double-blind, controlled trial comparing continuous infusion vs patient-controlled intraperitoneal injection of local anaesthetic. *British journal of anaesthesia,* 112**,** 328-336.

PERNIOLA, A., MAGNUSON, A., AXELSSON, K. & GUPTA, A. 2014. Intraperitoneal Local Anesthetics Have Predominant Local Analgesic EffectA Randomized, Double-blind Study. *The Journal of the American Society of Anesthesiologists,* 121**,** 352-361.

PLUMB, D. C. 2005. *Plumb's veterinary drug handbook*, PharmaVet.

RIVIERE, J. E. & PAPICH, M. G. 2017. *Veterinary pharmacology and therapeutics*, John Wiley & Sons.

ROY, K. K., SUBBAIAH, M., NAHA, M., KUMAR, S., SHARMA, J. B. & JAHAGIRDAR, N. 2014. Intraperitoneal bupivacaine for pain relief after minilaparoscopy in patients with infertility. *Archives of Gynecology and Obstetrics,* 289**,** 337-340.

SAVVAS, I., PAPAZOGLOU, L. G., KAZAKOS, G., ANAGNOSTOU, T., TSIOLI, V. & RAPTOPOULOS, D. 2008. Incisional block with bupivacaine for analgesia after celiotomy in dogs. *Journal of the American Animal Hospital Association,* 44**,** 60-66.

SCHULTE-STEINBERG, H., WENINGER, E., JOKISCH, D., HOFSTETTER, B., MISERA, A., LANGE, V. & STEIN, C. 1995. Intraperitoneal versus interpleural morphine or bupivacaine for pain after laparoscopic cholecystectomy. *Anesthesiology: The Journal of the American Society of Anesthesiologists,* 82**,** 634-640.

SCOTT, D. B., LEE, A., FAGAN, D., BOWLER, G., BLOOMFIELD, P. & LUNDH, R. 1989. Acute toxicity of ropivacaine compared with that of bupivacaine. *Anesthesia and analgesia,* 69**,** 563-569.

TRANQUILLI, W. J., THURMON, J. C. & GRIMM, K. A. 2013. *Lumb and Jones' veterinary anesthesia and analgesia*, John Wiley & Sons.

VIÑUELA-FERNÁNDEZ, I., JONES, E., WELSH, E. M. & FLEETWOOD-WALKER, S. M. 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. *The Veterinary Journal,* 174**,** 227-239.

6 Appendix

Appendix 6.1: Patients list

Dog Number	Rx	Serial Number	Name	Age	Weight	Breed
1	R	5928417	Kippie	7 M	5.6	Jack Russel Terrier
2	R	5860717	Bella	6 M	2.2	Toy French Poodle
3	R	6112818	Lulu	10 M	9.2	Scottish Terrier
4	R	6102618	Babsie	12 M	2	Yorkshire Terrier
5	R	6149418	Lucy-Mae	24 M	4.4	Yorkshire Terrier
6	R	5698117	Lexi	10 M	3.2	Yorkshire Terrier
7	R	6254118	Keeda	12 M	4.6	Dachshund
8	R	6276918	Lily	48 M	4.6	Yorkshire Terrier
9	R	6276718	Foxie	84 M	4.4	Yorkshire Terrier
10	R	6277018	Trixie	48 M	3.6	Yorkshire Terrier
11	S	6070818	Bella	8 M	17.6	Basset Hound
12	S	5559017	Saartjie	6 M	3.8	Yorkshire Terrier
13	S	6102718	Ayra	24 M	2.2	Yorkshire Terrier
14	S	6149618	Storm	36 M	3.2	Yorkshire Terrier
15	S	6149518	Skylie	36 M	6.4	Yorkshire Terrier
16	S		Scruffy	10 M	2.6	Yorkshire Terrier
17	S	6254218	Pipper	48 M	2.4	Chihuahua
18	S	6276818	Cherry	48 M	6.2	Yorkshire Terrier
19	S	6320118	Missy	9M	7.8	Jack Russel Terrier
20	S	6330918	Tosca	18M	28	Weimarner

