Use of Analgesic Combination Morphine-Lidocaine-Ketamine in Holstein Calves Undergoing Ventral Midline Herniorrhaphy

THESIS

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Abstract

Abdominal surgery is commonly performed in cattle for both diagnostic and therapeutic purposes. A recent survey of veterinarians in the United States revealed that abdominal surgery is thought to be the most painful of the commonly performed surgical procedures in cattle. Pain is thought to play an important role in post-operative performance in cattle undergoing abdominal surgery. However, pain evaluation in ruminants is difficult, and recent research suggests that assessment of pain in ruminants requires measurement of both physiologic and behavioral parameters. Combination analgesic techniques may be superior to individual techniques in calves following abdominal surgery. Pain and incisional algometry scores were evaluated for a total of 120 hours in calves following routine umbilical herniorrhaphy.

Twenty-one calves presenting for umbilical herniorrhaphy were randomly assigned to one of two treatment groups: 1) BAN: Flunixin meglumine 1.1 mg/kg IV following intubation and at 24 hours post-op 2) MLK: Co-infusion of morphine (4.75 mcg/kg/hr), lidocaine (2.11 mg/kg/hr) and ketamine (0.42 mg/kg/hr) for 24 hours beginning immediately following intubation. Co-infusion was discontinued at 24 hours. A modified sheep pain scoring system, as well as an algometer to measure incisional pain were used by one blinded evaluator to assess comfort at 14 timepoints during the 5 day

study period. There were no significant differences in heart rate, respiratory rate, or pain score between groups during the study period or during CRI administration. Incisional algometry scores were significantly different between groups during the CRI administration, with cattle in the MLK group having higher nociceptive thresholds than cattle in the BAN group($p=0.019$). During the entire study period, there was not a significant difference between groups, however there was a trend towards higher thresholds in the MLK group ($p=0.098$).

In addition to the pain scoring, blood samples were taking at 31 time points during the study period for pharmacokinetic analysis of the drugs used as well as serum cortisol analysis. Serum cortisol values were not significantly different between groups over the study period ($p=0.390$). However, significant differences were noted between groups during the CRI administration ($p<0.001$), with MLK animals having higher serum cortisol during this period than BAN animals. Additionally, time is a significant factor in cortisol concentration ($p=0.001$), with cortisol tending to decrease over time, and increase during periods of more intensive handling.

In conclusion, we found that pain scores were similar among groups both during the CRI administration and during the entire study period, and that cortisol and incisional algometry scores were significantly different between groups during the CRI administration.

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Fields of Study

Major Field: Comparative and Veterinary Medicine

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Chapter 1: Introduction

In recent years there has been an increased focus on animal welfare as well as identification and alleviation of pain in farm animals.[1] The mainstream media is increasingly critical of production animal welfare, and consumers are becoming cognizant of these issues. The AVMA's position on pain in animals states that the AVMA "believes that animal pain and suffering are clinically important conditions that adversely affect an animal's quality of life. Drugs, techniques, or husbandry methods used to prevent and control pain must be tailored to individual animals and should be based, in part, on the species, breed, age, procedure performed, degree of tissue trauma, individual behavioral characteristics, degree of pain, and health status."[2]

Abdominal surgery is considered by veterinarians to be the most painful surgical condition of cattle, and over 90% of veterinarians surveyed in a recent study use at least a single dose of an analgesic drug in the peri-operative period.[3] Despite veterinarians' concerns regarding cow comfort following abdominal surgery, pain management protocols for use in cattle following these procedures are extremely limited.[3] According to the aforementioned survey, the most common drug used in cattle in the post-operative period in the United States is flunixin meglumine, a non-steroidal anti-inflammatory drug. However, flunixin meglumine is not labeled for analgesic use in cattle. In fact, the

only drug labeled for analgesic or anesthetic use in cattle is 2% lidocaine, which has limited label uses and dosages. To date, there have been limited studies looking at assessment or relief of pain secondary to abdominal procedures in cattle.[4,5] Because abdominal surgical procedures are commonly performed in cattle, it is imperative that research be conducted to focus on both identification and alleviation of peri-operative and post-operative pain in these animals. Commonly performed abdominal procedures in cattle include standing exploratory laparotomy, correction of abomasal displacements, cesarean section and herniorrhaphy. Mitigation of painful stimuli leads to improved gastrointestinal function, return to health and decreased production losses.

The goal of this project is to provide data that will allow veterinarians to make evidence-based decisions regarding treatment of peri-operative and post-operative pain in cattle admitted to a veterinary hospital for abdominal surgery. The large majority of pain management research in cattle has focused on NSAIDs, with alternative therapies having only been sporadically evaluated.[4,6-8] There are no studies evaluating the use of intravenous constant rate infusions (CRIs) of pain medications to alleviate post-operative surgical pain in cattle. At this time, morphine-lidocaine-ketamine as a continuous rate infusion is used at the OSU-VMC in clinical patients both to relieve pain post-operatively and to treat ileus. It is our clinical impression that these patients benefit significantly from administration of these drugs.[9] The benefits and risks of the use of analgesic drugs in cattle continues to be an important topic of current research in pain management of farm animal patients.[6-8,10-18]

As previously mentioned, there is a paucity of objective data examining post-surgical pain of any type in cattle. The current study was designed to examine the efficacy of an intravenous CRI of MLK during the peri-operative period, using pain-scoring and incisional algometry data. Additionally we expected to be able to validate a method of pain assessment in individually housed cattle in the post-operative period, using both physiologic and behavioral parameters.

The primary goal of this study is to determine whether an intravenous CRI of MLK is more effective at reducing post-operative pain experienced by cattle undergoing abdominal surgery compared to 2 doses of flunixin meglumine. We hypothesize that cattle receiving a 24-hour MLK CRI will experience significantly less pain than cattle that receive two doses of flunixin meglumine post-operatively .

A pain scoring scale using both behavioral and physiologic parameters to assess post-surgical pain was used in these animals. Pressure tonometry was used as an objective measure of incisional pain. Cortisol levels were examined in the calves as well. Currently no drugs are labeled for analgesia in cattle in the United States, and this work may help in establishing withdrawal times for extralabel use of morphine, lidocaine, and ketamine when used as continuous rate co-infusions. Goals in the current study are to:

1. Develop and evaluate a standardized method for pain evaluation in hospitalized cattle undergoing abdominal surgical procedures.
2. Evaluate the analgesic efficacy of an MLK CRI in cattle undergoing abdominal surgery.

3. Complete a pharmacokinetic analysis of the three drugs (morphine, lidocaine, ketamine) in the bloodstream of study cattle during and after the CRI.

Chapter 2: Literature Review

2.1 Definitions of Pain

Pain is a complex sensation that can be defined, at the very least, as an unpleasant sensory and emotional experience associated with tissue damage.[19] Because of the complexity of the pain response, and the differences with which individuals and species experience pain, it is, at best, difficult to evaluate in domestic animals.

In domestic animals, there have been multiple attempts to define pain. It has been defined as an aversive sensory experience caused by actual or potential injury that elicits protective reactions, results in learned avoidance behavior, and may modify species-specific behavior, including social behavior. However, it has been acknowledged that pain is not the only stimulus that may elicit these behaviors.[20,21] It is well established that there is no reliable and universal indicator for pain in animals, making assessment of pain in animals extremely difficult.[22,23] Another definition of pain in domestic animals is an aversive sensory and emotional experience, which changes both behavior and physiologic responses in animals experiencing it.[22] In animals, it is difficult to separate certain other terms, such as suffering and stress, from pain. These conditions have been associated with many of the same physiological and behavioral changes seen in domestic animals in response to pain. It is not uncommon for these terms to be used synonymously in the

literature, and pain studies in domestic animals are invariably influenced by other factors, including stress.

2.2 Types of Pain

Surgical pain is unique in that it is being elicited in a controlled environment, particularly during elective surgical procedures. The surgical procedure, technique, and the surgeon's level of experience may all influence the magnitude of surgical pain. Pain control may play an important role in surgical outcomes by relieving the body of the burden of pain responses.[24]

2.2.1 Somatic vs. Visceral Pain

Surgery causes disruption of the skin, muscle and other tissues via the incision, potentially causing acute nociceptive pain via rapid relay of signals from nociceptors at the site of the injury through to the central nervous system. These nociceptors can detect somatic pain (from the skin, muscle, joints and bones), as well as visceral pain (from the internal organs). Somatic pain can be further divided into superficial and deep pain, with superficial pain arising from stimulation of receptors in the skin, and deep pain arising from underlying structures, such as periosteum. [23] Visceral pain arises from stretch receptors in the peritoneum and internal organs, which respond to changes in tension and shape.[25]

In humans, somatic pain (such as pain associated with a surgical incision) is reportedly a highly localized, sharp, sensation. In contrast, visceral pain is described as dull, diffuse, and poorly localized.[25,26] Visceral pain can also occur in response to tissue

manipulation, such as distension or traction, ischemia and inflammation, and may trigger autonomic responses.[27] The visceral sensory pathways are excited by inflammatory changes, which can result in peripheral and central sensitization.[25] Visceral pain is considered one of the most painful clinical conditions in cattle, and abdominal surgical procedures are considered by veterinarians to be the most painful surgical procedures experienced by farm animals.[3,28,29] Abdominal surgeries require both sharp dissection of skin and muscle, as well as the manipulation of internal organs and other tissues within the peritoneal cavity. Although abdominal procedures likely result in both somatic and visceral pain responses, there is little data in livestock species addressing the differences in the type, magnitude, and duration of these two responses.[30] Taylor and Weary found in their study looking at castration in piglets that there was a more vocal response during the portion of the castration associated with visceral pain (traction and severing of the spermatic cord) when compared to portions of the procedure associated with somatic pain (scrotal incision).[30]

2.2.2 Acute vs. Chronic Pain

Depending on duration, pain can be acute or chronic. Acute pain is expected to last throughout the expected healing process of an injury or surgically induced lesion. Definitions of chronic pain vary, but it can be most simply be defined as pain that persists beyond the expected healing time.[23] While acute pain generally responds to analgesic treatment, chronic pain may not respond to traditional analgesic therapies. Inflammation that occurs after injury can decrease excitability thresholds, further activating nociceptive fibers due to the local release of inflammatory mediators and cytokines. Local

inflammation can also stimulate peripheral nociceptors resulting in hyperalgesia (increased sensitivity to pain). Primary hyperalgesia develops at the site of the injury while secondary hyperalgesia develops in the surrounding uninjured tissue.[27] Allodynia, sensitivity to previously innocuous stimuli, in the area adjacent to the incision may also occur. Recently, a surgically induced traumatic reticuloperitonitis model of visceral pain was tested in cattle, with "agitation while lying" as the only behavioral outcome validated for assessing acute and chronic pain in this model.[4] Thus it may be extremely difficult to distinguish between acute and chronic pain in animal models.

2.3 Assessment of Pain in Cattle

Assessment of pain in cattle is difficult, as there is no current standard for pain assessment.[4,23,31] In its essence, assessment of pain is subjective, with both physiological and behavioral indices being used to provide indirect evidence that the animal in question is experiencing pain.[22] Those most qualified to assess pain in domestic animals include veterinarians, farmers, and animal caretakers.[22] These individuals will be familiar with at least the species and breed in question, and may even have knowledge of the individual animal. However, even among veterinarians, evaluation and assessment of painful conditions in cattle is largely subjective. A number of factors, including gender, % dairy/beef in practice, having been raised on a farm or participated in 4H or FFA during childhood, having graduated from a rural high school, age, or political affiliation may play a role in individual assessment of pain[3,28,29]

Assessment of pain in cattle has focused on behavioral responses to pain, physiologic responses such as plasma cortisol concentrations, and other parameters

including weight gain and feed intake. Behavioral responses to stress and pain are well documented in cattle. In cattle, documented behavioral responses to pain include subdued behavior, decreased feed intake and rumen motility, increased time lying down, and a failure to clean the nares.[10,12,16,21,31-34] Physiologic responses to pain include increased heart rate or respiratory rate, increased substance P, increased serum cortisol levels, decreased fecal production, and ileus.[5,10,31]

One additional tool, pressure algometry has been used for assessing pain in livestock. The algometer measures the amount of force applied to a surface (such as the skin), and the amount of force required to elicit a withdrawal response is recorded, and painful thresholds are determined using a withdrawal response. This technique has been used in both calves and adult cattle to measure pain responses.[16,35-37] Following disbudding, calves had significantly lower peri-incisional nociceptive thresholds when compared with the pre-surgery threshold.[16,36] Algometry has also been used to assess analgesic efficacy. Heinrich *et al* demonstrated that calves that received an NSAID at time of disbudding displaying a higher peri-incisional nociceptive threshold than calves that received a placebo solution.[16]

Although the technique appears to be useful, it is difficult to avoid responses associated with stressors due the handling required to accomplish the technique.[20] This may be dependent on the temperament of the animals being examined, and the previous handling history of the animals being examined.[20,21] Advantages of pressure algometry in a clinical setting include cost and ease of use. Potential disadvantages include intra- and inter-observer variability.