Appendix 6.2: Animal ethics certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Evaluation of intraperitoneal ropivacaine for peri-operative analgesia during ovariohysterectomy in bitches**
PROJECT NUMBER	**V095-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. M Khanzadeh**

STUDENT NUMBER (where applicable)	**U_17231249**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	***Canine***	
NUMBER OF SAMPLES	**20**	
Approval period to use animals for research/testing purposes		**September 2017 – September 2018**
SUPERVISOR	**Prof. GF Stegmann**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	30 October 2017
CHAIRMAN: UP Animal Ethics Committee	Signature	

S4285-15

Appendix 6.3: Research consent form

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Consent to take part in a research project

A research project is undertaken at the Veterinary Academic Hospital of the

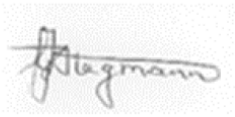
University of Pretoria to investigate abdominal local anaesthetic techniques for pain management in bitches during ovariohystertectomy (spay).

Consent from the owner is required by the Animal Ethics Committee of the University of Pretoria.

The dogs will be anaesthetised and spayed with the standard technique routinely used. In addition

local anaesthetic will be administered that will improve analgesia during surgery and the postoperative period

Prof. GF Stegmann (Project Leader) (012 5298279)



I (full name),_____, owner of _____
hereby give permission that my dog maybe used in in this clinical trial.

Signature .....Date .....

Appendix 6.4: Recording sheet

Patient details

Patient Number:……..... Patient Name: ……..… Species: …….

Breed: ……..... Age: …..... Weight: …….... Temperature: ……....

Physical examination

HR: ……. RR: ……. MM: …… CRT: …….. Auscultation: ……

Blood work

HT: …… TSP: …… Glu: …… Urea: …. ALT: …….. Creatinine: ……..

Time	Procedure	Drug	Dosage	Mg Total	Ml Total	Route
	Premed	Acepromazine	0.03 mg/kg			
	Premed	Morphine	0.3 mg/kg			
	Induction	Propofol	4-6 mg/kg			
	Rescue Analgesia	Morphine	0.2 mg/kg			
	Rescue Analgesia	Carprofen	4 mg/kg			
	Rescue Analgesia	Meloxicam	0.1 mg/kg			
	Antibiotic	Cefazolin	20 mg/kg			

Fluid therapy	Dosage	Total
Ringer lactate	10ml/kg/h	ml/h
Ringer bolus	10ml/kg	ml

Timings	
Incision:	Duration of Surgery:
1st Ovary:	Duration of Anaesthesia:
2nd Ovary:	Duration of Monitoring:
Uterine body:	Notes:
Ropivacaine:	
Normal Saline:	
Closure:	
End of procedure:	
Extubation:	
Discharge:	

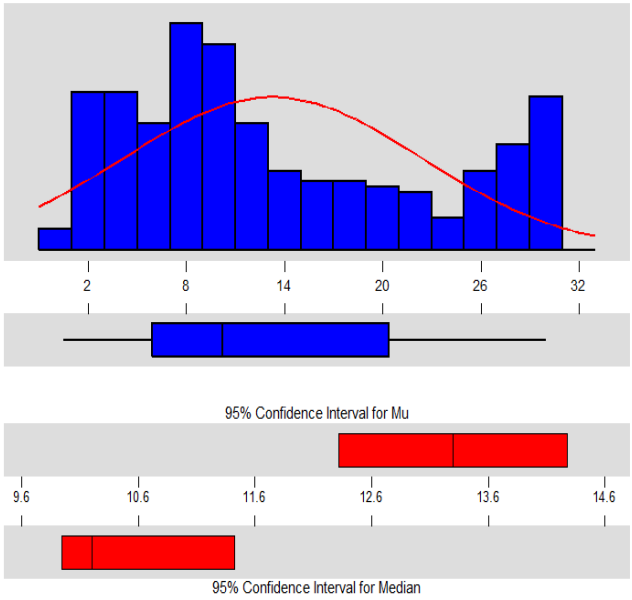
Appendix 6.5: Chart for recording pain scores measured by the mechanical algometer