Observing animals for behavioral or physiologic changes, such as changes in attitude, feed intake, HR, or RR can be useful in clinical and research settings with animals housed individually or in small groups. These measurements are not continuous, however, and may be subjective. Many social and environmental factors influence pain perception and responses in cattle. Cows subjected to social isolation, stressors, and illness all demonstrated significant physiologic and behavioral changes in response to these conditions.[20,21,33] Despite the lack of standardization, assessment of pain in cattle has been described for a variety of conditions.[4,5,12,18,22,31,38-41]

2.4 Analgesic Drug Use in Cattle

Pain management in livestock animals has become not only a concern for producers and practitioners, but a concern of the general public in recent years.[1] In cattle, economic and logistic factors play roles in whether or not appropriate pain management practices are instituted.[42] Despite the interest in developing affordable and effective pain management strategies in cattle, NSAIDs, particularly flunixin meglumine, remain the mainstay of pain control in cattle. In recent years, many studies have focused on use of alternative therapies, either combined with NSAIDS or alone, to treat pain elicited by commonly performed procedures, such as castration and dehorning, with apparent benefits coming from the NSAID and non-NSAID drugs.[6,7,10-16,43-46] Veterinarians have cited economics, lack of knowledge of appropriate pain assessment and management techniques, and regulatory issues as reasons for failing to provide appropriate analgesia.[3,28,47]

2.4.1 Flunixin Meglumine

NSAIDS exert their analgesic action by inhibiting cyclooxygenase (COX) enzymes. COX enzymes oxidize arachadonic acid to various compounds including prostaglandins. There are at least three isomers of the COX enzyme, with COX-1 generating prostaglandins that are involved with normal homeostatic mechanisms. COX-2 is an enzyme that is thought to be induced in response to injury, and is known to play a key role in nociception. A third isoform, COX-3, has been described in rodents and exerts a protective effect by initiating fever.[24] NSAIDs have differential activity depending on the COX enzyme inhibited. Non-specific COX inhibitors act on both COX-1 and COX-2 isoenzymes. Non-specific COX inhibitors include aspirin, flunixin, and phenylbutazone, with flunixin meglumine being the only NSAID carrying a label for use in cattle in the United States. More selective COX-2 inhibitors have been developed recently and are thought to be safer for long-term use (e.g., COX-2 inhibition is less likely to interfere with homeostasis of abomasal mucosa or renal perfusion). However, COX-1 may also play a key role in the pain experience, particularly visceral pain.[24]

Some NSAIDS are thought to have comparable efficacy to the pure μ-agonist opioids.[24] In cattle, veterinarians use NSAIDS commonly to relieve pain for a variety of conditions.[3,48] It should be noted, however, that flunixin meglumine is only labeled for control of fever associated with respiratory disease or mastitis and fever and inflammation associated with endotoxemia. Despite the general consensus that NSAIDS as a drug class do provide effective analgesia in cattle, flunixin meglumine lacks a label for this use. Additionally, flunixin meglumine is only labeled for intravenous use, which

may make use by owners or in herd situations difficult. Despite the widespread use of flunixin meglumine by practitioners, studies showing the analgesic effects of flunixin meglumine administered alone at the approved dose of 1.1-2.2 mg/kg are limited in the published literature.[12-15,49]

2.4.2 Morphine

Opioids are used often in veterinary medicine because of their ability to provide safe and effective analgesia. Their use in ruminant animals, however, has been limited due to cost and regulatory issues.[4,6]

The analgesic effects of opioids are associated with binding to μ, κ, δ receptors.[24] Drug binding activates receptor-linked potassium channels and inhibits voltage-gated calcium channels, thereby decreasing propagation of the pain signal. In addition to producing analgesia, μ-receptor activation is associated with respiratory depression, ileus, sedation, bradycardia, increased appetite, sedation, excitement or dysphoria and altered thermoregulation. Therefore, partial and mixed receptor opioids may have fewer adverse effects than pure μ- agonists. There are currently no narcotic analgesics approved for use in cattle in the United States. However, opioids are used off label with some regularity in food animal practice, with morphine and butorphanol being the two most commonly used opioids in farm animal species.[50] Morphine has been examined in farm animals in limited instances, with only a handful of reports describing the use of morphine in cattle.[4,51,52]

2.4.3 Lidocaine

Local anesthetics are used commonly in livestock animals, particularly as local or regional anesthetics via nerve blockade, or as caudal epidural agents.[3,12,53,54] Lidocaine is approved for use in cattle for these purposes (maximum of 15ml as epidural or maximum of 20ml as nerve block), and is the only drug labeled for analgesic use in cattle.[55] Lidocaine is not labeled for intravenous use in any veterinary species,. Lidocaine and other local anesthetic drugs act primarily by inhibiting Na^+ channels to impede nerve conduction by preventing depolarization of the nerve fiber. However, intravenous use of local anesthetics, particular lidocaine, has been described in multiple species. In addition to its analgesic effects, lidocaine is thought to have both pro-motility and anti-inflammatory effects.[56,57]

Intravenous lidocaine has been demonstrated to have pro-motility effects in horses with post-operative ileus or horses that were likely to experience post-operative ileus following gastrointestinal surgery.[9,58] Gastrointestinal motility of normal, non-injured gut could not be enforced by lidocaine administration.[59,60] Recent *in vitro* and *in vivo* studies have confirmed the direct contractility-enhancing effects of lidocaine on equine jejunal smooth muscle.[61] In one study, these effects were more pronounced in injured than in control tissues.[62] In addition to contractility-enhancing effects, several studies have shown the beneficial effects of lidocaine on creatine kinase release from cardiac and intestinal muscle challenged by ischemia and reperfusion, indicating membrane protecting effects as well.[62,63] Nevertheless, cellular mechanisms underlying these effects remain unclear.

In veterinary medicine, intravenous local anesthetics have been studied in anesthetized animals, and appear to have a minimum alveolar concentration (MAC) sparing effect in several species, including cattle.[64-66] Vesal *et al* documented a MAC sparing effect in calves undergoing umbilical surgery with a lidocaine infusion of 50 mcg/kg/min.[66] Similarly, in dogs, Muir *et al* showed a MAC sparing effect using a continuous rate infusion of lidocaine at 50 mcg/kg/min, with or without co-administration of other drugs.[67] Pharmacokinetics following a single intravenous injection of 1.5mg/kg, caudal epidural injection, and inverted L block have been reported in cattle.[68,69] Pharmacokinetics of lidocaine following continuous rate infusion in cattle have not been reported.

Local anesthetics do have a significant potential for toxicity which varies by species.[24] In cattle and dogs, the reported toxic dose is 10mg/kg, with 6mg/kg being the reported toxic dose in cats, and 5mg/kg being the reported toxic dose in goats.[24] In general, drugs with faster absorption rates and shorter durations of action are associated with greater risk of toxicity. When lidocaine is administered intravenously, signs of toxicity would involve both the central nervous system (CNS) and cardiovascular system, with CNS signs occurring prior to cardiovascular signs. Side effects are typically the result of overdose.

In humans, CNS toxicity follows a predictable progression, which begins with agitation, visual disturbance, and muscle twitching, and ends in coma. In animals, signs of CNS toxicity follow a similar progression, may beginning as muscle twitching or excitation that can quickly progress to convulsions, coma, and death.[24] CNS toxicity

results from unopposed excitatory pathways in the brain following the depression of inhibitory cortical neurons. The CNS toxicity may also be due to inhibition of neurotransmitter release, such as GABA. [70]

Signs of cardiovascular toxicity may be due to a direct effect on electrical conduction or secondary to CNS toxicity. Initial cardiovascular signs, which are secondary to CNS excitation, include increased HR, arterial blood pressure, pulmonary artery pressure, and cardiac output. However when plasma levels approach sufficient levels to achieve cardiovascular toxicity, a decrease in HR, arterial blood pressure, pulmonary artery pressure, and cardiac output occur. Other cardiovascular signs include dysrhythmias, ventricular fibrillation, and cardiac arrest.[24]

2.4.4 Ketamine

Ketamine is an NMDA-receptor antagonist that also binds μ-opioid and κ-opioid receptors.[71] The effect produced by ketamine has been termed "dissociative anaesthesia" in that, rather than general electroencephalographic (EEG) depression, there is EEG evidence of dissociation between the thalamocortical and limbic systems.[71] It is commonly used either alone or in combination with other drugs for sedation or general anesthesia in cattle and other ruminants.[7,72-74] It is reported to produce analgesia and dissociative anesthetic effects when administered to calves at a dose of 2 to 4 mg/kg IV.[75] In humans, sub-anesthetic ketamine administered at 0.1 to 1 mg/kg as an IV bolus is reportedly effective in managing acute postoperative pain.[76] It is reported that analgesic effects of ketamine are produced at 10-20% of the anesthetic dose. Plasma ketamine concentrations more than 1000 ng/mL are required to produce anesthetic effects, while

analgesic effects are associated with plasma concentrations less than 275 ng/mL.[77] Another study reported that plasma ketamine concentrations ranging from 40 to 150 ng/mL were associated with analgesia in humans.[78] In cattle, pharmacokinetic data following intravenous injection of ketamine have been reported, however no-studies specifically addressing the analgesic effects of ketamine administered by CRI in cattle have been published.[7,46,79-81]

2.4.5 Morphine-Lidocaine-Ketamine

In addition to being used individually, analgesic agents may be administered as a continuous rate, co-infusion**.** These co-infusions can provide multimodal analgesia due to multi-pathway and multi-pharmacologic actions. Drugs administered concomitantly can exert greater effects than when only one or the other drug is administered, and drugs may have additive or synergistic effects[70] Benefits of these CRIs include decreased incidence of side effects of due to the fact that drugs can be delivered at reduced dosages. Several drug combinations used in cattle can provide synergistic effects when administered intravenously. MLK causes a MAC sparing effect when administered to isoflurane-anesthetized dogs.[67]

To the author's knowledge, there has not been a comparison of analgesic effects in cattle associated with MLK infusion to that associated with parenteral NSAID administration. Clinically, it is unclear whether or not the combination of an infusion provides better analgesia than an NSAID following umbilical surgery in calves.

Chapter 3: Materials and Methods

3.1 Study Design

This study was a blinded, prospective, case controlled clinical trial. Pain was assessed post-operatively in cattle that underwent herniorrhaphy at the Ohio State University Veterinary Medical Center. Cattle were randomly assigned to one of two treatment groups. The first treatment group (BAN) received two doses of flunixin meglumine at the label dose, and the second treatment group (MLK) received a 24 hour CRI of MLK starting immediately after induction of general anesthesia for routine umbilical herniorrhapy. Cattle were pain scored by one investigator (AKH) using a modified sheep pain scale established for research sheep undergoing orthopedic procedures .[82,83] All of the study team was blinded to treatment assignments except for AJN and BM. The evaluator was blinded to the treatment regimen.

3.2 Animals

The Ohio State University Institutional Animal Care and Use Committee (APPROVAL # 2012A00000088; July 13, 2012) and the OSU Clinical Research Advisory Committee approved this protocol (APPROVAL #2012V12; July 25, 2012). Consent was obtained from the owner prior to enrollment in the study. Patients that underwent routine herniorrhaphy surgery while hospitalized at the Ohio State University

Veterinary Medical Center were included in the study. All animals enrolled in the study were weaned Holstein heifer and steer calves <205 kilograms (body weight) admitted to the Veterinary Medical Center for surgical correction of an umbilical hernia. Animals were randomly assigned to one of two treatment groups (BAN or MLK). All calves were obtained from a single premise and were examined by one investigator upon admission and prior to surgery. Animals were included in the study if they were determined to be free of concurrent disease prior to surgery.

3.3 Treatment Groups

Group 1 (BAN, 10 animals): Standard peri-operative group (flunixin meglumine): Two doses of flunixin meglumine at 1.1mg/kg intravenously (IV). The first dose was given peri-operatively within 30 minutes of the completion of surgery, and the 2nd dose was given 24 hours later. Because the study was blinded, these cattle received a CRI of 0.9% sterile saline at the same rate as the Group 2 cattle (0.11 ml/kg/hr).

Group 2 (MLK, 10 animals): Morphine-Lidocaine-Ketamine: CRI of MLK at a rate of 0.11ml/kg/hr. The CRI was prepared as follows: 1000 ml of 2% lidocaine, 4g ketamine (100mg/ml; 40 mL), and 45mg morphine (15mg/ml; 3 mL) per bag (1043 mls total volume within assigned bag). Each bag then held a total volume of 1043 millileters. The concentrations of drug per bag were as follows: 19.2 mg/ml lidocaine, 3.84 mg/ml ketamine, and .043 mg/ml morphine. When delivered at 0.11 ml/kg/hr, the dosing rates were as follows: 2.1 mg/kg/hr (35 mcg/kg/min) lidocaine, 420 mcg/kg/hr (7 mcg/kg/min) ketamine, and 4.4 mcg/kg/hr morphine. This constant rate infusion (CRI) was started after induction of anesthesia and placement of an orotracheal tube (approximately 5

minutes after induction), The CRI was provided at the same rate for a total period of 24 hours.

3.4 Surgical Procedure and Anesthesia

A physical examination was performed on each calf the day of the surgery. Temperature, HR, RR, weight, mucous membrane color, hydration status, packed cell volume (PCV), and total protein (TP) were recorded prior to surgery. Hernia size was also recorded.

Food, but not water, was withheld for a minimum of 12 hours prior to anesthesia. A 14 gauge catheter (Abbocath) was inserted into one jugular vein for drug and fluid administration. A second catheter was placed in the opposite jugular vein for sampling purposes. Cattle were induced with intravenous (guaifenesin and ketamine, given to effect, and the total dose was recorded. Cattle were orotracheally intubated and maintained using isoflurane. The MLK or sham CRI was initiated following intubation and the time was recorded in the anesthetic record. Blood sampling began during the anesthetic episode. Patients were clipped and prepped in routine aseptic fashion and open herniorrhaphy was performed. Any abnormal findings, such as urachal remnant, abscess, or adhesions were recorded.

3.5 Pain Scoring and Clinical Evaluation of Patients

Post-operatively, one blinded investigator examined all patients. Body temperature, heart rate, respiratory rate, and ruminations were measured and recorded. Any abnormal physical examination findings were recorded.

Patients were pain scored a total of 14 times during the 5-day study period. Pain scoring was performed by a single observer (AKH). If the criteria for inclusion in the study were met, then the animal was enrolled in the study. All animals enrolled in the study were scored using a modified pain scoring algorithm original reported in sheep (Table 1). In addition, pressure algometry was performed using a ForceOne FDIX digital force algometer).

Variable	**0**	**1**	**2**	**3**
Mental assessment	Normal and alert	Lethargic, depressed appearance, ears drooping	Head down, very lethargic, ears drooping	Non-responsive
Respiratory Rate	Normal	Mildly increased (>40bpm)	Moderately increased (> 60 bpm)	Open mouth breathing
Heart Rate	Normal (60-80bpm)	Mild Elevation (80-90 bpm)	Moderate Elevation (90-100bpm)	Severe Elevation (>100 bpm)
Recumbency	Normal	Slightly Delayed Rising	Requires Encouragement to Stand	Unwilling or Unable to Stand
Incision	Normal (no swelling/discharge)	Mild Swelling /No Discharge	Moderate Swelling and/or Serosanguinous Discharge	Severe Swelling and/or Purulent Discharge
Rumen	Normal	Decreased Motility	No Criteria	Absent Motility
Appetite	Normal	Mildly Reduced Interest	Moderately Reduced Interest	Inappetant
Fecal Production	Normal	Mild Decrease	Moderately Decreased	Absent

Table 1: Pain Scoring Scale

Cattle were scored on all 8 categories and assigned a pain score number (0-24). Cattle were pain scored within 30 minutes of being placed in a hospital stall after surgery, and at the following time points: 4h, 8h, 12h, 18h, and 24h. After the CRI was discontinued, animals were pain scored at the following time points: 4h, 8h, 12h, 24h, 36h, 48h, 60h, 72h, and 96h. The total study period was 5 days (120 hrs).

Pressure Algometry: After pain scoring, each animal had peri-incisional pressure sensitivity measurements taken using a ForceOne FDIX digital force algometer. This instrument was applied to 4 sites around the incision to objectively evaluate the force (lbs force) required to elicit withdrawal or avoidance behaviors. These behaviors were defined as reactions that were repeatable, including kicking, vocalization, or moving away from the investigator. The measurement recorded by the algometer were collected and used to compare between the two groups at each time point. These values were averaged for each calf at each observation time. Algometry was performed on calves prior to surgery as well as during the study period. Pain score and algometry data was analyzed for each group, and each group was analyzed for differences.

3.5 Blood Sampling

Blood samples were drawn at pre-determined intervals after initiation of the CRI. These time points included 0 (pre-infusion), 1, 2, 4, 8, 12, 18, and 24 hours. The infusion was stopped at 24 hours and blood samples were taken at the following time points following conclusion of the CRI: 3,6, 12, 18, 30, 45, 60, 75, 90 minutes, 2, 2.5, 3, 4, 5, 6, 8,12, 24, 36, 48, 60, 72, and 96 hours. A total of 31 blood

samples (4 mL/sample point) per calf were taken. Samples were taken following pain scoring and pressure algometry measurements. Blood was centrifuged and serum stored in quadruplicate cryovials at -70C. Serum cortisol determinations were performed using Immulite, bead based assays through the OSU VMC Clinical Pathology Laboratories. Aliquots of serum were submitted to the CYCADS laboratory at the Iowa State University where they were analyzed for the CRI drugs, flunixin and the data subjected to PK and PK-PD analysis.

3.6 Drug analysis

Plasma concentrations of lidocaine, morphine, and ketamine along with associated metabolites were determined using liquid chromatography coupled with mass spectrometry (LC-MS/MS) following enzyme hydrolysis and workup by solid phase extraction (SPE). The LC-MS/MS system consisted of an Agilent 1100 pump, autosampler, and column compartment (Agilent Technologies, Santa Clara, CA, USA) coupled to an ion trap mass spectrometer (LTQ , Thermo Scientific, San Jose, CA, USA). Plasma samples, 0.5 mL, were subjected to overnight hydrolysis at 37.5^{o}C after the addition of 0.5 mL acetate buffer, pH 4.5 containing 2000 units/mL of beta-glucuronidase (Helix pomatia). After hydrolysis, 2 mL of 0.1 Molar ammonium acetate buffer, pH 4.7 was added and the samples were centrifuged at 2000 x g for 20 minutes. An internal standard mixture (10 uL @ 5ng/uL) consisting of six deuterated compounds was added prior to each sample prior to enzyme hydrolysis. The samples were then applied to a conditioned Strata X-C strong cation SPE column (100 mg, 3 mL, Phenomenex, Torrance CA). After washing with buffer and methanol the drugs of interest were eluted from the

SPE column with two 0.75 mL portions 5% ammonium hydroxide in acetonitrile. The eluate was dried at 50°C with a stream of nitrogen in a Turbovap evaporator. The dry residue was reconstituted with 100 µL of 25% (v/v) acetonitrile in water and vortexed followed by 50 µL of water and vortexed. The tube contents were transferred to an autosampler vial fitted with a glass insert. The injection volume was set to 10 µL. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.25 mL/min. The mobile phase began at 10% B and held for 1 minute with a linear gradient to 95% B at 9 minutes, which was maintained for 1.5 minutes, followed by re-equilibration to 10% B. Separation was achieved with a ACE C18 column (ACE 3 C18, 150 mm x 2.1 mm, 3 µm particles, Mac-Mod Analytical, Chadds Ford, PA, USA) maintained at 40°C. Drugs detected by the mass spectrometer consisted of morphine and its internal standard d3-morphine. Lidocaine metabolites consisted of 3-hydroxylidocaine and 4-hydroxylidocaine as well as norlidocaine (MEGX) and internal standards for these metabolites were d_{10}-3-hydroxylidocaine and d_5-norlidocaine. Lidocaine-d_{10} was used as the internal standard for lidocaine. Ketamine was paired with d_4-ketamine as an internal standard while d_4-norketamine was used as the internal standard for norketamine and dehydronorketamine. Full scan MS of the pseudomolecular ion of each drug/metabolite yielded from one to six ions for identification and quantitation. Lidocaine and its two hydroxyl metabolites fragmented to an ion at m/z of 86 while the corresponding d_{10} internal standards fragmented to a single ion at m/z 96. Six fragment ions were used for quantitation of morphine while 2-4 ions were used for quantitation of the ketamine species. Sequences consisting of plasma

blanks, calibration spikes, QC samples, and bovine plasma samples were batch processed with a processing method developed in the Xcalibur software (Thermo Scientific, San Jose, CA, USA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. Plasma concentrations of parent drug and metabolites in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. The standard curves in bovine plasma were linear from 0.25 to 2000 ng/mL with correlation coefficients exceeding 0.995.

3.7 Statistical Analysis

Data were analyzed for differences in pre-operative values (HR, RR, Temp, PCV, TP, Age, Body Weight) using independent sample t-tests. Gender distribution and complication rates were analyzed using a Fisher's exact test. Pain score, incision score, HR, RR, and cortisol data was analysed using a linear mixed model with animal ID as a random factor. Before analysis, data were analyzed for normality of distribution, and were transformed if needed. Data was analyzed using the SPSS Statistical Software Program.

Individual serum concentration-versus time data were tabulated and mean ± SD for all 10 calves for each analyte (morphine, lidocaine and ketamine) serum concentration versus time curves after CRI infusions were prepared. The time (h) serum morphine, lidocaine and ketamine concentrations were maximal was determined from the serum concentration versus time curves for each calf. Serum concentration vs. time data from

each animal given the MLK CRI were subjected to non-compartmental analysis using a computer software program (Phoenix, WinNonlin v 6.2; Pharsight – Certara, Cary, NC, USA) with data weighted $1/C^2$ where C is the actual serum florfenicol concentration.

Zero time serum drug concentration intercept (C_0) of i.v. disposition curve were determined by back-extrapolation from the first 2 analyte concentration values back to T=0. Maximum serum concentration (C_{max}) was taken as the maximum observed concentration, occurring at the corresponding time (T_{max}) taken directly from the individual animal concentration versus time curves.

The first order rate constant associated with the terminal portion of the concentration versus time curve (λ_z) was estimated by linear regression of time versus log concentration after discontinuation of the CRI dosing. These slopes incorporated at minmum of 3 terminal data points of individual concentration-vs.-time data. The terminal phase half-life (HL_λ_z) was calculated by $ln\ 2/\lambda_z$. The area under the curve (AUC) to the last measured time point was calculated by the trapezoidal rule and was extrapolated to infinity by adding the area from C_{last} to infinity ($AUC_{0\rightarrow tlast} + C_{last}/\lambda_z$). The percentage of the AUC extrapolated to infinity was recorded for each drug quantitated. The total body clearance of morphine, lidocaine and ketamine after CRI administration was calculated from the i.v. dose (mg) and $AUC_{0\rightarrow\infty}$. The area under the first moment of the concentration vs. time curve (AUMC) from time of dosing to infinity was calculated using the product of concentration x time versus time. The volume of distribution based upon the terminal phase (V_z) after the i.v. bolus dose was determined from i.v. dose/λ_z•$AUC_{0\rightarrow\infty}$, and the steady state volume of distribution for the i.v. bolus dose was

calculated from V_{ss} =(i.v. dose/$AUC_{0\rightarrow\infty}$)•MRT. Mean residence time (MRT) extrapolated to infinity was calculated by determining the ratio $AUMC_{0\rightarrow\infty}/AUC_{0\rightarrow\infty}$. Steady plasma concentrations of morphine, lidocaine and ketamine were estimated by determining the ratio of dose rate (mg/hr)/CL_{total}

Chapter 4: Results

4.1 Animals

All animals included in the study were Holstein calves sourced from a single premise. Treatment groups did not differ significantly with regard to age, body weight, sex distribution or pre-operative PCV, TP, HR, RR, or temperature ($p>.05$). (Table 2)

	Banamine	MLK
Age (days)	160.7 ± 27.6	148.8 ± 29.9
Weight (kg)	150.5 ± 35.7	146.2 ± 36.7
Sex	9 Heifer, 2 Steer	5 Heifer, 5 Steer
PCV (%)	31.8 ± 2.4	32 ± 3.4
TP (g/dl)	7.1 ± 0.4	6.8 ± 0.3
HR (beats/min)	89.1 ± 9.4	88.2 ± 19.8
RR (breaths/min)	40.7 ± 9.9	52.7 ± 18.5
Temp (°F)	102 ± 0.7	101.8 ± 0.5

Table 2: Pre-operative demographics and physical exam findings

4.2 Pain Score

The maximum pain score possible was 24. Pain scores did not differ significantly by group. Average pain scores over time are shown in Figure 1. Average pain scores by group are shown in Table 3.