Time:

Position ↓	**Pain Intensity** →	1	2	3	Average
Sternal Recumbancy (Right abdominal fossae)					
Sternal Recumbancy (Left abdominal fossae)					
Dorsal Recumbancy (Umbilicus)					
Dorsal Recumbancy (1/3 of linea alba)					
Right side of linea alba					
Left side of linea alba					

Appendix 6.6: Normality assessment

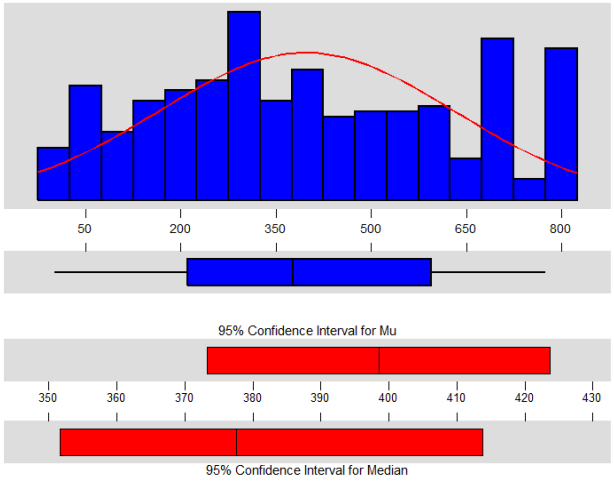
Descriptive Statistics



Variable: Pressure

Anderson-Darling Normality Test	
A-Squared:	10.029
P-Value:	0.000
Mean	13.2984
StDev	9.0437
Variance	81.7894
Skewness	0.566603
Kurtosis	-9.6E-01
N	328
Minimum	0.5000
1st Quartile	5.9025
Median	10.2000
3rd Quartile	20.3750
Maximum	30.0000
95% Confidence Interval for Mu	
12.3161	14.2808
95% Confidence Interval for Sigma	
8.4006	9.7944
95% Confidence Interval for Median	
9.9430	11.4228

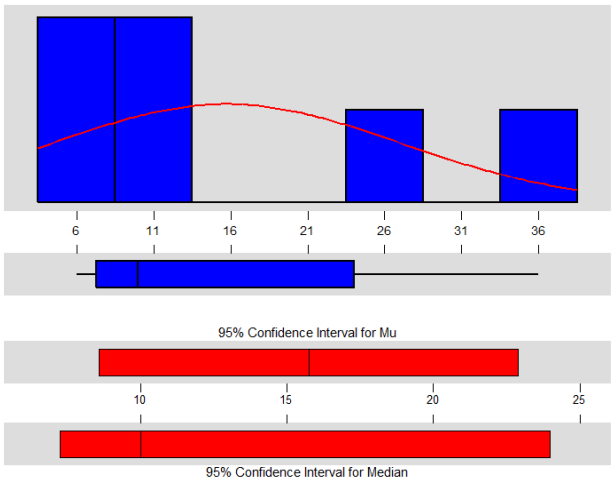
Descriptive Statistics



Variable: Rank_pressur

Anderson-Darling Normality Test	
A-Squared:	3.762
P-Value:	0.000
Mean	398.524
StDev	232.255
Variance	53942.3
Skewness	0.115802
Kurtosis	-1.12699
N	328
Minimum	1.500
1st Quartile	211.250
Median	377.500
3rd Quartile	595.250
Maximum	775.000
95% Confidence Interval for Mu	
373.296	423.753
95% Confidence Interval for Sigma	
215.737	251.533
95% Confidence Interval for Median	
351.645	413.867