4.3 Incision Score

Algometry scores were recorded over time and did not differ significantly between groups (Figure 2). Pre-op algometry data was significantly different than post-op algometry data. ($P<0.05$). There was no significant difference between groups over the study period, however there was a trend towards MLK animals having higher nociceptive thresholds than BAN animals ($p=0.098$). During the CRI administration, there was a significant difference between groups ($p=0.019$). During this time period, animals in the MLK group had higher nociceptive thresholds than animals in the BAN group.

4.4 Heart rate and Respiratory Rate

Both heart rate and respiratory rate were plotted over time and did not differ significantly between groups over the course of the study period, or at any individual time points (Fig. 3 and 4)

	BAN	MLK
Pain Score (0-24)	2.9 ± 1.6	2.9 ± 1.8
HR (beats/min)	92.2 ± 13.9	91.7 ± 15.4
RR (breaths/min)	46.9 ± 12.1	46.7 ± 13.2
Algometry score (lbf)	1.8 ± 1.7	2.1 ± 2.4

Table 3: Average HR, RR, Incision Score, and Pain Score

4.5 Serum Cortisol

Serum cortisol was graphed over time and was not significantly different between groups during the study period ($p=0.390$). However, there was a significant difference between groups during CRI administration ($p<0.001$), with MLK animals had higher

serum cortisol during this period than BAN animals. Time was a significant factor in cortisol concentration ($p=0.001$), with cortisol tending to decrease over time and increase during periods of more intensive handling. Mean serum cortisol concentrations over time can be seen in Figure 5.

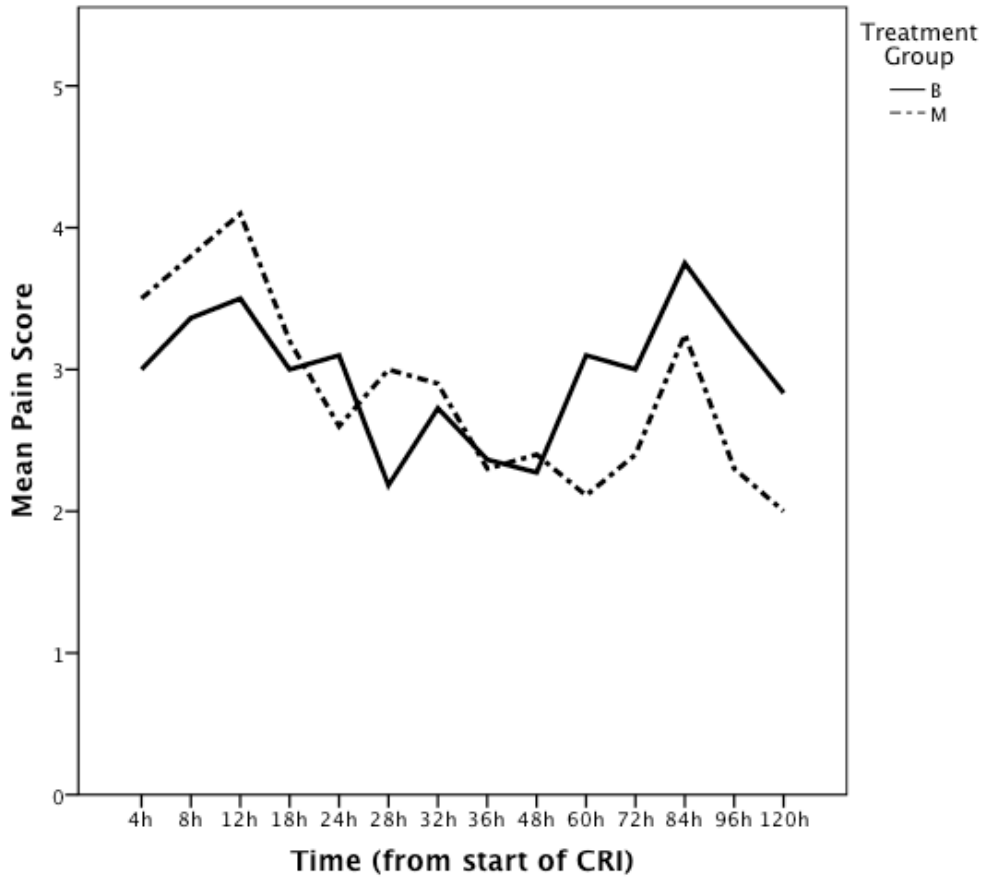

Figure 1: Mean Pain Score versus Time

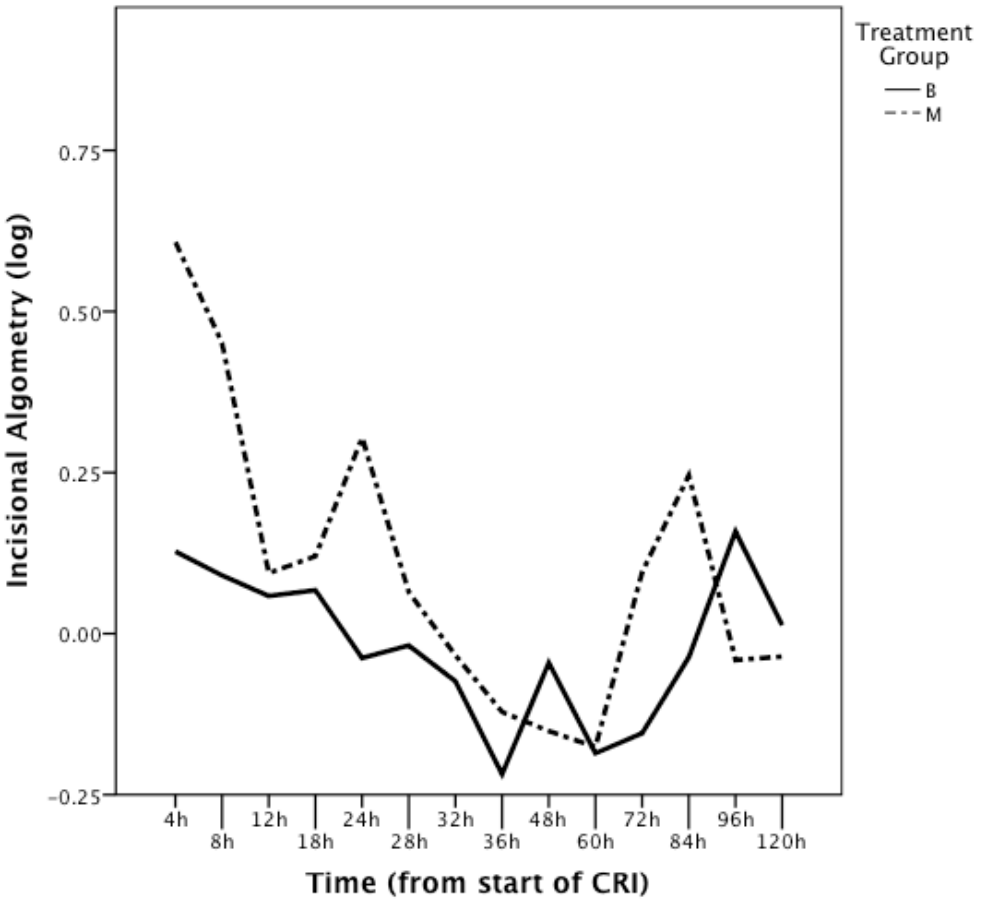

Figure 2: Algometry Value versus Time

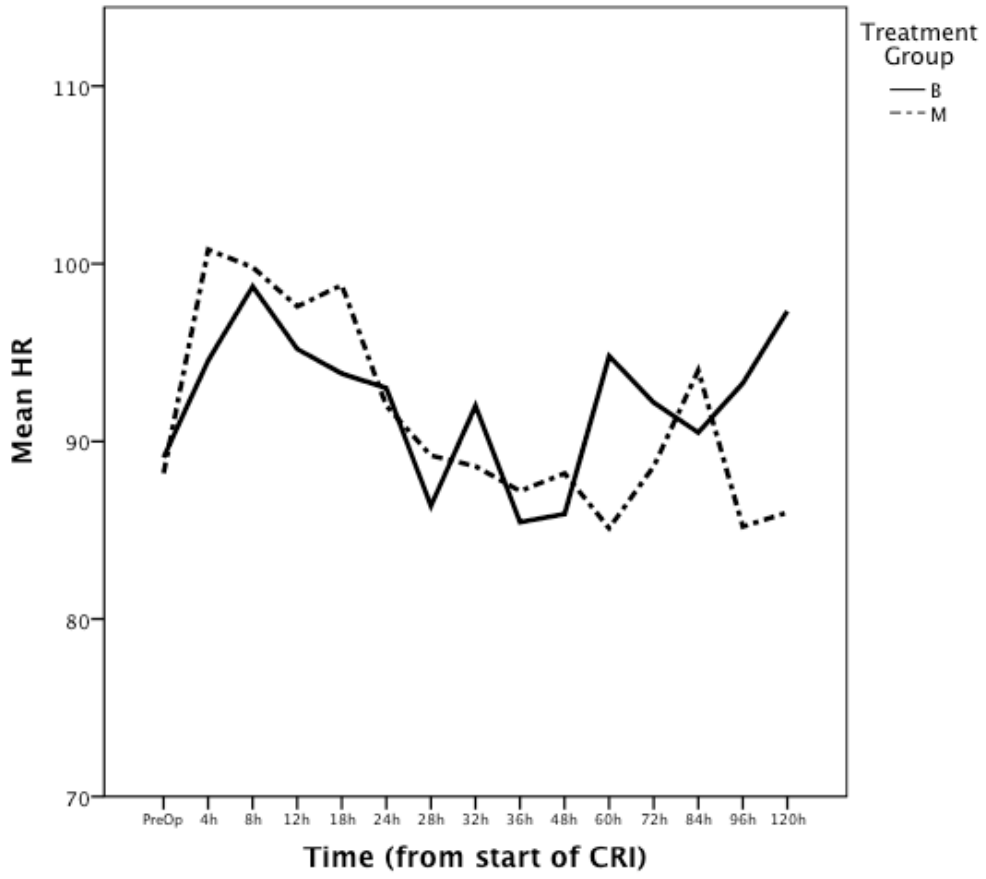

Figure 3: HR Data versus Time

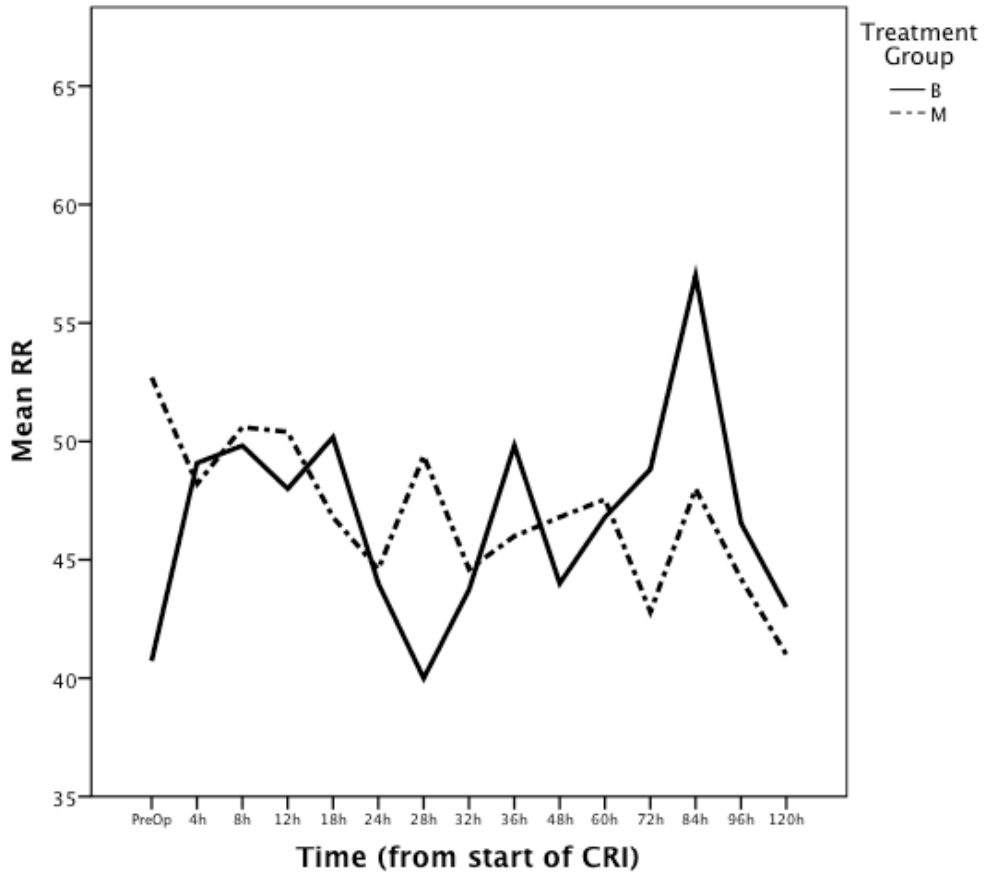

Figure 4: RR Data versus Time

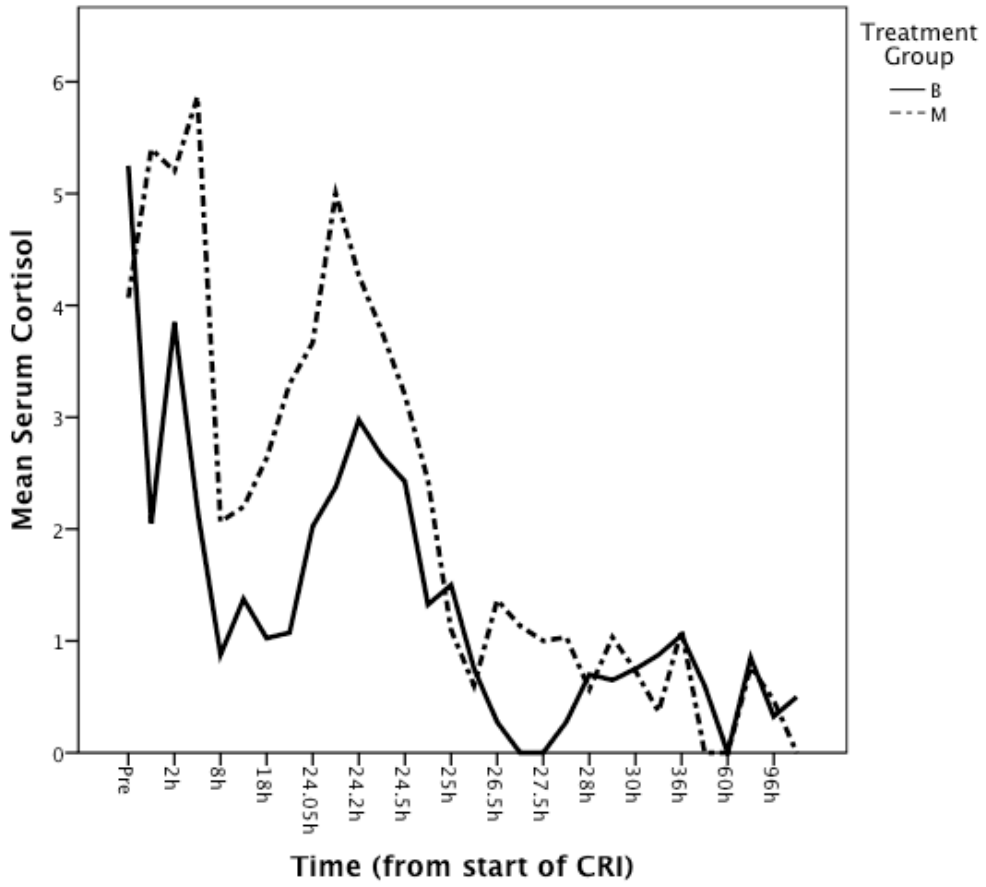

Figure 5: Serum Cortisol versus Time

4.6 Complications

Complication rates were not significantly different between groups. There were 5 calves that developed mild seromas during the study period (3 BAN, 2 MLK). One calf developed thrombophlebitis of one jugular vein (MLK). One calf died during CRI administration. This calf was in the MLK group and received an inadvertent bolus of drug by an inexperienced technician.

4.7 Pharmacokinetic Data

Serum concentrations of morphine, lidocaine, and ketamine were established during and after CRI of MLK in all MLK group animals. The concentration in each animal over time can be seen in Tables 4.3-4.5. Average serum levels for each drug are summarized in Figure 6. Pharmacokinetic data as well as average concentrations over time are illustrated in Figures 7-9. Raw data for concentrations of drugs and their metabolites can be seen in Tables 4-11 in the Appendix.

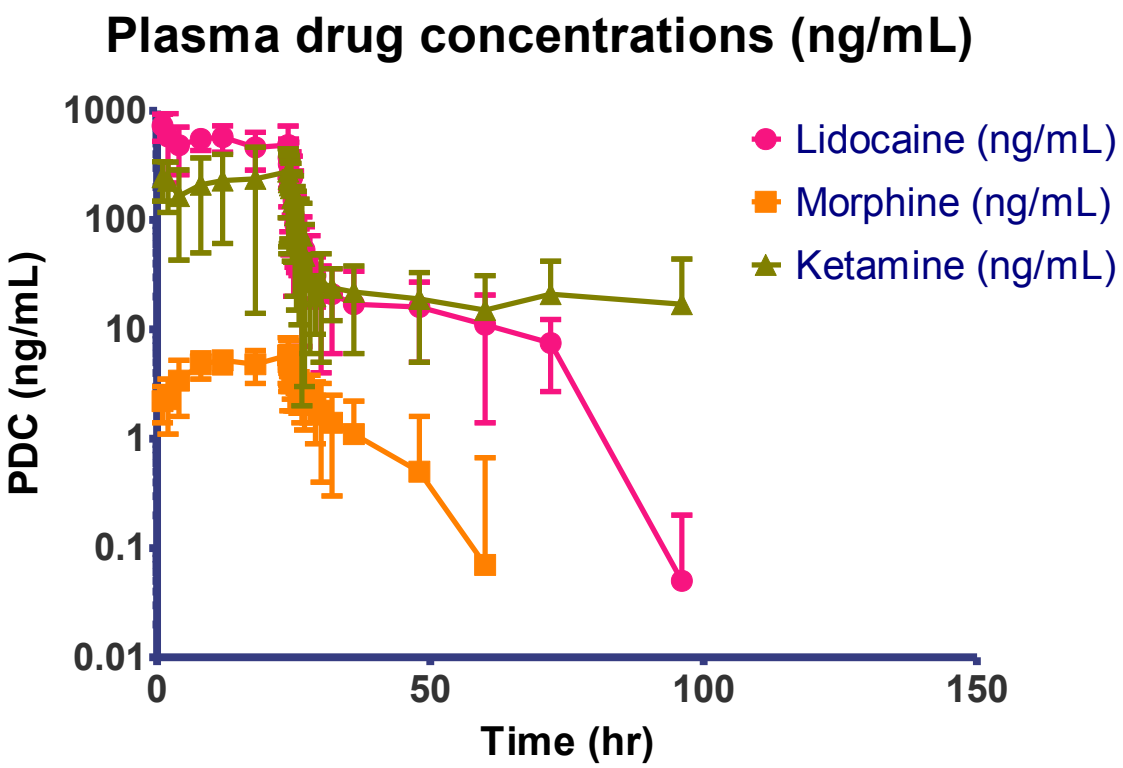

Figure 6: Serum Drug Concentrations Morphine-Lidocaine-Ketamine

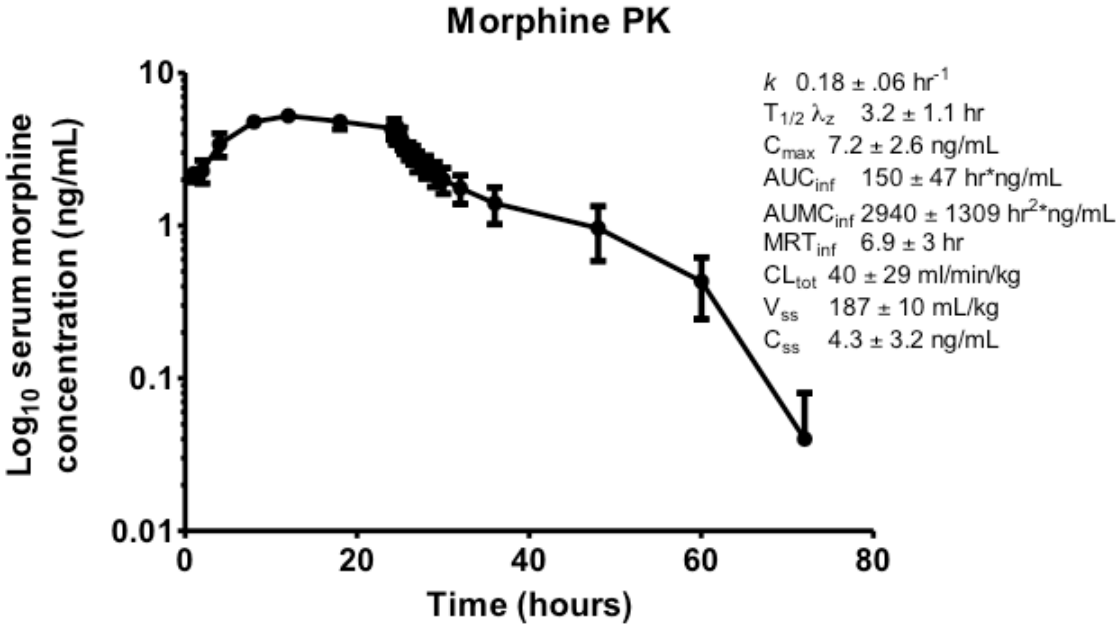

Figure 7: Morphine serum concentration versus time data and associated average non-compartmental statistical moment analysis of this data

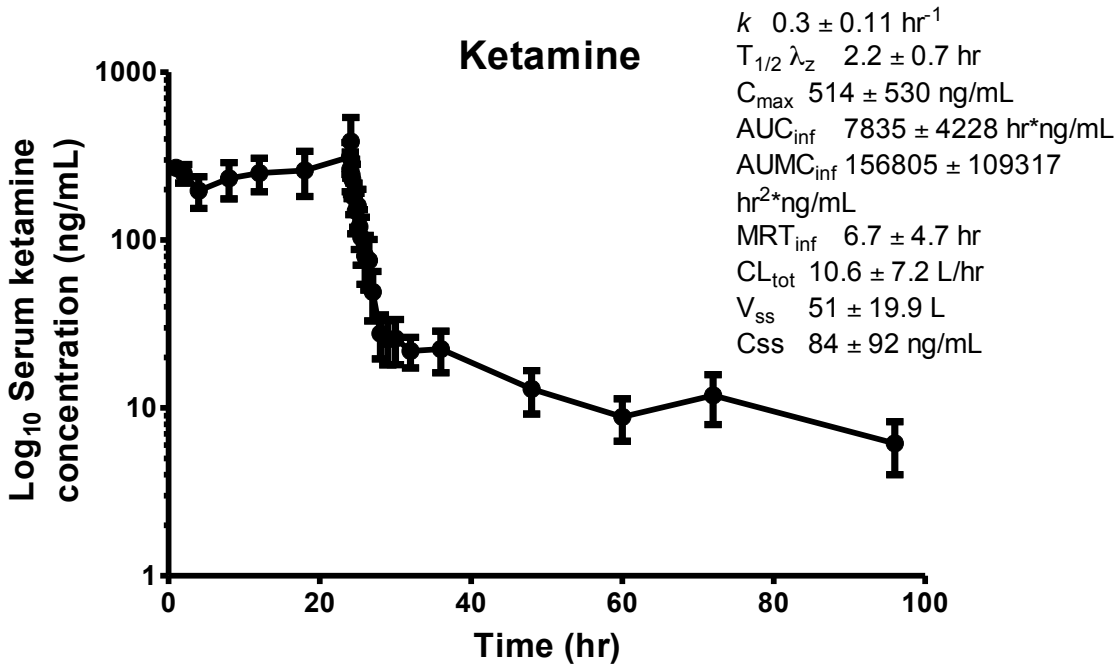

Figure 8: Ketamine serum concentration versus time data and associated average non-compartmental statistical moment analysis of this data