Descriptive Statistics



Variable: Age_month

Anderson-Darling Normality Test

A-Squared:	1.111
P-Value:	0.004
Mean	15.7500
StDev	11.2664
Variance	126.932
Skewness	1.07700
Kurtosis	-3.5E-01
N	12
Minimum	6.0000
1st Quartile	7.2500
Median	10.0000
3rd Quartile	24.0000
Maximum	36.0000

95% Confidence Interval for Mu

8.5917	22.9083

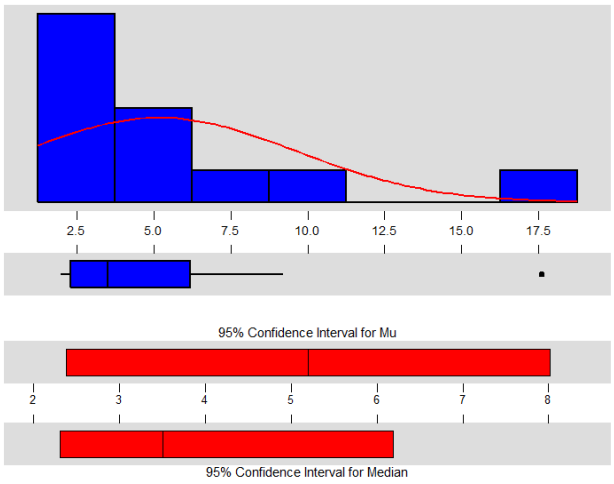
95% Confidence Interval for Sigma

7.9811	19.1290

95% Confidence Interval for Median

7.2631	24.0000

Descriptive Statistics



Variable: Weight_kg

Anderson-Darling Normality Test

A-Squared:	1.276
P-Value:	0.001
Mean	5.20000
StDev	4.44113
Variance	19.7236
Skewness	2.30251
Kurtosis	5.78890
N	12
Minimum	2.0000
1st Quartile	2.3000
Median	3.5000
3rd Quartile	6.2000
Maximum	17.6000