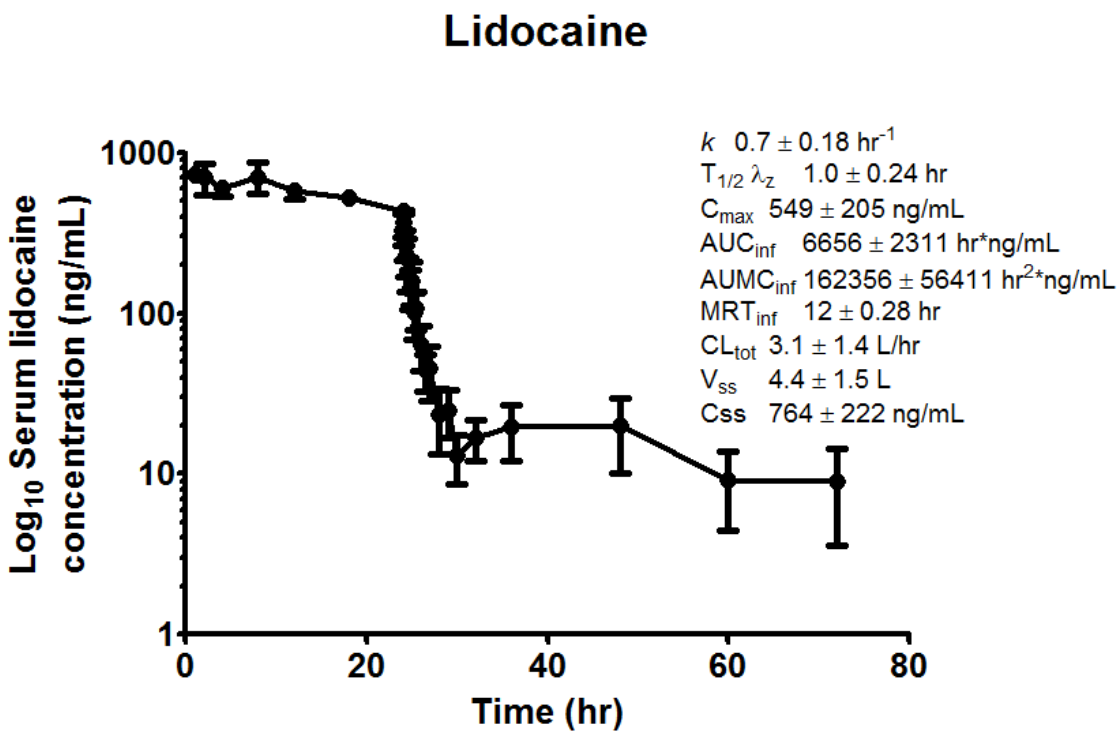

Figure 9: Lidocaine serum concentration versus time data and associated average non-compartmental statistical moment analysis of this data

Chapter 5: Discussion

The response to pain created by routine herniorrhaphy was similar between the BAN and MLK groups. The results of our study do not support our hypothesis that there would be a significant decrease in pain experienced by cattle receiving MLK compared to those receiving 2 post operative doses of flunixin meglumine. Pain scores, as well as other measured values obtained during the study period, including HR, RR, incisional algometry score, and serum cortisol were not different between groups when analyzed over all of the observation time points included in this study ($p>0.05$). However, during the CRI, there were statistical differences in the algometry assessments and serum cortisol values in the MLK group in comparison to the BAN group ($p=0.019$, $p<0.001$).

The primary finding in this study is that post-operative pain levels are similar between treatment groups, with BAN and MLK providing equivalent levels of analgesia following routine ventral midline umbilical herniorrhaphy in calves.

A second significant study finding was that serum cortisol decreased over time during the study period in both treatment groups. However, it was noted that cortisol increased with periods of more intensive handling.

Finally, we were able to establish pharmacokinetic data following continuous rate infusion of the MLK drug combination in cattle. This has not previously been examined in cattle.

In our practice, constant rate infusion of MLK is used most often in patients with gastrointestinal disease or with pain-induced ileus. It is our clinical impression that continuous rate infusion of MLK improves animal comfort levels by relieving visceral pain, and has a positive effect on gastrointestinal motility, without causing significant sedation or dysphoria. Continuous rate infusion of lidocaine has been shown to have a positive effect of gastrointestinal motility in horses[9,58] and is thought to have a similar effect in cattle.[84] Reduction in ileus may be due to both direct pro-motility effects of lidocaine and the indirect effects of pain reduction on gastrointestinal motility. Our study, however, focuses solely on the analgesic efficacy of a MLK CRI in calves. Morphine, lidocaine, and ketamine have all been shown, either alone or in combination with other drugs, to provide effective analgesia or anesthesia in cattle undergoing surgical procedures.[32,46,53,85] NSAIDs, however, remain to be the most commonly used analgesic agent to treat surgical pain in cattle, and have been shown to be effective.[3,48] Results of our study indicate that two post operative doses of flunixin meglumine and a post operative MLK CRI provide post-operative analgesia that were not statistically different based on the parameters measured during this study.

To date, no studies in veterinary medicine have examined the use of MLK as a CRI in the post-operative period. The evaluation of the drug combination as a CRI in animals is limited to a small number of studies looking at its effect on MAC of inhalant anesthetics in anesthetized dogs and horses.[67,86-88] MLK infusion was shown to decrease MAC in dogs under anesthesia without significant hemodynamic effects.[67] Similar studies have been performed using alternative drug combinations for continuous rate

infusion in both dogs and horses under general anesthesia. Lidocaine and ketamine, alone or in combination, decreased the MAC of sevoflurane in anesthetized dogs.[89] In horses, ketamine infusion reduced halothane MAC and produced beneficial hemodynamic effects.[90] Lidocaine administered as a continuous rate infusion of 100 mcg/kg/min following a 2mg/kg loading dose has been associated with a decreased heart rate in anesthetized calves, and may be a concern in compromised patients.[91] The dosage rate of lidocaine administered in the current study was 35 mcg/kg/min, and was not associated with a decreased heart rate during the study period.

Lidocaine administered as a CRI until the end of surgery has been associated with higher ataxia and poor quality of recovery following isoflurane or sevoflurane anaesthesia in horses.[65] In this study, the MLK CRI was discontinued just prior to the isoflurane vaporizer being turned off and was re-started when the calf was placed in its stall (Avg time off of CRI=15 min+/- 4.63min). Despite continuation of the CRI, no adverse effect was observed at recovery and all calves recovered uneventfully. It is difficult to compare surgical recoveries between cattle and horses, however, because recovery from anesthesia in cattle is rarely associated with complications, particularly the catastrophic complications that are seen with horses.[92,93] Our findings that MLK infusion did not adversely affect anesthetic recovery in calves is consistent with findings in a previous study looking at lidocaine infusion in calves undergoing umbilical surgery.[66] In that study, calves were administered a CRI of 50 mcg/kg/min following an initial loading dose of 2mg/kg. These calves, however, were not continued on a CRI post-operatively, nor were their pain levels assessed in the post-operative period.

Another primary finding of our study is that serum cortisol levels were noted to decrease over time during the study period. It is not surprising that the pre-operative serum cortisol levels were elevated, as the calves were restrained in a chute for catheter placement and sampling. Additionally, handling and catheter placement on the day of surgery was performed by a large number of individuals with variable experience handling cattle and placing intravenous catheters. Effects of acute stressors, or social isolation and restraint, on behavioral, adrenocortical and nociceptive responses have been described in cattle.[20,21] Herskin et al[20] looked at the effect of acute stressors (social isolation in novel surroundings, fixation by the head in the home stall, and the provision of novel neighbors/stall) in 24 dairy cows kept in tie stalls. All stressors led to signs of hypoalgesia as indicated by slower and reduced responses toward nociceptive laser stimulation after exposure to the acute stressors, however social isolation had the strongest effect. Additionally, social isolation or fixation by the head led to increased plasma concentration of cortisol. The behavioral responses were affected by treatments as well. Later, Herskin et al[21] looked at effects of social isolation or restraint in loose-housed dairy cows, and found that cows experiencing social isolation had the most significant negative effects, including hypoalgesia and increased plasma cortisol concentrations. In our study, it is impossible to know whether changes in serum cortisol over time are due to decreased pain levels or effects of stressors. Because the pain scores did not decrease over time, it is likely that stressors are at least partially responsible for changes in serum cortisol levels. Interestingly, serum cortisol levels decreased significantly over the first 24 hours, and then increased significantly and suddenly at the

24hour time point. This is most likely due to the fact that the cattle were restrained and handled intensively during initial post-CRI sampling period. Although care was taken to mitigate the effects of stressors during the study period, it is not possible to eliminate stressors in the population under the conditions of the study.

Results of the pharmacokinetic analysis revealed that all drugs reached steady state during the CRI administration.. In contrast to previous studies, we were able to detect concentrations of lidocaine metabolite MEGX in the blood of cattle.[68] The significance of this metabolite is not known. Levels of ketamine were still detectible in the serum at 96 hours following discontinuation of the CRI. Because use of MLK is considered extra-label drug use, withdrawal periods have not been established. This research indicates that withdrawal periods > 96 hours would need to be considered. Serum concentrations appeared to be adequate to achieve analgesic levels in post-operative cattle. However, because the drugs were used in combination we cannot determine each individual drug's contribution to analgesic level achieved.

The level of morphine administered was significantly lower than those used in other studies, and increasing the amount of morphine in the combination may result in superior analgesia.[4,52,53,87] Another approach may have been to increase the number of cattle in the study and look at varying dosage rates.

Limitations of the current study include the difficulty of assessing pain in animals, particularly ruminants. A recent journal issue devoted to pain management in ruminants included four articles devoted solely to assessment of pain in livestock animals.[34,38,40,94] Despite using a pain scoring system that allowed for methodical evaluation of pain in

study subjects, the pain scores are largely subjective assessments with limitations. A single observer was responsible for pain scoring and incisional algometry measurement in the present study. The observer (AKH) performed all pain and algometry scoring to eliminate inter-observer variation. The technique for pain scoring patients was modified from a sheep pain scoring scale used following orthopedic procedures.[82,83] The majority of pain studies in cattle have focused on routine procedures, such as castration and dehorning. Few studies have looked at visceral or incisional pain in cattle.[4,5] In the current study, we felt that the pain scoring system used provided an adequate clinical picture of pain level. Incisional algometry scores complemented our pain scoring system. Neither method revealed a difference between groups, leading to the rejection of our hypothesis.

Another study limitation was the type of surgery performed and lack of untreated control group. Although open umbilical herniorrhaphy involves both entry into the peritoneal cavity and some manipulation of internal organs and tissues, animals did not appear extremely painful in the post-operative period. It is possible that this is due to the fact that both treatment groups were given analgesic drugs. We considered an untreated control group to be below the standard of care for animals undergoing surgery of the abdominal cavity, thus an untreated control group was not included in the current study. Additionally, it should be noted that umbilical herniorrhaphy is an elective procedure that is not commonly associated with marked pre-operative pain. Patients with other surgical conditions, such as an intestinal blockage, may experience significant pre-operative pain and possible up-regulation of pain responses. Use of the combination in animals

undergoing more invasive surgical procedures, such as resection and anastomosis, or in clinically ill animals may provide different results.

The current study indicates that morphine-lidocaine-ketamine continuous rate infusion provides analgesia that is not statistically different than cattle receiving two doses of flunxin meglumine post operative following umbilical herniorrhaphy in cattle. Though cost and technical support required for safe administration make this protocol impractical for field or herd based use, the combination may be considered in individual animal cases and should be examined in clinically ill animals experiencing intractable pain or ileus.

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Appendix

Time	11785	6553	6567	46	3992	1153	90	1495	1511	9623	Average	SD
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0
1	2.3	1.0	2.6	2.9	1.0	2.6	1.6	3.5	2.3	2.0	2.2	0.8
2	3.2	1.0	3.1	2.3	0.9	1.8	0.9	4.8	2.8	2.0	2.3	1.2
4	1.8	3.2	3.5	4.3	0.7	3.9	2.6	7.9	2.9	3.3	3.4	1.8
8	6.2	4.6	3.4	5.0	2.6	5.4	3.7	7.3	4.3	5.2	4.8	1.3
12	7.0	5.7	3.4	5.2	2.7	6.0	6.1	5.7	5.2	5.3	5.2	1.2
18	7.0	6.3	3.5	2.5	3.1	7.5	4.1	4.8	5.3	3.9	4.8	1.6
24	6.5	8.4	3.1	5.5	3.2	9.7	3.4	4.1	4.2	10.3	5.8	2.6
24.05	6.2	4.5	3.0	5.4	2.6	7.3	3.2	4.6	4.2	8.7	5	1.9
24.1	6.2	4.4	3.1	11.3	2.6	5.6	2.9	4.1	4.3	8.0	5.3	2.6
24.2	6.0	4.1	3.0	6.9	2.5	5.9	2.8	4.0	4.0	7.6	4.7	1.9
24.3	6.2	3.7	2.9	6.5	2.4	5.5	2.6	3.7	3.8	7.1	4.4	2.6
24.5	8.3	3.6	2.7	5.3	2.4	5.6	2.5	3.5	3.5	6.2	4.4	1.7
24.75	6.1	3.2	2.6	4.9	2.3	5.3	2.2	3.1	3.2	6.1	3.9	1.6
25	5.5	3.0	2.4	4.3	2.1	5.3	2.1	3.0	3.1	5.1	3.6	1.8
25.25	5.1	2.8	2.2	4.3	1.9	5.1	1.9	2.8	3.0	4.3	3.3	1.5
25.5	4.7	2.7	2.2	4.0	1.7	5.2	1.7	2.3	2.7	3.9	3.1	1.3
26	4.5	2.4	1.9	3.7	1.6	5.4	1.6	2.1	2.5	3.5	2.9	1.2
26.5	3.9	2.3	1.6	3.5	1.4	5.3	1.5	1.8	2.2	2.9	2.6	1.2
27	3.8	2.2	1.3	3.3	1.4	5.2	1.4	1.3	2.0	2.4	2.4	1.2
28	3.4	2.3	5.0	2.7	1.0	5.1	1.3	1.1	1.7	2.2	2.6	1.2
29	3.4	2.2	1.2	2.6	1.0	4.3	1.0	0.8	1.5	2.7	2.1	1.2
30	3.7	1.7	1.0	2.2	0.7	3.4	0.8	0.4	1.4	2.5	1.8	1.4
32	3.9	1.8	0.7	1.5	0.4	2.9	0.5	0.0	1.3	1.3	1.4	1.1
36	3.8	1.6	0.6	0.9	0.0	1.8	0.0	0.0	0.8	1.2	1.1	1.1
48	1.6	1.1	0.0	0.0	0.0	0.7	0.0	0.0	1.3	0.4	0.5	1.1
60	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.07	0.6
72	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.1
96	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0	0