95% Confidence Interval for Mu

2.3782	8.0218

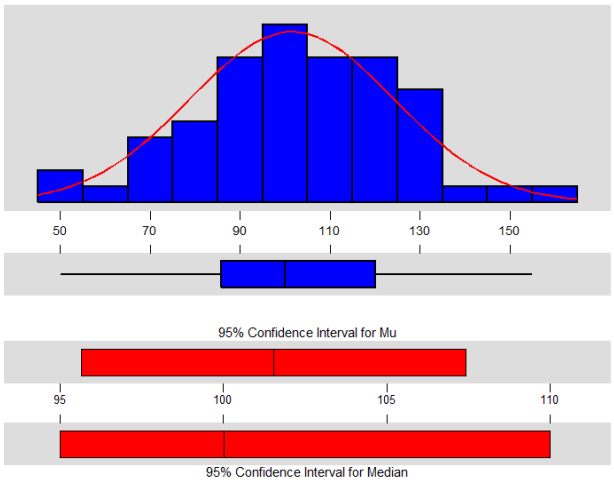
95% Confidence Interval for Sigma

3.1461	7.5405

95% Confidence Interval for Median

2.3052	6.1895

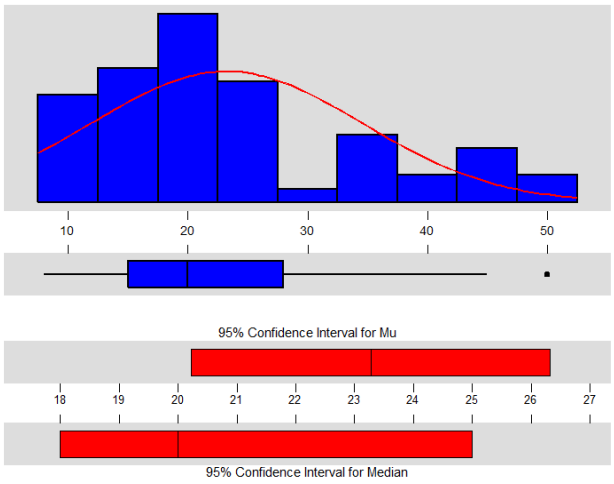
Descriptive Statistics



Variable: HR

Anderson-Darling Normality Test	
A-Squared:	0.260
P-Value:	0.702
Mean	101.533
StDev	22.741
Variance	517.134
Skewness	-7.3E-02
Kurtosis	-1.6E-01
N	60
Minimum	50.000
1st Quartile	85.750
Median	100.000
3rd Quartile	120.000
Maximum	155.000
95% Confidence Interval for Mu	
95.659	107.408
95% Confidence Interval for Sigma	
19.276	27.736
95% Confidence Interval for Median	
95.000	110.000

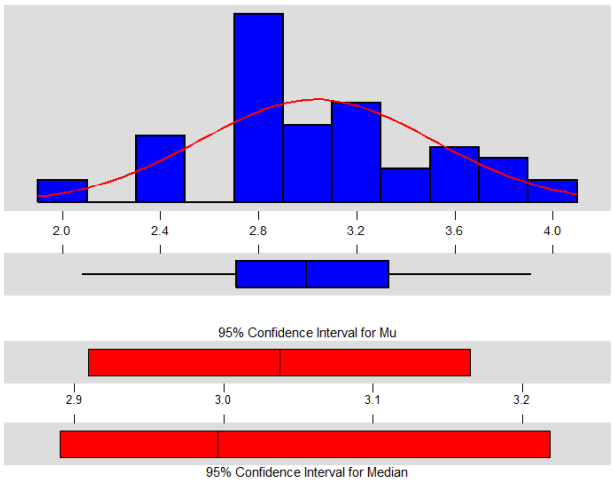
Descriptive Statistics



Variable: RR

Anderson-Darling Normality Test	
A-Squared:	2.137
P-Value:	0.000
Mean	23.2727
StDev	11.2833
Variance	127.313
Skewness	0.928991
Kurtosis	-3.5E-02
N	55
Minimum	8.0000
1st Quartile	15.0000
Median	20.0000
3rd Quartile	28.0000
Maximum	50.0000
95% Confidence Interval for Mu	
20.2224	26.3230
95% Confidence Interval for Sigma	
9.4990	13.8993
95% Confidence Interval for Median	
18.0000	25.0000

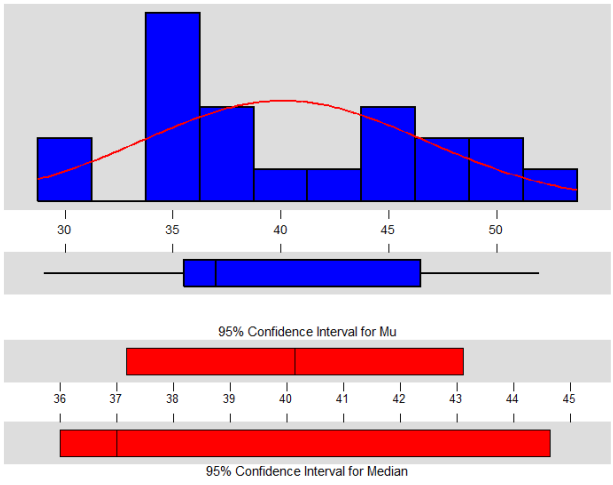
Descriptive Statistics



Variable: ln_RR

Anderson-Darling Normality Test	
A-Squared:	0.636
P-Value:	0.092
Mean	3.03734
StDev	0.47374
Variance	0.224431
Skewness	6.57E-02
Kurtosis	-5.4E-01
N	55
Minimum	2.07944
1st Quartile	2.70805
Median	2.99573
3rd Quartile	3.33220
Maximum	3.91202
95% Confidence Interval for Mu	
2.90927	3.16541
95% Confidence Interval for Sigma	
0.39883	0.58358
95% Confidence Interval for Median	
2.89037	3.21888