Table 4: Serum morphine concentrations

Time	6553.00	6567.00	46.00	3992.00	1153.00	11785.00	90.00	1495.00	1511.00	9623.00	Average	STDEVP
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
1.00	358.05	902.45	657.55	426.00	946.75	883.93	975.75	1075.44	683.15	696.53	760.56	225.86
2.00	191.90	1023.41	127.09	160.46	237.11	913.03		764.28	1159.76	825.82	600.32	392.09
4.00	505.86	790.37	427.40		637.58		901.57	620.69	345.95	435.49	583.12	179.24
8.00	0.00	789.11	416.96	438.88	572.16		678.07	582.77	407.20	524.04	489.91	209.83
12.00	794.88	729.21	290.58	315.70	483.25	1000.35	614.00	499.81	411.37	581.88	572.10	210.60
18.00	645.01	700.62		362.00	566.77	631.39	371.05	483.37	308.32	470.43	504.33	131.82
24.00	575.60	507.51	1023.58	367.34		437.92	512.49	483.37	247.21	529.29	520.48	200.77
24.05	400.36	420.68	814.44	241.38		263.88	275.15	358.46	206.86		372.65	181.83
24.10	334.44	377.14		229.08	813.86	196.33	191.40	248.69	188.80		322.47	196.76
24.20	312.91	254.71	1464.15	175.71	647.58	217.35	164.51	171.55	134.86		393.70	406.02
24.30	226.40	257.15	943.87	163.51	563.05		103.08	110.15	112.80		310.00	278.10
24.50	146.31	163.33	385.12	107.10	354.59		70.64	85.28	99.14	693.93	233.94	195.70
24.75	93.26	132.53	291.40	94.92	347.26		44.40	59.81	63.25	456.16	175.88	141.41
25.00	81.98	82.70		88.47		253.63	32.98		39.52	341.70	131.57	109.51
25.25	80.87	74.40	101.73	76.67	133.84	127.78	30.50	33.01	35.37	223.15	91.73	56.09
25.50	56.33	53.29	134.91	51.91		223.00	27.70	23.42	23.29	147.49	82.37	65.98
26.00	39.47	31.36	81.68	42.71	81.06	173.18	14.95	19.59	13.74	76.50	57.42	46.12
26.50	34.20	18.59	113.03	29.76	92.20	35.83	10.96	15.81	10.89	165.33	52.66	50.05
27.00	19.51	13.19	78.21	23.73		21.86	7.40	6.52	6.47	80.16	28.56	27.76
28.00	15.98			14.79	34.03	13.50	8.52	3.46	3.95	62.53	19.59	18.55
29.00	11.24		45.11		44.72	21.40	5.21		3.02	26.35	89.01	176.79
30.00	11.04	7.59	58.68		3.86	61.32	5.00	3.49		17.28	21.03	22.90
32.00	8.60	3.27	42.89		24.05	16.31	3.48	2.64	5.60	17.42	13.81	12.50
36.00	5.62	2.00	39.55		65.66	9.81	0.00		3.54	31.98	19.77	22.12
48.00	3.67	0.00	24.92		17.14		0.00	0.00		28.53	10.61	11.68
60.00	8.97	0.00	31.52		12.28	3.97	0.00	0.00			8.11	10.56

Table 5: Serum lidocaine concentrations

Time	6553	6567	46	3992	1153	11785	90	1495	1511	9623	avg	STDEVP
0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.00
1.00	13.93	15.21	20.5	0.9	2.3	2.9	1.1	3.7	2.4	2.2	6.52	6.80
2.00	9.26	27.80	14.7	1.6	2.6	1.1	2.1	2.9	3.7	3.6	6.94	8.02
4.00	5.13	6.80	9.7	0.3	1.9	1.4	1.6	2.7	1.9	1.7	3.31	2.82
8.00	5.18	3.35	5.3	1.0	1.3	2.3	1.1	3.0	1.4	1.6	2.54	1.54
12.00	9.78	4.75	10.7	2.5	2.2	2.0	4.2	4.2	2.9	2.0	4.52	3.02
18.00	6.99	5.77	4.1	2.6	2.7	3.0	1.4	4.4	2.8	2.4	3.61	1.63
24.00	5.63	5.65	7.3	2.4	1.4	2.9	2.9	4.2	2.1	2.8	3.72	1.79
24.05	5.45	5.45	7.4	2.5	1.6	2.7	2.8	4.2	2.1	3.2	3.73	1.76
24.10	5.08	5.42	7.8	2.4	1.5	2.5	2.7	4.3	2.1	3.9	3.77	1.83
24.20	5.16	5.47	8.6	2.4	1.5	3.0	2.7	4.3	1.8	4.1	3.91	2.03
24.30	4.46	5.52	8.9	2.4	1.3	2.7	2.6	4.3	1.8	4.0	3.80	2.11
24.50	3.65	4.72	8.0	2.6	1.5	2.6	2.8	3.9	1.6	3.4	3.47	1.79
24.75	3.10	4.85	7.3	2.6	1.2	2.1	2.7	4.2	1.8	3.1	3.29	1.67
25.00	3.20	4.64	7.0	2.7	1.2	1.7	2.5	3.8	2.1	2.4	3.12	1.60
25.25	3.34	4.09	6.9	2.2	1.1	1.6	2.2	3.7	2.0	2.1	2.91	1.61
25.50	3.29	3.82	5.7	1.8	1.1	1.5	1.9	3.3	1.8	2.1	2.63	1.32
26.00	3.64	3.61	4.6	2.1	1.6	1.2	1.6	3.0	1.5	2.0	2.48	1.09
26.50	4.50	3.30	4.2	2.0	1.1	1.7	1.6	2.5	1.4	1.6	2.38	1.16
27.00	4.27	3.02	5.0	1.5	1.5	2.3	2.0	2.1	1.1	1.2	2.40	1.25
28.00	3.96	2.47	5.8	1.3	1.1	3.3	1.9	1.9	1.0	1.4	2.41	1.44
29.00	2.32	2.20	3.7	1.4	0.8	2.7	2.0	1.6	1.2	2.2	2.01	0.80
30.00	2.06	2.27	2.6	1.0	0.4	1.6	1.3	1.0	1.3	0.8	1.44	0.64
32.00	3.75	1.10	2.2	0.4	0.2	0.7	0.8	0.4	0.6	0.5	1.07	1.05
36.00	1.68	0.89	1.2	0.0	0.0	0.2	0.4	0.0	0.2	0.0	0.45	0.56
48.00	0.92	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.09	0.28
60.00	0.18	0.00	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.13	0.34
72.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
96.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00

Table 6: Serum 4-OH-lidocaine concentrations

Time(hr)	6553	6567	46	3992	1153	11785	90	1495	1511	9623	3-OH-Lidociane	STDEVP
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0.00	0.00	0.00	0	0
1.00	1.33	1.59	2.79	6.78	25.80	18.92	10.88	23.68	21.72	20.43	13.39187	9.253444
2.00	1.30	3.57	3.06	8.68	21.82	25.85	15.09	12.81	30.65	29.28	15.21047	10.57452
4.00	1.41	1.40	2.76	1.72	8.51	7.20	7.67	9.46	8.70	8.07	5.690295	3.228116
8.00	1.22	0.79	1.93	3.03	4.96	7.53	4.44	7.03	5.48	6.64	4.304215	2.329322
12.00	2.73	1.20	4.68	6.19	6.78	9.48	8.50	7.71	9.01	5.77	6.206314	2.570623
18.00	2.43	1.82	1.64	5.47	8.02	6.65	3.39	6.71	6.84	7.34	5.031731	2.333157
24.00	1.88	1.67	3.27	4.80	4.04	7.91	5.28	6.52	4.90	9.08	4.934256	2.287024
24.05	1.98	1.68	3.37	4.87	4.95	7.48	5.05	6.25	4.59	11.83	5.20432	2.773402
24.10	1.86	1.64	3.50	4.80	4.28	7.11	4.72	6.85	4.56	12.57	5.187705	2.984994
24.20	1.84	1.69	3.80	4.80	4.13	7.03	4.50	6.35	3.92	12.92	5.09976	3.053287
24.30	1.69	1.65	3.64	4.65	3.91	9.22	4.39	5.95	3.67	11.18	4.996132	2.904971
24.50	1.41	1.51	3.67	4.96	3.92	7.60	4.24	5.76	3.35	9.60	4.601729	2.411865
24.75	1.18	1.62	3.74	4.92	3.59	7.04	4.12	5.74	3.90	8.28	4.41428	2.088848
25.00	1.22	1.49	3.35	4.68	3.51	5.32	3.57	5.11	4.74	6.83	3.981873	1.640166
25.25	1.26	1.42	3.30	3.84	3.35	4.47	3.19	4.91	4.29	5.83	3.586687	1.361079
25.50	1.18	1.29	3.20	3.18	3.27	4.25	2.84	4.34	4.30	5.79	3.365546	1.33803
26.00	1.32	1.34	2.48	3.86	4.71	3.81	2.42	3.91	3.05	5.07	3.198139	1.233912
26.50	1.69	1.30	2.27	3.65	3.26	3.16	2.23	3.26	3.26	4.44	2.852963	0.906054
27.00	1.53	1.18	2.49	2.63	4.60	4.17	2.85	2.78	2.39	3.17	2.779131	0.990338
28.00	1.45	0.79	2.79	2.60	3.58	6.04	2.66	2.69	2.12	3.49	2.819485	1.341335
29.00	0.84	0.78	1.65	2.66	2.70	7.94	2.74	2.32	2.63	4.51	2.8781	1.970769
30.00	0.83	0.91	1.08	2.23	1.89	6.23	1.83	1.51	3.06	2.63	2.219311	1.505121
32.00	1.38	0.38	0.78	1.16	1.22	3.47	1.31	0.85	1.50	2.18	1.423451	0.820302
36.00	0.50	0.26	0.27	0.66	0.50	2.13	0.80	0.00	0.81	0.39	0.631432	0.55499
48.00	0.00	0.00	0.00	0.00	0.00	1.37	0.00	0.00	0.18	0.00	0.155106	0.408343
60.00	0.00	0.00	0.00	0.00	0.00	0.45	0.00	0.00	0.00	0.00	0.045269	0.135807
72.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0	0

Table 7: Serum 3-OH-lidocaine concentrations

Time (hr)	6553.00	6567.00	46.00	3992.00	1153.00	11785.00	90.00	1495.00	1511.00	9623.00	MEGX	STDEVP
0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.64	0.00	0.00	0.00	0.46	1.39
1.00	13.12	40.91	96.77	41.28	43.14	53.70	28.28	114.59	65.52	93.59	59.09	31.19
2.00	10.74	84.61	31.29	30.42	31.59	81.53	47.06	34.05	75.24	151.35	57.79	39.21
4.00	6.54	9.17	13.32	2.03	8.38	7.40	8.66	9.37	11.60	10.63	8.71	2.92
8.00	12.68	4.87	8.56	2.54	3.78	23.09	3.96	7.31	4.89	6.47	7.81	5.80
12.00	8.73	3.28	5.08	1.45	2.50	7.19	3.10	5.70	5.24	7.11	4.94	2.21
18.00	5.41	4.12	1.61	3.26	3.08	8.26	2.43	6.88	3.69	6.79	4.55	2.07
24.00	8.88	3.18	10.79	2.99	9.62	3.68	3.90	5.36	3.41	23.32	7.51	5.96
24.05	3.88	3.46	10.25	2.47	14.28	3.16	3.38	5.03	3.31	26.02	7.52	7.15
24.10	3.63	3.44	20.99	2.11	3.73	2.96	2.48	7.44	3.89	26.54	7.72	8.23
24.20	2.88	2.65	16.93	2.18	4.04	4.14	2.35	6.44	2.30	24.98	6.89	7.36
24.30	2.45	2.37	10.67	1.80	2.86	159.19	1.76	5.73	2.19	16.49	20.55	46.44
24.50	2.68	1.98	6.43	0.00	1.65	42.16	1.67	3.09	2.77	13.31	7.57	12.07
24.75	2.00	1.91	5.77	1.09	1.55	6.79	0.00	4.13	2.28	6.67	3.22	2.32
25.00	1.80	1.66	15.36	0.00	2.03	3.94	0.00	1.22	1.60	6.27	3.39	4.36
25.25	1.84	1.32	1.87	0.00	0.00	3.77	0.00	3.18	1.66	4.79	1.84	1.57
25.50	1.37	1.13	1.93	0.00	0.00	3.77	0.00	2.03	1.34	5.80	1.74	1.74
26.00	1.37	0.00	0.00	0.00	0.00	3.49	0.00	1.94	0.00	2.29	0.91	1.22
26.50	1.39	0.00	1.00	0.00	0.00	2.10	0.00	1.40	1.24	3.32	1.05	1.05
27.00	0.00	0.00	1.20	0.00	0.00	1.92	0.00	0.00	0.00	2.59	0.57	0.93
28.00	0.00	5.59	1.96	0.00	0.00	0.00	0.00	0.00	0.00	2.05	0.96	1.73
29.00	0.00	2.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.68
30.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.70	0.17	0.51
32.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
36.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
48.00	0.00	1.05	0.00	0.00	0.00	2.68	0.00	0.00	3.64	0.00	0.98	1.33
60.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 8: Serum MEGX-lidocaine concentrations

Time(hr)	6553	6567	46	3992	1153	11785	90	1495	1511	9623	Average	STDEVP
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
1.00	154.6	357.5	263.9	173.0	349.1	245.2	310.9	335.8	225.4	276.0	269.13	67.33
2.00	117.8	360.1	165.7	132.3	244.5	257.5	215.0	347.6	424.4	236.4	250.13	95.92
4.00	71.0	481.0	183.6	63.2	181.0	39.4	252.8	255.0	114.8	167.2	180.90	122.98
8.00	211.3	646.0	145.8	162.1	114.0		232.2	332.6	90.8	161.2	232.91	160.94
12.00	90.9	674.7	157.7	120.1	191.8	277.3	230.3	429.4	108.2	224.3	250.46	169.87
18.00	106.8	911.6	30.4	152.3	296.6	183.4	285.9	323.9	118.8	190.5	260.01	234.32
24.00	1300.1	644.9	417.7	161.4	291	178.4	300.9	344.3	144.5		420.36	343.65
24.05	83.5	695.6	334.3	142.3		126.7	255.3	331.6	145.0	1395.1	389.92	396.21
24.10	66.0	658.0	1504.4	136.8	315.5	106.1	227.5	310.8	145.3	1208.6	467.90	476.54
24.20	66.1	518.7	513.6	110.4	311.6	92.8	193.6	282.7	117.2	953.2	315.98	263.87
24.30	49.1	515.4	359.1	122.9	263.0		181.9	233.7	114.8	613.6	272.63	179.43
24.50	44.6	405.5	177.8	100.7	201.4		151.4	237.2	122.8	485.5	214.10	135.87
24.75	27.7	404.7	128.2	84.0	194.0	544.7	129.5	136.8	90.9	364.3	210.49	159.94
25.00	19.8	390.5	144.8	76.9	241.2	154.1	104.2	106.2	64.0	292.3	159.41	109.20
25.25	18.7	352.4	72.6	69.3	99.7	89.2	70.8	121.6	61.7	242.3	119.83	95.50
25.50	16.2	322.0	68.7	47.3	87.3	128.6	55.9	80.7	42.5	212.4	106.16	88.83
26.00	13.8	283.7	71.3	39.3	77.2	105.8	41.3	59.0	29.1	160.8	88.14	76.60
26.50	15.6	262.5	65.8	30.9	81.8	29.4	38.0	45.4	26.9	169.9	76.61	75.09
27.00	7.2	164.0	47.5	19.9	69.2	19.6	21.3	23.3	18.6	104.7	49.54	47.48
28.00	4.2		71.0	13.4	30.7	14.2	13.8	19.7	12.3	66.6	27.32	23.18
29.00	5.9		27.7	14.2	37.3	18.0	10.8	14.1	6.9	36.6	19.06	11.32
30.00	17.0	90.3	40.0	18.9	9.8	76.6	10.3	10.2	12.9	28.5	31.44	27.68
32.00	16.1	53.9	32.6	17.7	29.9	20.9	9.9	8.0	15.6	26.4	23.10	12.80
36.00	8.3	29.1	29.1	19.5	62.8	8.0	4.2	7.4	8.3	33.4	21.00	17.30
48.00	4.6	15.2	22.2	24.9	15.2		2.1	4.5		36.1	15.60	11.05
60.00	5.4	14.2	24.2		15.9	4.8	2.4	4.1	4.2	14.9	10.01	7.08
72.00	2.9	14.0	35.1	49.6	19.8	3.0	0.0	5.8	0.0	27.2	15.75	16.11
96.00	0.0	11.1	5.2	0.0	10.8	6.2	0.0	3.2	0.0	0.0	3.65	4.27

Table 9: Serum ketamine concentrations

Time (hr)	6553	6567	46	3992	1153	11785	90	1495	1511	9623	Average	STDEVP
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
1.00	325.6	296.3	307.9	186.8	484.8	393.8	482.9	477.3	367.4	376.6	369.94	91.05
2.00	250.8	319.3	322.8	191.7	415.8	371.0	480.2	712.0	387.7	364.7	381.59	134.54
4.00	231.7	547.3	320.4	92.4	509.4	270.2	567.9	539.0	312.5	339.0	372.98	152.11
8.00	212.0	471.3	258.1	136.0	289.9	343.5	396.5	466.1	238.5	222.4	303.43	106.94
12.00	183.8	459.6	241.6	110.6	334.1	375.7	326.2	569.2	216.3	289.5	310.65	128.19
18.00	246.9	696.1	44.6	150.9	452.5	298.2	347.1	471.0	205.4	228.4	314.12	177.19
24.00	201.7	556.3	225.6	168.3	206.2	376.9	376.3	540.8	248.7	321.9	322.26	132.26
24.05	197.5	625.2	237.0	180.6	231.8	343.1	349.9	499.1	260.1	415.7	333.99	136.58
24.10	199.1	633.7	234.3	174.5	211.5	323.5	336.6	523.3	258.7	542.3	343.77	155.72
24.20	197.9	541.9	257.7	176.3	242.7	314.5	325.1	521.8	241.3	550.2	336.94	138.46
24.30	156.8	558.7	262.5	184.5	176.2	185.0	318.6	471.4	245.2	504.6	306.36	143.14
24.50	125.7	498.6	217.8	160.4	223.7	346.5	298.2	487.4	257.3	473.9	308.94	130.56
24.75	90.7	490.7	192.9	128.1	177.4	334.1	269.9	323.2	204.7	447.6	265.92	125.74
25.00	78.4	534.9	173.0	115.5	176.6	248.5	232.3	303.1	172.6	381.1	241.59	128.69
25.25	71.8	430.5	136.1	102.8	152.7	211.8	183.5	338.1	157.4	366.4	215.11	114.91
25.50	68.6	411.5	129.4	84.3	137.7	206.0	158.9	260.7	128.7	352.9	193.88	108.50
26.00	53.7	432.5	93.2	72.5	117.0	197.6	138.4	192.6	104.4	320.1	172.21	113.88
26.50	46.5	451.1	79.0	60.9	87.1	138.6	125.9	165.5	97.4	242.7	149.46	114.29
27.00	38.0	299.1	59.7	45.3	77.1	138.0	91.7	108.2	75.0	178.7	111.08	74.69
28.00	25.3	251.1	44.0	31.2	48.7	96.4	63.7	111.5	58.6	91.6	82.21	62.45
29.00	20.2	216.0	26.2	17.4	36.5	87.2	43.3	65.0	27.9	64.2	60.40	56.13
30.00	24.7	174.9	21.5	15.9	27.5	55.6	25.9	36.8	28.6	42.4	45.37	44.53
32.00	19.3	110.8	7.1	6.0	14.9	22.1	15.5	15.6	19.5	16.5	24.74	29.09
36.00	8.8	47.7	0.0	0.0	5.4	12.9	0.0	8.9	10.3	7.1	10.10	13.29
48.00	0.0	13.0	0.0	0.0	0.0	0.0	0.0	5.0	9.5	6.2	3.37	4.57
60.00	0.0	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.4	1.43	2.97
72.00	0.0	7.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9	1.32	2.67
96.00		5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	1.12	2.10

Table 10: Serum norketamine concentrations

Time (hr)	6553	6567	46	3992	1153	11785	90	1495	1511	9623	Average	STDEVP
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00	0.00
1.00	84.6	42.6	59.8	53.1	53.2	123.0	68.4	58.5	108.8	79.4	73.14	24.64
2.00	78.6	50.1	60.0	52.9	57.7	93.6	74.6	100.1	121.4	78.3	76.76	21.81
4.00	76.8	33.6	53.1	19.1	86.4	93.0	70.2	63.5	88.7	60.9	64.50	22.84
8.00	58.0	23.2	38.8	26.8	57.8	73.1	46.7	51.9	51.7	39.1	46.70	14.37
12.00	54.9	21.5	40.4	33.1	42.0	94.7	38.2	61.2	40.7	51.8	47.84	18.94
18.00	63.5	24.7	8.5	27.3	41.8	79.6	40.0	67.3	30.3	29.8	41.29	21.06
24.00	48.6	32.7	38.8	36.0	41.7	77.5	47.6	59.2	39.6	51.4	47.29	12.56
24.05	68.8	32.9	41.9	32.9	42.6	75.0	48.8	58.2	42.0	54.9	49.80	13.57
24.10	66.2	34.2	39.4	31.9	38.1	68.6	46.3	55.8	38.5	74.1	49.31	14.82
24.20	53.8	36.9	44.9	30.9	36.1	103.0	43.6	61.1	37.6	83.0	53.09	22.12
24.30	58.1	32.5	39.9	34.7	35.0	140.5	40.4	61.5	41.1	89.6	57.33	32.32
24.50	47.9	31.6	36.3	32.2	35.2	130.1	43.3	64.1	41.2	80.9	54.28	29.29
24.75	40.5	33.6	32.5	24.7	33.6	118.6	39.8	54.8	35.1	81.5	49.48	27.59
25.00	39.8	25.0	34.1	27.5	37.9	75.6	35.7	44.9	31.5	59.5	41.17	14.73
25.25	34.5	24.2	23.0	24.9	27.8	64.3	29.5	50.6	24.5	60.4	36.38	15.10
25.50	32.9	29.1	26.6	18.9	25.3	60.5	27.5	43.5	23.5	59.5	34.73	14.03
26.00	31.1	28.5	19.1	18.9	23.9	56.0	23.8	41.3	18.9	49.8	31.12	12.76
26.50	29.4	28.0	15.9	14.3	17.8	59.5	21.9	38.9	17.2	46.8	28.98	14.31
27.00	20.4	22.4	13.3	11.0	17.7	40.3	17.7	26.6	17.0	32.4	21.86	8.51
28.00	18.0	15.9	9.4	10.9	15.4	29.5	17.5	24.4	14.7	19.7	17.54	5.67
29.00	13.9	15.3	7.4	4.8	14.2	29.0	12.2	13.9	8.9	18.4	13.81	6.35
30.00	13.3	15.8	5.6	3.8	10.0	20.5	6.1	9.9	8.4	13.9	10.73	4.91
32.00	8.9	10.4	2.9	2.7	6.3	18.0	3.8	4.2	4.4	7.1	6.87	4.43
36.00	4.8	3.7	1.7	1.4	2.0	9.0	2.2	1.1	2.8	1.2	2.99	2.29
48.00	1.3	0.8	0.4	0.8	0.8	2.1	0.4	0.7	2.2	0.5	1.01	0.62
60.00	0.5	0.0	0.3	0.0	0.4	1.1	0.4	0.4	0.3	0.3	0.37	0.29
72.00	0.0	0.0	0.0	0.4	0.3	0.8	0.0	0.4	0.0	0.0	0.19	0.26
96.00	0.0	0.0	0.0	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.08	0.16

Table 11: Serum dehydronorketamine concentration