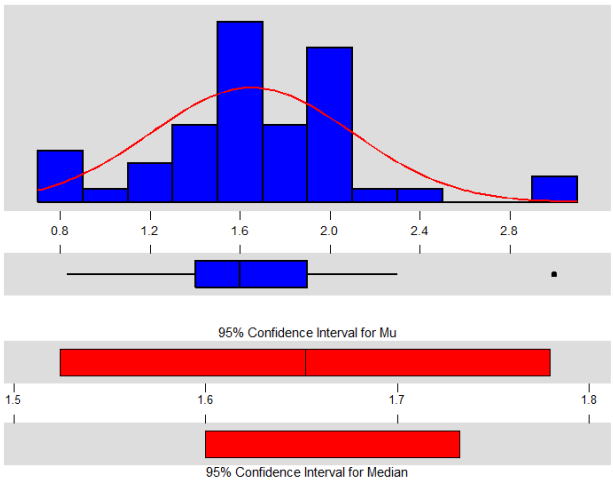
Descriptive Statistics



Variable: ETCO2

Anderson-Darling Normality Test	
A-Squared:	0.613
P-Value:	0.096
Mean	40.1429
StDev	6.5367
Variance	42.7286
Skewness	0.242714
Kurtosis	-1.06213
N	21
Minimum	29.0000
1st Quartile	35.5000
Median	37.0000
3rd Quartile	46.5000
Maximum	52.0000
95% Confidence Interval for Mu	
37.1674	43.1183
95% Confidence Interval for Sigma	
5.0010	9.4395
95% Confidence Interval for Median	
36.0000	44.6530

Descriptive Statistics



Variable: ETIso

Anderson-Darling Normality Test

A-Squared:	0.991
P-Value:	0.012
Mean	1.65200
StDev	0.44987
Variance	0.202380
Skewness	0.660435
Kurtosis	1.91601
N	50
Minimum	0.83000
1st Quartile	1.40000
Median	1.60000
3rd Quartile	1.90000
Maximum	3.00000

95% Confidence Interval for Mu

1.52415	1.77985

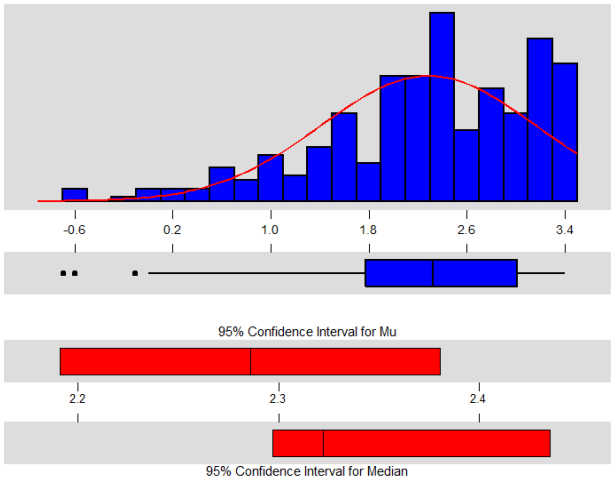
95% Confidence Interval for Sigma

0.37579	0.56059

95% Confidence Interval for Median

1.60000	1.73283

Descriptive Statistics



Variable: ln_pressure

Anderson-Darling Normality Test

A-Squared:	4.499
P-Value:	0.000
Mean	2.28580
StDev	0.87318
Variance	0.762439
Skewness	-8.2E-01
Kurtosis	0.456731
N	328
Minimum	-0.69315
1st Quartile	1.77533
Median	2.32239
3rd Quartile	3.01425
Maximum	3.40120

95% Confidence Interval for Mu

2.19095	2.38065

95% Confidence Interval for Sigma

0.81108	0.94565

95% Confidence Interval for Median

2.29682	2.43560